DISSOCIATION STUDIES OF POLYOMA TEMPERATURE SENSITIVE MUTANT VIRIONS

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by

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II. LITERATURE REVIEW

INTRODUCTION

Polyoma virus, an endemic virus of mice, was discovered by Gross (45), to be the agent responsible for inducing parotid tumors in newborn mice and was subsequently isolated and adapted to tissue culture by Stewart (72, 73). It was found to be the first oncogenic virus capable of inducing a wide range of tumors in its natural host, the mouse, but was also able to cross the species barrier and induce tumors in other rodents as well (29). Polyoma virus is both structurally and antigenically similar to the various papilloma viruses as well as SV40, BK, and JC virus. It has therefore been placed in the family Papovaviridae. Because polyoma virus is able to exist and reproduce widely in its natural environment only rarely inducing tumors, but frequently induces tumors upon introduction to a non-permissive environment, it has been extensively used to study the mechanisms of both viral replication and transformation. One method commonly employed to study these mechanisms is observing the effects caused by various types of mutations on the polyoma genome. In this way it is possible to study the role which each protein plays in the viral replication or transformation cycle. To do this several classes of polyoma mutants have been developed; deletion mutants, host-range mutants, and temperature sensitive (ts) mutants. The use of these mutants, coupled with the recent advancement of techniques in the field of molecular biology, has led to a wealth of new information on both viral replication and transformation. The following review will summarize the recent knowledge of polyoma research and the temperature sensitive class of polyoma virus mutants. Other current reviews of the literature are listed in the bibliography (5, 20, 31, 39, 80).

PRODUCTIVE INFECTION

Productive infection results from the exposure of polyoma virus to permissive cells leading to replication of the virus and death of the cell. The events of viral replication can be divided into several stages: 1) Absorption, penetration, and uncoating; 2) Replication and transcription of viral DNA and biosynthesis of proteins; and 3) assembly and maturation of progeny virions leading to cell death and lysis. The first stage of infection was studied by Mackay and Consigli (55), using electron microscopy autoradiography and thin sectioning techniques. They found that after an infectious virion attaches to the cell membrane it is engulfed in a monopinocytic vesicle and transported directly to the nucleus. It then crosses the nuclear membrane and is released free in the nucleoplasm as a complete virus particle. The capsid structure is lost shortly after crossing the nuclear membrane because intact particles are not seen to accumulate. The events following nuclear penetration were further elucidated by Winston et. al. (82), after isolating nuclei from infected cells. From these nuclei they were able to extract a viral uncoating intermediate sedimenting at 190s. Under electron microscopy this complex was shown to be a viral nucleoprotein core associated with a host contributed structure visualized to be derived by fragmentation of the nuclear matrix. The viral nucleoprotein complex isolated by this method was found to preferentially retain VP2 and VP3 but was otherwise similar to polyoma nucleoprotein complexes isolated by other methods (12, 44, 56). Both transcription and DNA replication have been shown to occur in conjunction with this type of structure (13, 22).

The next stage of the replication cycle; replication, transcription, and translation, may be further subdivided into events occurring before and

after viral DNA synthesis. Viral DNA synthesis begins to occur at approximately 12-15 hours post infection, but before this there is transcription of early viral mRNA (67). These viral specific mRNAs have been found to code for the three tumor ("T") antigen proteins of polyoma. The exact roles which these proteins play during the lytic cycle of polyoma infection are still in question. Some functions have been attributed to these proteins even though the exact mechanism of action remains obscure. Large "T" antigen has been found to induce cellular DNA synthesis (17, 77), repress early mRNA synthesis (18), initiate viral DNA replication (7, 76), and promote late mRNA transcrption (66). Small "T" antigen is thought to induce constituative factors in cells which are necessary for permissive growth of the virus (71). A role for middle "T" antigen in lytic infection has been postulated by Benjamin (5), to be involved in virus assembly. Shortly after viral DNA replication begins the presence of late mRNAs can be found in the cytoplasm (4). These late mRNAs code for the structure proteins VP1, VP2, and VP3. The structural proteins begin to accumulate in the cytoplasm of the cell and are subsequently transported to the nucleus (51).

Very little is known about the last stage of polyoma virus replication; assembly and maturation. However, it is believed to be similar to the assembly of Simian virus 40 (SV 40). Using methods of extracting assembly intermediates from the nuclei of SV40 infected cells it has been found that there is an orderly assemblage of structural proteins around the viral chromatin, rather than insertion of viral chromatin into preassembled capsid structures as previously proposed (32, 49, 62). The first step in assembly is the conversion of the 75s viral DNA complex to a 200s assembly intermediate by the addition of structural proteins and loss of histone H1, possibly by replacement with VP3 (17, 32). At this time hyperacetylation of host contributed histones also occurs (17). The 200s assembly inter-

mediate is then converted to a 240s particle which by electron microscopy has an identical appearance to and cosediments with mature virus particles. These immature intracellular 240s particles however are sensitive to salt, EDTA, and detergents, and have a lower specific infectivity than mature virus particles (17). It is assumed that the final stabilization of the immature 240s particle is brought about by the formation of interchain disulfide bonds and possibly modification of capsid proteins.

GENOME

Polyoma DNA is a small double stranded covalently closed circular molecule with a molecular weight of $3.4 \times (10)^6$ daltons or 5290 nucleotide pairs. Using restriction enzymes a genetic map of the polyoma genome has been constructed (39). One of the DNA strands, designated the early strand, codes for the "T" antigens. The other strand codes for the structural proteins and is designated the late strand. Recently two separate groups have sequenced the entire polyoma genome and from these data amino acid sequences of the proteins can be predicted (3, 21, 40, 69, 70). In the cases where the predicted amino acid sequences have been compared to actual sequences considerable agreement has been seen (47, 52).

PROTEINS

The protein constituents of polyoma virus were initially separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The virus contains three structural proteins; VP1, 42,500 daltons; VP2, 34,600 daltons; VP3, 22,800 daltons; and a dimer of VP1, 85,000 daltons which is believed to be a result of interchain disulfide bonding. VP1 accounts for 70% of the total protein content of the virus, with VP2 and VP3 representing 12% (12). The virus also contains the four host contributed histones H3,

H2A, H2B, and H4 associated with the viral DNA and accounting for approximately 18% of the virion protein (42, 56). The "T" antigen proteins have also been examined with SDS-PAGE and have the following molecular weights: large "T", 100,000 daltons; middle "t", 55,000 daltons; and small "t", 17,000 daltons (65).

In order to study the biosynthesis of the structural proteins McMillen and Consigli (56), injected rabbits with the SDS-PAGE separated proteins and extracted the antisera. These experiments lead to the interesting finding that antisera developed to the histone region of the gel was far more efficacious in hemagglutination inhibition (HAI) and neutralization assays than any of the antisera derived from the structural proteins. This phenomenon was later explained by the finding of Bolen and Consigli (8), that VP1 has an intrinsic fragility and breaks down into three major fragments of 29,000 daltons, 16,000 daltons, and 14,000 daltons of which the lower molecular weight fragments react with the anti-histone antisera. They were further able to determine using a capsid affinity column that the 16,000 dalton fragment reacted with the neutralizing antibodies. This then implies that these fragments of VP1 contain the region responsible for cell binding. Further studies by Anders and Consigli (2), have determined that the carboxy terminus of VP1 is responsible for cell binding.

The O'Farrell technique of isoelectricfocusing is another method which has recently been used to further characterize the polyoma structural proteins showing that VP1 consists of six isoelectric species designated A through F (6, 60). The species differences are due in part to different patterns of phosphorylation, presumably brought about by post-translational modifcation. The work of Bolen et. al. (6), has brought forward evidence that each species has a specific function in the structural or regulatory mechanisms of the polyoma virion. They showed that the most basic species,

VP1 A, is not present in purified capsids which lack DNA and histones. Also the VP1 A in polyoma virions is poorly labeled *in vitro* by iodination techniques which preferentially label the exterior of the virion. Furthermore, when replication complexes isolated form virions dissociated by the EGTA-DTT method of Brady *et. al.* (11), were analyzed the VP1 subspecies predominantly present was VP1 A (6, 12). Similar results were obtained using SV40, where it was also found that the presence of VP1 enhanced transcriptional activity by five-fold (9, 10). These data indicate that VP1 A is closely associated to the virion nucleoprotein complex and may perform a functional role in viral transcription.

By using the same principle as in the immunological studies performed with the VP1 fragments described above, Bolen *et. al.* (6, 8), were able to demonstrate that the neutralizing IgG antibodies reacted specifically to VP1 E and the HAI IgG antibodies reacted specifically to VP1 D and VP1 F. Bolen and Consigli (7, 8), were also able to demonstrate that the polyoma capsids do not compete for the same binding sites on the cell as the polyoma virions and that the capsids lack VP1 E. Therefore, it appears that the subspecies D, E, and F play an important role in the recognition of cell binding sites.

The VP2 and VP3 genes are overlapping genes located in the late region of the genome. They are read in the same reading frame and share a common C-terminus. Tryptic peptide analysis has shown that the VP3 protein is a subset of the larger VP2 protein (46). Isoelectricfocusing studies have shown that the VP2 and VP3 each consist of two species (74). While it has been found that both VP2 and VP3 of SV40 are phosphorylated it has not yet been shown with polyoma VP2 and VP3 (19, 75).

The majority of the information of the VP2 and VP3 functions comes from work with various mutants mapping in the VP2/3 regions. The growth of

mutants in this region is usually restrictive in some early event of infection or else is assembly defective. These findings have lead to speculation that VP2/3 has a direct role in the uncoating and/or assembly steps or replication; albeit evidence that in SV40 VP2 may be dispensible due to the isolation of viable VP2 deletion mutants (19). The most current evidence seems to indicate that VP2/3 may bind to transcription complexes and act as attenuators of transcription (54).

STRUCTURE

Electron microscopy of polyoma has shown it to be an icosahedral virus with a diameter of 45nm consisting of 72 capsomeres (53, 81). It was long thought that the 72 capsomeres were composed of 60 hexomeres containing 360 identical protein subunits (protomeres) and 12 pentameres containing 60 protomeres (34). Recent evidence of X-ray diffraction studies however have lead to the proposal that all 62 capsomeres are pentomeres and a model of a capsid consisting of 360 VP1 subunits was proposed (1, 63). This is consistent with the most recent evidence that VP2 and VP3 are not an integral part of the capsid structure. If this model is correct it could provide further support to the evidence that the differential phosphorylation of VP1 leads to specific subspecies functions, allowing the resulting subspecies to interact in a manner which conserves bonding specificity.

The importance of interchain disulfide bonds in maintaining the structural integrity of the capsid was shown by several laboratories (11, 30, 68, 79). They found that the disulfide linkages protected the viral DNA from digestion by micrococcal nuclease and the capsid structure from the denaturing conditions of sodium dodecyl sulfate. VP1 is the only protein containing cysteine residues and is therefore the only protein capable of forming disulfide linkages (21, 40, 47, 69, 70).

As shown by Brady et. al. (11), another integral component of the capsid structure is calcium and that by chelating the calcium with ethyleneglycol-bis-N,N'-tetracetic acid (EGTA) in the presence of a reducing agent dithiothreitol (DTT) it is possible to separate the virion into a nucleoprotein complex and capsomeres. DTT alone is unable to promote breakdown of the virion. This may explain the mechanism for the uncoating of the virion in the nucleus as the nucleus contains low concentrations of free calcium, calcium binding proteins, and has considerable reducing potential. Indeed there is evidence that the role of divalent cation stabilization may be a general principle in animal virus structure.

TEMPERATURE SENSITIVE MUTANTS

Temperature sensitive (ts) mutants of polyoma virus were first isolated by Freid (37), by exposing wild type virus to the chemical mutagen nitrous acid. The nitrous acid induced alterations in the base sequence of the DNA and clones of this mutagenized virus were isolated by plaquing the virus in permissive 3T3 cells at 32°C. By comparing the yield of the virus when grown in cells at the permissive temperature (32°C) to the nonpermissive temperature (39°C) Freid was able to isolate those clones showing a temperature sensitive trait. Because the mutations are due to an altered DNA base sequence the corresponding amino acid sequence coded for by that cistron will also be altered. Due to these alterations in the amino acid sequence the structure of the protein is altered in such a way that it is functional at the permissive temperature, but nonfunctional at the nonpermissive temperature. Therefore by shifting the cells from the permissive to the nonpermissive temperature it is possible to modify the expression of the mutationally altered gene product. This method of isolating polyoma ts mutants has made

it theoretically possible to isolate mutations in any gene which is essential for the lytic growth of the virus. It would not be possible however to isolate a virus with a mutation in a gene nonessential to the reproductive cycle of the virus.

The isolation of restriction endonucleases and development of a genetic map of polyoma DNA made it possible to utilize marker rescue experiments in mapping the ts mutants (33, 59). In these experiments wild type DNA is cleaved with specific nucleases and the fragments separated. Each successive fragment is then annealed to the mutant DNA, introduced into a permissive cell at the nonpermissive temperature, and scored for the growth of the wild type virus. If the wild type fragment corresponds to the segment of the mutant DNA containing the mutated base then the virus will be rescued under conditions normally nonpermissive for its growth.

The polyoma ts mutants have been divided rather broadly into two classes, early and late, based on whether the defective function occurs before or after the onset of viral DNA replication during an infectious cycle. The following information is a review of the current knowledge of two mutants of the early class, tsA and ts3, and one mutant of the late class, ts10.

tsA: Mapping studies of the tsA mutant using marker rescue techniques have placed the lesion in the early region of the polyoma genome, between 1 and 26 map units from the Eco R1 site (59). This region has been found to code for the carboxyl terminal portion of the large "T" antigen. Many of the polyoma mutants of this class have been shown to produce large "T" antigens of variable sizes and in quantities below that of wild type virus (48). None of the tsA mutants mapped to date have been found to have mutations in either the middle or small "t" antigens (78).

When tsA mutants are used to infect permissive cells at the restrictive temperature there is no detection of polyoma DNA synthesis (35), however, there is induction of cellular DNA synthesis as well as the surface changes which bring about increased agglutinability using wheat germ agglutinin or concanvalin A (25, 28, 38). The tsA mutant is not capable of transforming BHK cells at the nonpermissive temperature (36). If, however, these cells are first transformed at the permissive temperature and then subsequently raised to the nonpermissive temperature they remain stabily transformed (23, 24, 36). This implies that the polyoma tsA gene product is required for the initiation but not the maintenance of the transformed state. It has also be found that large "T" antigen of polyoma is a DNA-binding protein which binds specifically to the region near the origin of DNA replication (14). In temperature shift experiments polyoma DNA molecules that have already begun their replication cycle before the shift to the nonpermissive temperature are completed, but no new rounds of replication are initiated (14). This indicates that polyoma large "T" antigen plays a functional role in the initiation of polyoma DNA replication.

There is evidence that large "T" antigen also has a function in regulating late transcription and protein synthesis. When tsA is used to infect permissive cells at the restrictive temperature there is no synthesis of viral late mRNA or proteins. If cells are infected at the permissive temperature viral DNA replication and late viral mRNA transcription occurs. In temperature shift experiments of tsA infected cells a shift from the permissive to the restrictive temperature results in a rapid halt of viral DNA replication but late mRNA continues to be transcribed (64). It has not been established how the transient availability of a functional large "T" antigen is capable of establishing a steady transcription of late genes but the most

logical explanation is that cells infected by tsA mutants at the permissive temperature accumulate templates for late transcription which remain active after a shift to the restrictive temperature.

An overall summary of the above information shows that the polyoma tsA gene plays an important regulatory role in both viral replication where it is an initiator of viral DNA replication and a promotor of late transcription; and in transformation where it is an initiator of the transformed state.

ts3: Marker rescue experiments have shown that Hpa II fragment 3 can restore the function of the ts3 mutant (33). The Hpa II fragment 3 region of the polyoma genome predominantly codes for the N-terminus of VP2 but also includes a small portion of the N-terminus of VP3 (33, 78). Gonenne et. al. (43), have studied the ts3 mutant using two-dimensional electrophoresis. Isoelectricfocusing was performed in the first dimension followed by SDS-PAGE in the second dimension. Using this technique they found that one of the ts3 VP2 subspecies had an increased isoelectric point when compared to the wild type virus (43). This would indicate that the ts3 mutation lies in the N-terminal region of the VP2 protein. However, because the VP3 protein is a subset of the VP2 protein and the Hpa II fragment 3 does contain a small region of the VP3 gene the possibility of a mutation in the VP3 protein cannot be totally excluded.

The expression of the ts3 mutation occurs early after infection. At the restrictive temperature induction of cellular DNA synthesis, initiation of viral DNA synthesis, and viral induced cell surface changes are not seen (27). The ts3 defect can be overcome by a short incubation at the permissive temperature prior to a shift to the restrictive temperature (27). Also, the

growth of the ts3 mutant is not temperature sensitive when carried out with ts3 viral DNA (27). The ts3 mutant also expresses a type of host range phenomena in that the extent to which it exhibits temperature sensitive growth depends on the type of cells infected. The greatest temperature sensitivity is seen in BALB/3T3 cells followed by primary mouse kidney cells and secondary mouse embryo cells (27). A temperature restrictive growth pattern is not observed at all in the wild type polyoma transformed PY6/3T3 cell line (27). Because of the above findings it has been hypothesized that the defect in the VP2/3 of the ts3 mutant somehow results in a failure of nuclear penetration and uncoating and the host range phenomena is due to differences in the expression of certain cellular genes affecting the permissibility of each cell line. An alternative explanation is that VP2/3 are involved in some early step of viral transcription which the ts3 mutant is unable to perform. Evidence for this theory has recently been supported by the work of Llopis and Stark (54), indicating that VP2 and/or VP3 act as attenuators of viral transcription. In this model the ts3 mutant would have an attenuator function which binds to the transcriptional complex more tightly than the wild type VP2/3 and thereby would delay the expression of early events in the replicative cycle.

ts10: Using the technique of marker rescue mapping, the ts10 polyoma mutant is rescued by the Hpa II fragments 6 and 1, mapping between 26.6 and 54.4 map units on the polyoma genome (59). This region of the genome codes for the major structural protein VP1 (51). Using the predicted amino acid sequence for VP1, as derived from the nucleotide sequence of polyoma DNA by Soeda et. al. (78), it can be deduced that the ts10 lesion occurs somewhere following the first 40 amino acids of the N-terminus. SDS-PAGE

analysis by this laboratory has not observed any change in the molecular weights of the ts10 structural proteins. Forst and Bourgaux (41), however did observe a change in the migration pattern of VP2 and VP3 which suggests that these structural proteins are slightly larger then the wild type VP2 and VP3 (41). These reported differences in the molecular weights of the VP2/3 of the ts10 mutant may possibly be due to strain differences. However, since the marker rescue experiments indicate that the functional lesion of the ts10 mutant is located in the VP1 protein the significance of the VP2/3 finding is unknown.

Heat inactivation studies of the ts10 late class of polyoma and many of the late mutants of complimentation groups B, C and BC of SV40 have shown these mutants to be more heat labile then other ts mutants (15, 16, 24). In the case of the ts10 virion it is approximately 1000 times more susceptible to heat inactivation when compared to wild type and tsA virions (24). The isolated ts10 DNA does not exhibit this heat sensitivity, lending evidence to the fact that the VP1 structural protein plays an important role in maintaining the infectivity of the virion structure.

When ts10 infects nonpermissive cells at the restrictive temperature there is no reduction in the transforming ability (61). The ts10 mutant is also capable of inducing large "T" antigen synthesis, cellular DNA synthesis, viral DNA synthesis, and cell surface changes in permissive cells at the restrictive temperature (61). The structural protein VP1 however is not made at the restrictive temperature (61). It has not been determined if this is due to a lack of production of the 16s mRNA coding for VP1 or a failure of translation of the 16s mRNA.

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III

DISSOCIATION STUDIES OF POLYOMA TEMPERATURE SENSITIVE MUTANT VIRIONS

ABSTRACT

The virion structure of three temperature sensitive (ts) mutants of polyoma virus; tsA, ts3, and ts10 were compared to wild type virus. By comparing structural susceptibility to β-mercaptoethanol (ME) in hemagglutination assays ts10 was found to be highly sensitive to the reducing agent followed respectively by ts3, tsA, and wild type polyoma. Treatment of each mutant with ethyleneglycol-bis-N,N'-tetracetic acid (EGTA) and ME at pH 8.5 resulted in dissociation of the virions into a DNA-protein complex and capsomere subunits. Velocity sedimentation analysis could detect no differences in the DNA-protein complexes of the mutants compared to the wild type DNA-protein complex. Velocity sedimentation analysis of the 18s, 12s, and 5s capsomere subunits revealed that the ts10, 18s and 12s species were highly susceptible to the experimental dissociation conditions followed respectively in sensitivity by ts3, tsA, and wild type polyoma. Raising the temperature of dissociation from 25°C to 40°C resulted in a relative decrease in the 18s and 12s species and a concomitant increase in the 5s species. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of capsomere species dissociated at 25°C and 40°C revealed a decrease of VP1 in the 18s and 12s species and an increase of VP1 in the 5s species when dissociation occurred at 40°C. Isoelectricfocusing analysis of the ts10 VP1 could detect no differences in the pI's of the six ts10 VP1 species as compared to the wild type VP1 species. It was found that the ts10 VP1 contained a relatively greater proportion of the phosphorylated species D, E, and F and a lesser proportion of the nonphosphorylated species B and C as compared to the wild type VP1 species.

INTRODUCTION

Polyoma virus contains three structural proteins: VP1 (42,500 daltons), VP2 (34,600 daltons), and VP3 (22,800 daltons), (8). Of these three proteins recent work has shown that the major structural protein VP1, associated with the divalent cation Ca^{++}, is the only constituent responsible for the icosehedral capsid structure of polyoma virus (1, 21). Brady et. al. (6), developed a method by which chelation of the Ca^{++} with ethyleneglycol-bis-N, N'-tetracetic acid (EGTA), and reducing the interchain disulfide bonds with the reducing agent dithiothreitol (DTT), it was possible to dissociate the virion structure into capsomeres and a DNA-protein complex in vitro under physiological conditions. Treatment with EGTA or DTT alone did not lead to dissociation. The importance of the interchain disulfide bonds in protecting the structural integrity of the capsid from denaturing conditions and the DNA from nuclease digestion has been shown by several laboratories (6, 13, 21, 25).

It has also been shown that VP1 is involved in roles which are non-structural, including regulation of transcription and identification of the receptor sites for hemagglutination and infection (3, 4, 5). All of these functions are not due to a single VP1 protein however, but to a family of six subspecies identified using the O'Farrell technique of two-dimensional electrophoresis (3, 20). These six subspecies possibly differ due to post-translational modifications such as phosphorylation.

In order to achieve a more in depth understanding of how Ca^{++}, interchain disulfide bonds, intrachain disulfide bonds, and post-translational modifications of VP1 interact to produce a stable infectious virion we have in this study utilized the above techniques to characterize the interaction

of the structural proteins in the virions of three temperature sensitive (ts) mutants of polyoma virus. Two mutants, ts10 and ts3, were specifically chosen because of previous studies describing interesting phenotypic alterations attributed to mutations in their structural proteins. The VP1 mutant ts10 is extremely heat sensitive, exhibiting an 1000 fold greater loss of infectivity following a six minute exposure to 68°C than either tsA or wild type virus (10). The isolated ts10 DNA does not exhibit this heat sensitivity. The inference has been made that the ts10 VP1 mutation results in an altered capsid structure which is highly sensitive to environmental conditions. The defect of the VP2 mutant ts3 is expressed early after infection for at the restrictive temperature induction of cellular DNA synthesis, initiation of viral DNA synthesis, and cell surface changes are not seen (11). The ts3 mutant will not compliment the growth of other temperature sensitive mutants, however, isolated ts3 DNA does not express a temperature sensitive trait (12). Due to the above observations it has been hypothesized that the defect in the VP2 of the ts3 mutant somehow results in a failure of nuclear penetration and uncoating. The third mutant used in this study was tsA. The tsA mutant has a defect in the large "T" antigen but possesses no known mutations in the structural proteins and was therefore chosen to represent an additional control between the wild type virus and the structural mutants ts10 and ts3.

In this study we have shown that both the virion structure and the capsomere subspecies of ts10 are highly sensitive to the presence of reducing agents. The ts3 mutant exhibits an intermediate sensitivity and tsA behaves similar to the wild type virus. Examination of the six VP1 species of ts10 indicate that there are significant alterations in the distribution of these species in the ts10 virion.

MATERIALS AND METHODS

Cell and virus propagation. These studies were performed using a small plaque strain wild type virus and the temperature sensitive mutants tsA, ts3, and ts10 obtained from Dr. Walter Eckhart. The virus was grown in primary mouse kidney cells at a multiplicity of infection of 10. Virus absorption was allowed to occur at 39°C for a period of 1 hour at which time the temperature sensitive mutant cultures were shifted to the permissive temperature of 32°C. The infected cultures were maintained in serum free Dulbecco's modified Eagle's medium (DMEM). The preparation of primary mouse kidney cells has been described (24).

Virus purification. Virions were purified from infected cell lysates as described previously (18). CsCl equalibrium gradients used to purify the virus were described more fully in the publication of Brady et. al. (6).

Preparation of radioactively labeled virus. The preparation of (^{3}H)-deoxyribosylthymine (^{3}H)dT labeled virus has been previously described (19). Purified virus was labeled in vitro with ^{125}I or ^{131}I by the method of Frost and Bourgaux (14).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of capsomere proteins. Isolated capsomeres were precipitated by the addition of cold trichloroacetic acid (TCA) to a final concentration of 25% and incubated for 2 h at 0°C. The precipitates were collected by centrifugation at 10,000 rpm (Sorvall HB-4 rotor) for 30 min. Each pellet was washed with one volume of 25% TCA and subsequently with 2 volumes of ice-cold acetone and air dried. Solubilization of the precipitates has been described (19). The

SDS-PAGE slab gels consisted of a 15% acrylamide running gel with a 0.2% bis acrylamide crosslinker and a 5% acrylamide stacking gel. Samples were loaded and subjected to electrophoresis at 15mA until the samples were concentrated at the interface, at which time the current was raised to 30mA for the duration of the run. Electrophoresis was stopped when the tracking dye eluted from the running gel. The running buffer consisted of 0.1% SDS-0.38M glycine in Tris buffer (0.05M, pH 8.4). After electrophoresis the gels were fixed by allowing the gel to soak overnight in 50% methanol-7.5% acetic acid. The dried radioactively labeled gels were exposed to Kodak medical X-ray film (NS-2T). The film was developed with D-19 Kodak developer for 5 min, washed in 1% acetic acid for 3 min, and then transferred to Kodak fixer for 10 min.

Isoelectricfocusing of ^{125}I and ^{131}I labeled VP1. Equal molar amounts of ^{125}I-labeled mutant and ^{131}I-labeled wild type virion proteins were first isolated by SDS-PAGE tube gels prepared identical to the slab gels described above. The tube gels were sliced into 2 mm segments and the radioactivity of each segment was determined by counting in a gamma counter. The peak segments containing the ^{125}I-labeled VP1 protein of ts10 were then mixed with the ^{131}I-labeled VP1 segments of the wild type virus and subjected to isofocusing by the O'Farrell method (20). The ampholines used for isoelectricfocusing (IEF: combining pH 3.5 to 10 and pH 5 to 8 ampholines) yielded very shallow pH gradients between pH 5 and 8. pI's were determined by slicing the focusing gels immediately after electrophoresis and measuring the pH of the individual gel segments in distilled water. The radioactivity within the gel slices were measured in a LKB minigamma.

Dissociation of the virus and analysis of the virion components. Stock solutions of each component of the dissociation buffer (5M NaCl,

200mM ethyleneglycol-bis-N,N'-tetracetic acid (EGTA), and 0.05M Tris pH 8.7) were prepared and stored at 4°C, with the exception of 1.0M β-mercaptoethanol (ME) which was prepared fresh for each assay. Specific experimental conditions for virion dissociation are described in the figure legends for each experiment. Virions or products derived from dissociated virions were layered onto either 5 to 20% or 10 to 30% (wt/vol) sucrose gradients containing 5mM EGTA, 0.15M NaCl, 0.01M Tris-hydrochloride (pH 8.5), and 0.25% Triton X-100. Centrifugation was in an SW50.1 rotor at 40,000 rpm (4°C) for 40 min to determine dissociation, 2 h to isolate DNA-protein complexes, or 12 h to isolated capsomeres as described for each experiment.

Hemagglutination assays. The hemagglutination assay used in this study was described previously by Consigli et. al. (9). In each experiment the hemagglutination titer of purified stocks of the wild type and tsA, ts3, and ts10 mutants were initially determined and all stocks diluted with 0.01M Tris (pH 7.4) to have the same initial hemagglutination titers. All experiments were performed at both room temperature (25°C) and 40°C. For experiments at the nonpermissive temperature (40°C) virus stocks were equilibrated in a water bath maintained at 40°C for a period of 15 min before exposure to dissociation reagents. At the time periods indicated for each experiment aliquots were removed and serial dilutions (1:2) were made in phosphate buffered saline (PBS). Guinea pig erythrocytes were added and the hemagglutination titers determined after incubation at 4°C for 3 h.

Quantitative assays. Protein concentrations were determined with the Biorad assay using bovine serum albumin as the standard. Samples labeled with β-emitters were quantitated in a toluene-Triton (3:1) scintillation fluid with a Beckman LS-33 liquid scintillation counter. ^{125}I and ^{131}I-labeled samples were determined in a LKB Minigamma counter.

RESULTS

Comparison of the structural sensitivity of the capsids to ME. Initial experiments showed that all of the viruses lost their hemagglutinating activity in the presence of the standardized dissociation mixture 1mM EGTA, 0.1M ME, 0.15M NaCl, in 0.05M Tris buffer (pH 8.5) but that the loss occurred at different rates indicating that there existed differences in the susceptibilities of the capsid structures to the dissociation mixture. Therefore to compare the sensitivity of the structural integrity of the intact ts mutant virions hemagglutination assays were performed at the permissive and nonpermissive temperatures in the presence of various concentrations of EGTA, ME, and EGTA-ME. No loss of hemagglutination activity was seen under any of the experimental conditions tested in which the disulfide reducing agent ME was omitted from the assay (data now shown). However the mutants did exhibit a differential sensitivity to the concentration of ME. Fig. 1 shows the affect of 0.1M ME on the hemagglutination activity of the mutants at both the permissive (25°C) and the nonpermissive (40°C) temperatures. In these experiments all virus stocks were adjusted to have the same initial titer and the affect of ME on the integrity of the capsids was measured by comparing the survival of hemagglutination activity as a function of time and temperature. The VP1 mutant, ts10, exhibited the greatest degree of sensitivity by loosing 75% of its hemagglutinating activity following one minute of exposure to 0.1M ME at 25°C. The VP2 mutant, ts3, was intermediate in sensitivity, loosing 50% of its hemagglutinating activity under identical conditions. The tsA and wild type virions were the least sensitive. The large "T" mutant, tsA, did not exhibit a loss in hemagglutination activity until after five minutes of exposure to 0.1M ME at 25°C and the wild type virus did not loose hemagglutinating activity until after ten minutes of exposure under these conditions.

To investigate the possibility of structural changes occurring in the ts mutants at the nonpermissive temperature, the temperature was raised to 40°C and the experiment repeated. There was no loss in hemagglutination titer by any of the viruses when untreated control samples were incubated at 40°C. However, all of the viruses were more sensitive to reducing agents when incubated at 40°C. After one minute of exposure to 0.1M ME at 40°C the hemagglutination activity of ts10 was reduced by 87.5%; ts3 by 75%; tsA by 25%; and wild type by 25%. Aften ten minutes of incubation the ts10 and ts3 mutants retained only 6% of their original hemagglutination titer and tsA and wild type 12.5% of their original titers. When the incubation temperature was raised from 25°C to 40°C all of the viruses exhibited an increased susceptibility to the presence of the reducing agent. It is unclear whether this is due to a change in the capsid structure at the higher temperature leading to increased exposure or weakening of the VP1 disulfide linkages or if it is due to an increased efficacy of the ME at the higher temperature. Similar results were obtained however when dithiothreitol (DTT) was used as the reducing agent (data not shown). From these experiments it is suggestive that the mutants show a consistent difference in their sensitivity to the reducing agent β-mercaptoethanol which is temperature independent.

Comparison of the EGTA-ME dissociation of the wild type and the temperature sensitive mutants. Following dissociation of the (^{3}H)dt-labeled virions the dissociation products (DNA-protein complex and capsomere subunits), as well as the intact virions, were compared on parallel 10 to 30% sucrose gradients (Fig. 2). All of the intact untreated mutants had sedimentation coefficients of 240s; identical to that of wild type virus. After treatment of the virions with the EGTA-ME dissociation buffer there was a

decrease in the sedimentation rates of all four viruses. No appreciable radioactivity could be detected in the sedimentation region of the intact virions. The dissociated (^{3}H)dt-labeled virion products migrated as a single broad peak with a sedimentation coefficient of approximately 48s, and occasionally a smaller peak migrating at approximately 21s; the sedimentation coefficient of naked polyoma DNA. The amount of radioactivity migrating in this lighter 21s peak was found to vary with each virus preparation and also increased with prolonged storage of the virus at 4°C.

SDS-PAGE was performed on samples of ^{125}I-labeled intact and dissociated viruses to determine if dissociation with EGTA-ME would result in changes in the molecular weights of the structural proteins. Samples of the virus were dissociated at both 25°C and 40°C using the standardized dissociation mixture. After a two hour incubation period at the respective temperatures the samples were then analyzed by SDS-PAGE. The protein migration patterns of each mutant were identical to those of the wild type virus (data not shown).

Comparison of the DNA-protein complexes. To examine the stability of the DNA-protein complexes of the temperature sensitive mutants, (^{3}H)dt-labeled mutants and wild type polyoma were dissociated at both the permissive and the nonpermissive temperatures using equimolar amounts of protein in each dissociation reaction. The samples were then analyzed in parallel 5 to 20% sucrose gradients (Fig. 3). In each case the major peak of radioactivity was seen to migrate with a sedimentation coefficient of 48s and a minor peak of naked polyoma DNA sedimenting at 21s. In addition, no significant changes were detected as a result of dissociation at the nonpermissive temperature as opposed to the permissive temperature. The 48s DNA-protein complex released by the action of the dissociation buffer on the polyoma virion was described

in detail previously by Brady et. al. (7). Briefly it was found to consist of approximately equal amounts of the cell contributed histones and the virally coded VP1. The presence of VP2 and VP3 could be detected in very low amounts. The association of VP1 with the 48s DNA-protein complex was found to be conditionally dependent upon the environment, and the VP1 was lost under conditions of high ionic concentrations.

Comparison of the capsomere subspecies. To examine the stability of the capsomere subunits of the temperature sensitive mutants, ^{125}I-labeled mutants and wild type polyoma were dissociated at both the permissive and nonpermissive temperatures using equimolar amounts of protein in each dissociation reaction. To isolate the capsomere subspecies the samples were centrifuged in parallel 5 to 20% sucrose gradients as described in Fig. 4. The capsomeres of all three mutants separated into the 18s, 12s, and 5s capsomere subspecies as previously described for wild type polyoma by Brady et. al. (7). As can be seen in Fig. 4, while the dissociation of the virus at 40°C does not lead to a change in the sedimentation values of the capsomere subspecies, it does lead to a change in the relative proportions of each species present. Table 1 shows a comparison that was made between the proportion of radioactivity present in each capsomere subspecies of each virus dissociated at room temperature compared to the same virus dissociated at 40°C. In each case dissociation at 40°C resulted in a loss of the 18s and 12s subspecies and a concomitant increase in the 5s subspecies. By comparing the net change in the capsomere subspecies of each virus dissociated at 25°C to 40°C a pattern similar to that of the hemagglutination sensitivity (Fig. 1), of the mutants was seen. The VP1 mutant ts10 exhibited the greatest shift from the 18s and 12s subspecies to the 5s subspecies; followed

respectively by ts3, tsA, and wild type polyoma. SDS-PAGE analysis of the isolated capsomeres from the 25°C and 40°C dissociations were compared for each virus. It was found that in each case the 40°C 5s subspecies contained an increased amount of VP1 proportional to the loss of the VP1 observed in the 18s and 12s subspecies. Fig. 5 represents the autoradiogram of the ts10 capsomeres isolated after dissociation at both 25°C and 40°C. These findings are consistent with evidence from this laboratory suggesting that the 18s species is a dimer of the 12s species resulting from interchain disulfide linkages between VP1 proteins and the 12s species is a result of intrachain disulfide linkages within VP1 proteins. As these linkages are reduced the sedimentation coefficients of the resulting VP1 capsomere structures are seen to shift progressively from the 18s and 12s species to the 5s species.

Isoelectricfocusing of the ts10 VP1. Recently it has been determined by isoelectrofocusing studies that the VP1 of polyoma actually consists of six separate species (20). Bolen et. al. (3), were able to determine that these species differed in modifications such as phosphorylation and they were able to assign functions to the separate species. Since the previous experiments have indicated that the VP1 of ts10 is highly sensitive to reducing agents and as the ts10 was the only VP1 mutant employed in this study an effort was made to determine if there were any alterations in the VP1 species of ts10 as compared to the wild type VP1. To do this equimolar amounts of ^{125}I-labeled ts10 VP1 and ^{131}I-labeled wild type VP1 were mixed together and isofocused using the O'Farrell technique (Fig. 6). The VP1 of ts10 contained six species which comigrated with the same isoelectric points as the six species of wild type VP1, however the six species were present in different proportions. Table 2 summarizes the relative proportions of the species present in each virus. VP1 species A is associated exclusively with the DNA-protein complex and is not found in capsids lacking DNA (3). This species is found in the

same proportion in both ts10 and wild type virus. While the nonphosphorylated species B and C constitute the majority of VP1 in both the wild type and the ts10, the ts10 mutant had a significantly lower proportion of these two species. VP1 species B and C are believed to be the major species involved in forming the capsid structure. The phosphorylated polyoma VP1 species D, E, and F have been found to be responsible for the important functions of specific cell binding and hemagglutination. These three species constitute a greater proportion of the ts10 VP1 compliment than is present in the wild type.

DISCUSSION

Our goal in these studies was to utilize some of the recent techniques used in advancing the knowledge of the structure of the polyoma virion to investigate how mutations in the structural proteins effect function in the virion structure. To do this we chose two temperature sensitive mutants (ts3 and ts10) which would be representative of mutations in each of the structural proteins (VP1 and VP2/3) and one mutant which has a mutation in the large "T" antigen but no known mutations in the structural proteins (tsA). These experiments were designed to specifically examine the virion structure and have shown that the tsA mutant, as expected, exhibits characteristics which are virtually identical to the wild type virus. The VP1 mutant ts10 was found to have a structure similar to the wild type virus in biophysical characteristics but markedly different from the wild type in its sensitivity to certain environmental conditions. The VP2 mutant ts3 exhibited characteristics which were consistently intermediate between the wild type and the ts10. Initially it was determined that the virion structure of all of the viruses were the same in regards to velocity sedimentation and SDS-PAGE

analysis. In addition all of the viruses could be dissociated with EGTA-ME into the characteristic DNA-protein complex and capsomere subunits. This indicates that any mutation present in the structural proteins of the mutants examined are not of a nature to result in major structural changes to the capsid. Closer examination of the intact virion and its dissociation products revealed that the mutations in the structural proteins did result in altered abilities of the capsid structure to withstand certain environmental conditions.

Previous experiments with ts10 have shown that it was 1000 times more heat sensitive than either wild type or tsA virions (10). Experiments in this study have shown that when ts10 virions were subjected to low concentrations of reducing agents the structure of the virion was altered in such a way as to result in decreased hemagglutination of guinea pig erythrocytes, and that increasing the environmental temperature resulted in an increased loss in hemagglutination activity (Fig. 1). This phenomenon was also observed to a lesser degree with the other viruses and has been previously reported with wild type polyoma by Hare and Chan (16). Reducing agents alone are not able to completely destroy the hemagglutination activity or dissociate the virion but these experiments do imply the importance which disulfide bonds play in maintaining the correct three-dimensional orientation of the structural proteins within the virion.

When EGTA is added in the presence of a reducing agent the virus further dissociates into capsomeres and a DNA-protein complex. The capsomeres can be separated into three species; 18s, 12s, and 5s. Further evidence in this laboratory indicates that the faster sedimenting 18s species are doublets of the 12s capsomeres possibly held together by interchain disulfide bonds and that the 12s structure is a result of intrachain disulfide linkages.

If this is the case then the temperature sensitivity of the 18s and 12s species of ts10 would be consistent with the hemagglutination studies which showed an increased sensitivity of the VP1 disulfide bonds in ts10.

Analysis of the ts10 VP1 subspecies by isoelectrofocusing did not reveal an actual charge change in any of the six species as compared to the wild type VP1. It did however show that the overall proportions of the VP1 species of ts10 were different from those of the wild type (Fig. 6). It is interesting to note that the VP1 species A which is found associated with the DNA-protein complex was present in the same proportion of both ts10 VP1 and wild type VP1 for no differences were found between the ts10 and wild type DNA-protein complexes. The proportional differences between the VP1 species of the ts10 mutant compared to the wild type VP1 are also interesting. The fact that the ts10 virus apparently contains a lower relative proportion of the nonphosphorylated species B and C which Bolen et. al. (3), attributed to being the major species responsible for maintaining the virion structure may be significant in light of our observations indicating the instability of the ts10 capsid. While it is apparent that the mutation occurring in the ts10 VP1 does not result in a change in the overall production of the various VP1 species it is possible that the mutation does affect the rate of production of the various species. Because the ts10 virion contains a greater relative proportion of the minor phosphorylated species D, E, and F it would be interesting to determine if the ts10 virion contains an increased amount of phosphorylation as compared to the wild type virus. An understanding of the real significance between the differences in the isofocusing patterns of the ts10 and wild type VP1 must wait until there is a more thorough understanding of the post-translational modifications of polyoma proteins.

The recent work by Anders and Consigli (2) have further characterized

the wild type VP1 species. They have presented evidence that the receptor moiety lies in the carboxy terminal region of VP1 and that the major phosphorylation site resides in the central portion of the VP1 molecule; most probably on threonine residues between amino acid numbers 143 and 159. Since we have presented evidence in this research that there are no charge differences between ts10 and wild type VP1 it is suggestive that the ts10 mutation is located anterior to amino acid 143. Marker rescue experiments and restriction enzyme analysis of the ts10 genome have resulted in the conclusion that the ts10 mutation occurs somewhere following the first 40 amino acids of the N-terminus. It would therefore appear that the ts10 mutation occurs somewhere between amino acids 40 and 143. All of the experiments presented in this study point to the indication that there is a weaker disulfide bonding pattern in the VP1 linkages of the ts10 virion. Looking at the predicted VP1 sequence of Soeda et. al. (23), it can be seen that there are two cysteine residues between amino acids 40 and 143. As the majority of temperature sensitive mutations are due to amino acid substitutions it can be easily visualized that a possible substitution in this area could result in a change in the tertiary or quarternary structure of the VP1 protein preventing the proper conformation required for the establishment of a full complement of disulfide linkages.

The exact function of the VP2 and VP3 structural proteins have not yet been determined. Current evidence suggests that they are most likely attenuators of viral transcription and are not covalently attached to the viral capsids (17). This does not exclude the possibility that they may interact with the capsids through noncovalent bonding and in some way protect the disulfide bonds. Isofocusing analysis of ts3 has found that one of the VP2 species has an increased isoelectric point (15). This change in pI may result

in a partial exposure of some of the disulfide bonds in the ts3 capsid structure which would explain the intermediate sensitivity ts3 exhibits to reducing agents.

In this research we have further established the possible importance of the role of disulfide bonding in the polyoma virion. We have presented evidence indicating that without the proper arrangement of disulfide linkages to maintain the capsid structure there is a loss of integrity within the virion. Because of the recent work indicating that the polyoma VP1 is a highly complex family of proteins performing multiple functional roles in the polyoma virion the ts10 mutant is an ideal key to achieving a greater understanding of this complex system. Future research with the ts10 mutant in this laboraory is aimed at obtaining a greater understanding of the role disulfide linkages have in both interchain and intrachain VP1 interactions and how the post-translational modifications are affected.

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Fig. 1. Susceptibility of the capsid structure of wild type polyoma and temperature sensitive mutants to 0.1M ME as measured by survival of hemagglutination activity. (A), susceptibility at the permissive temperature (25°C); (B), susceptibility at the nonpermissive temperature (40°C). All samples contained 0.15M NaCl and 0.1M ME in 0.01M Tris buffer (pH 7.4). Symbols: ● wild type; ■ tsA; □ ts3; o ts10.

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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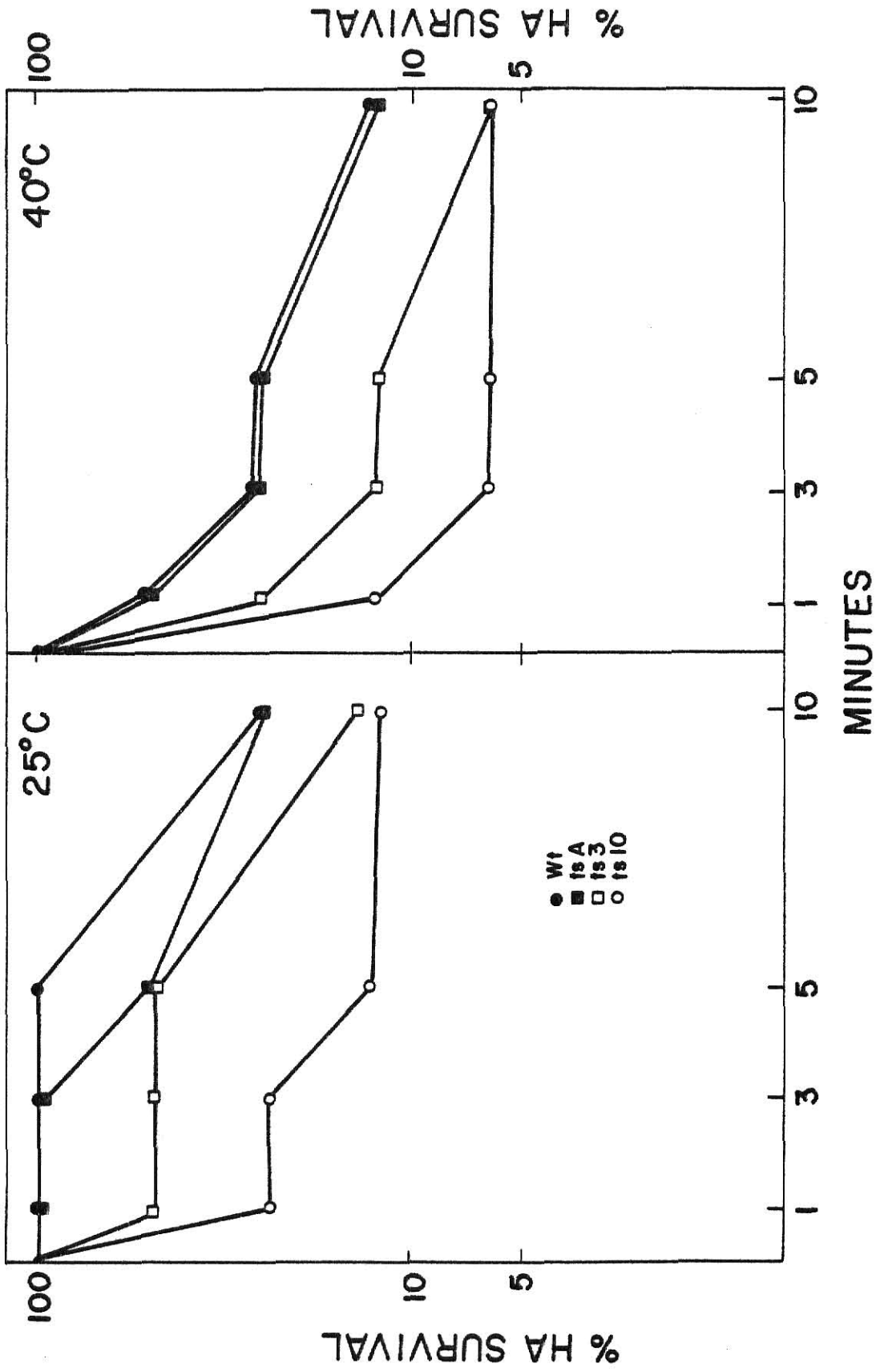
% HA SURVIVAL
100
10
5
40°C
25°C
1
3
5
10
MINUTES
wt
ts A
ts 3
ts 10

Fig. 2. Sedimentation of wild type and temperature sensitive mutant polyoma virions and EGTA-ME dissociated virions in sucrose gradients. (^{3}H)dT-labeled virions were exposed to 1mM EGTA, 0.1M ME, 0.15M NaCl, in 0.05M Tris buffer (pH 8.5) for 30 min at room temperature. Samples were layered onto 10 to 30% sucrose gradients and centrifuged in a SW50.1 rotor at 40,000 rpm for 40 min (4°C). (A), wild type polyoma; (b), tsA; (C), ts3; (D), ts10. Symbols: ●, (^{3}H)dT intact virions; o, (^{3}H)dT dissociated virions.

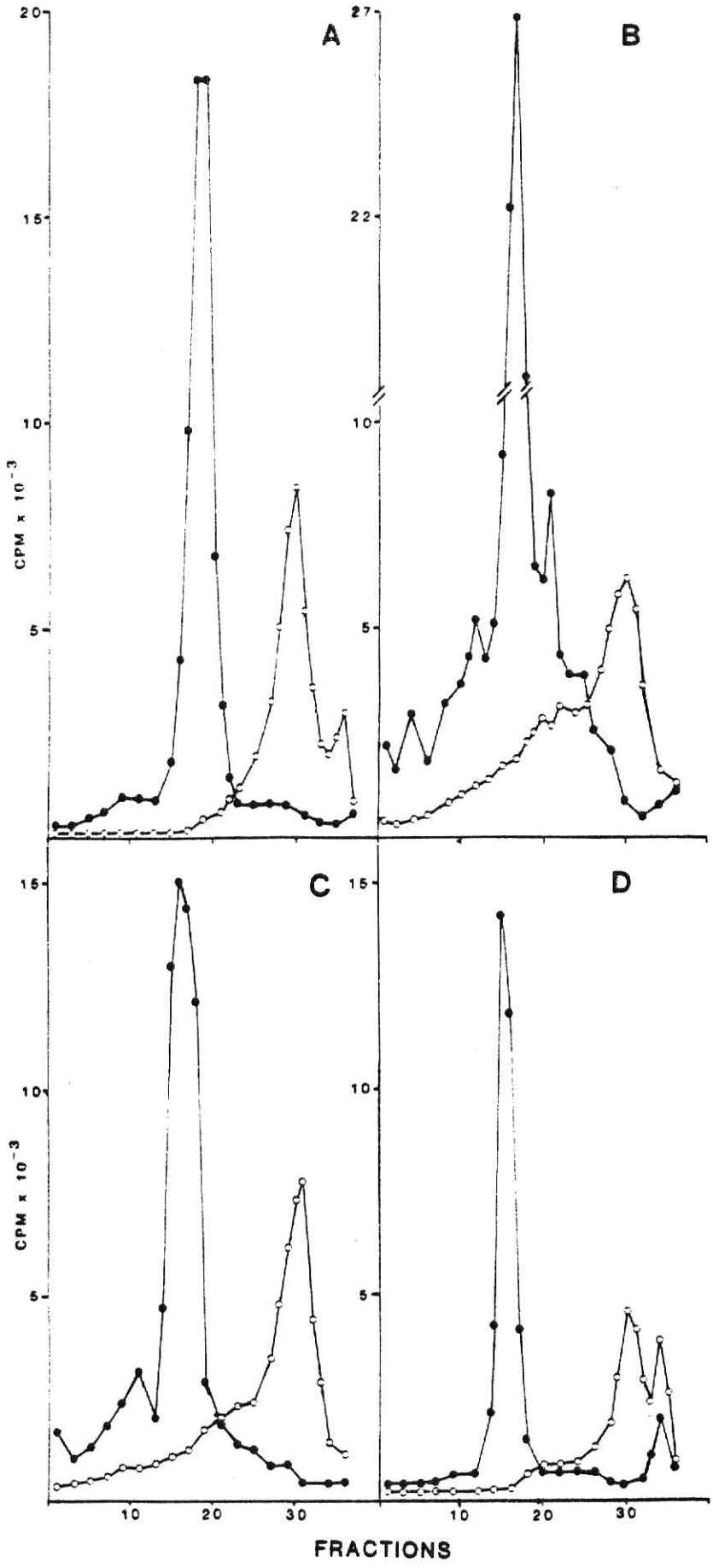
A
B
C
D
CPM x 10⁻³
FRACTIONS
20
15
10
5
27
22
10
20
30

Fig. 3. Sedimentation of EGTA-ME dissociated (^{3}H)dT-labeled wild type and temperature sensitive mutant polyoma virions in sucrose gradients. Virions were dissociated as in Fig. 2. Samples were layered onto 5 to 20% sucrose gradients and centrifuged in a SW50.1 rotor at 40,000 rpm for 2 h (4°C). (A), wild type polyoma; (B) tsA; (C), ts3; (D), ts10. Symbols: o,25°C dissociation; ●, 40°C dissociation.

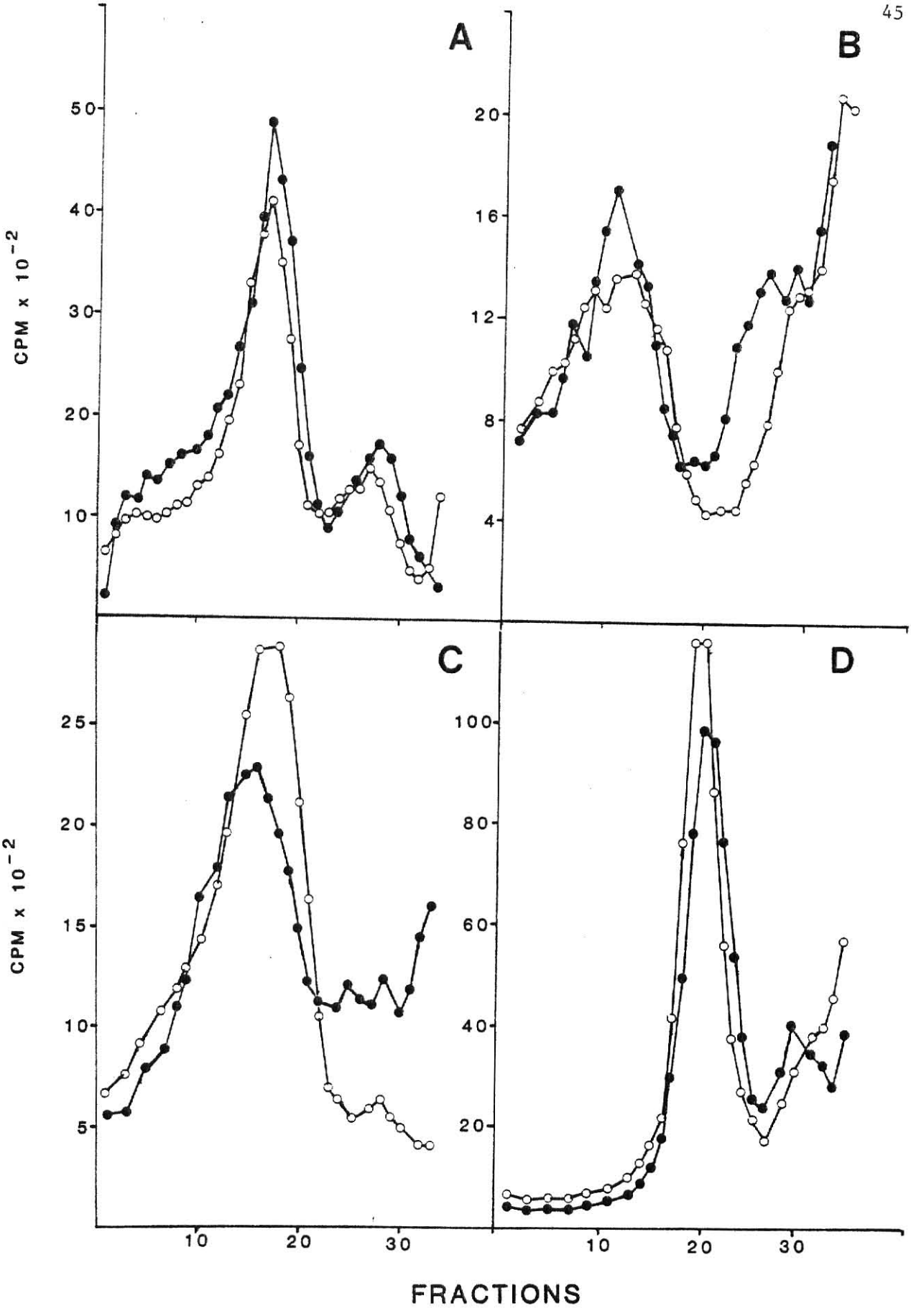
A
B
C
D
CPM x 10⁻²
CPM x 10⁻²
FRACTIONS
50
40
30
20
10
20
16
12
8
4
25
20
15
10
5
100
80
60
40
20
10
20
30
10
20
30

Fig. 4. Sedimentation of EGTA-ME dissociated ^{125}I-labeled wild type and temperature sensitive mutant polyoma virions in sucrose gradients. Virions were dissociated as in Fig. 2. Samples were layered onto 5 to 20% sucrose gradients and centrifuged at 40,000 rpm for 12 H (4°C) in a SW50.1 rotor. (A), wild type polyoma; (B), tsA; (C), ts3; (D) ts10. Symbols: o, 25°C dissociation; ●, 40°C dissociation.

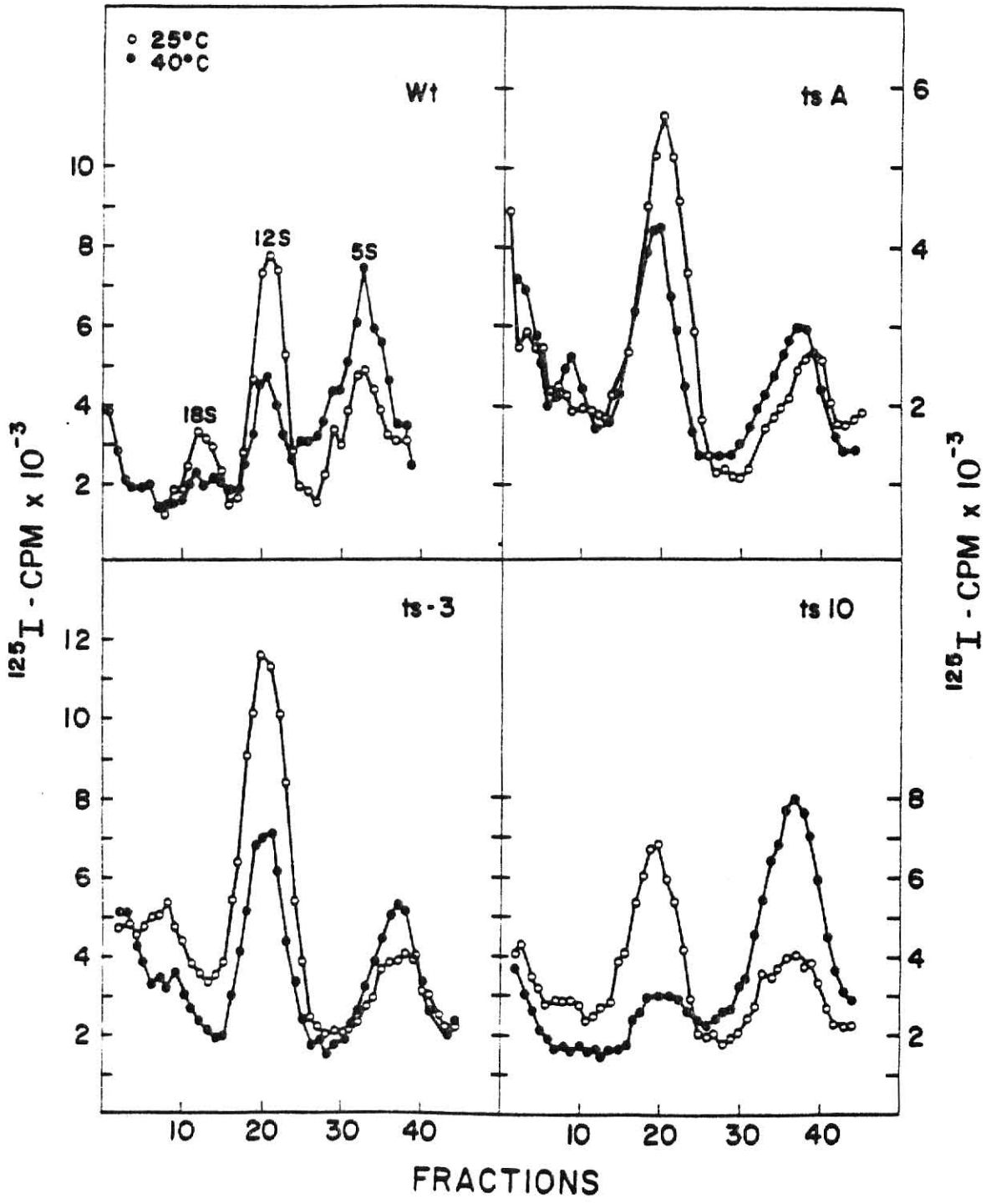
○ 25°C
● 40°C
Wt
ts A
ts-3
ts 10
18S
12S
5S
^{125}I - CPM x 10^{-3}
FRACTIONS

Fig. 5. Autoradiograph of the ^{125}I-labeled ts10 capsomere subspecies isolated as in Fig. 4. Samples were prepared for electrophoresis as described in materials and methods. (1), intact wild type polyoma virions; (2), 18s ts10 capsomeres dissociated at 25°C; (3), 18s ts10 capsomeres dissociated at 40°C; (4), 12s ts10 capsomeres dissociated at 25°C; (5) 12s ts10 capsomeres dissociated at 40°C; (6) 5s ts10 capsomeres dissociated at 25°C; (7) 5s ts10 capsomeres dissociated at 40°C; (8), intact ts10 virions.

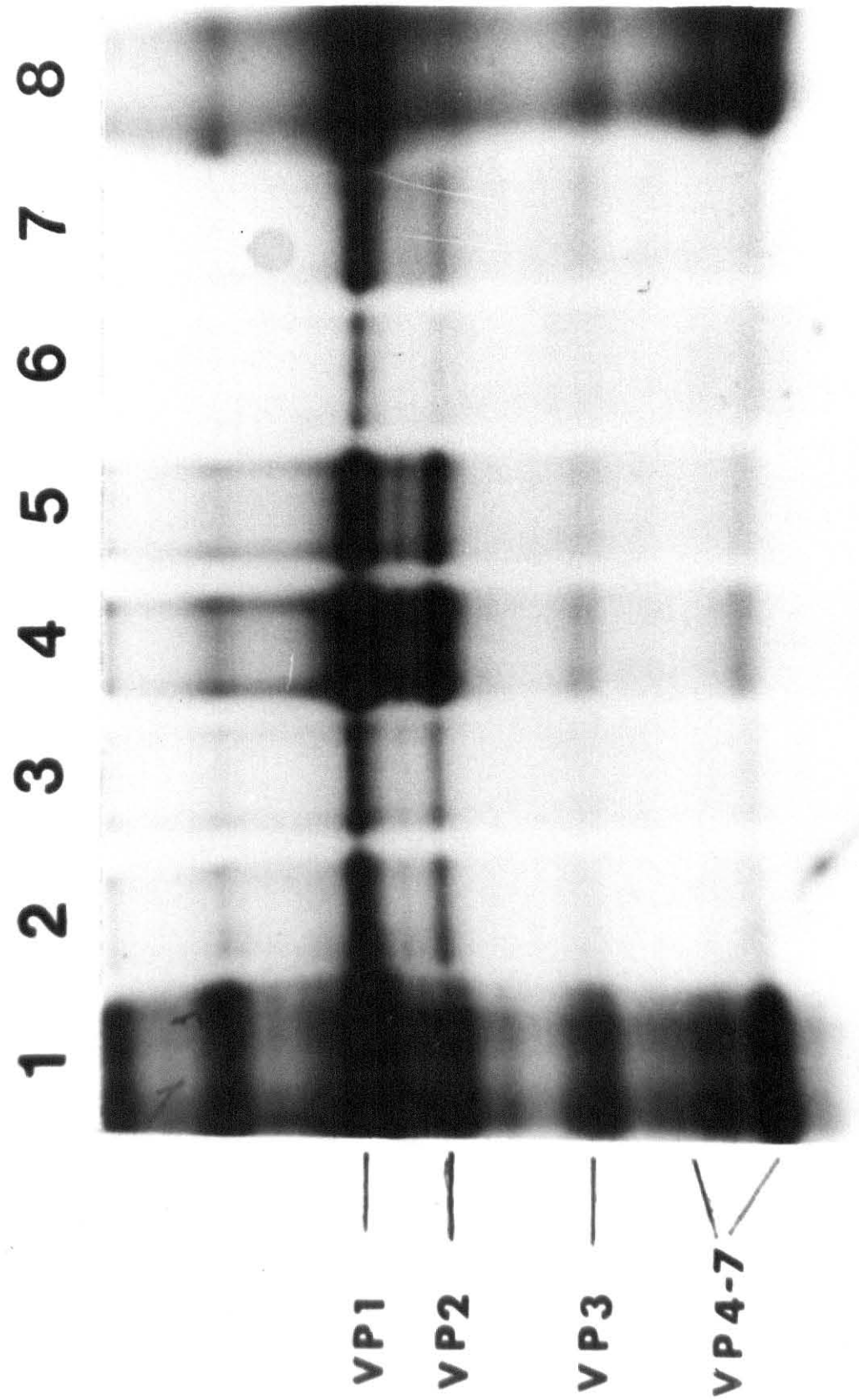
1
2
3
4
5
6
7
8
VP1
VP2
VP3
VP4-7

Fig. 6. IEF of ^{125}I-labeled ts10 VP1 and ^{131}I-labeled wild type VP1. Equal concentrations of ts10 VP1 and wild type VP1 were mixed together and subjected to IEF as described in materials and methods. The gel was then sliced into 2 mm segments and the radioactivity was quantitated in a gamma counter. Symbols: o, ^{131}I-labeled wild type VP1; ●, ^{125}I-labeled ts10 VP1; □, pH.

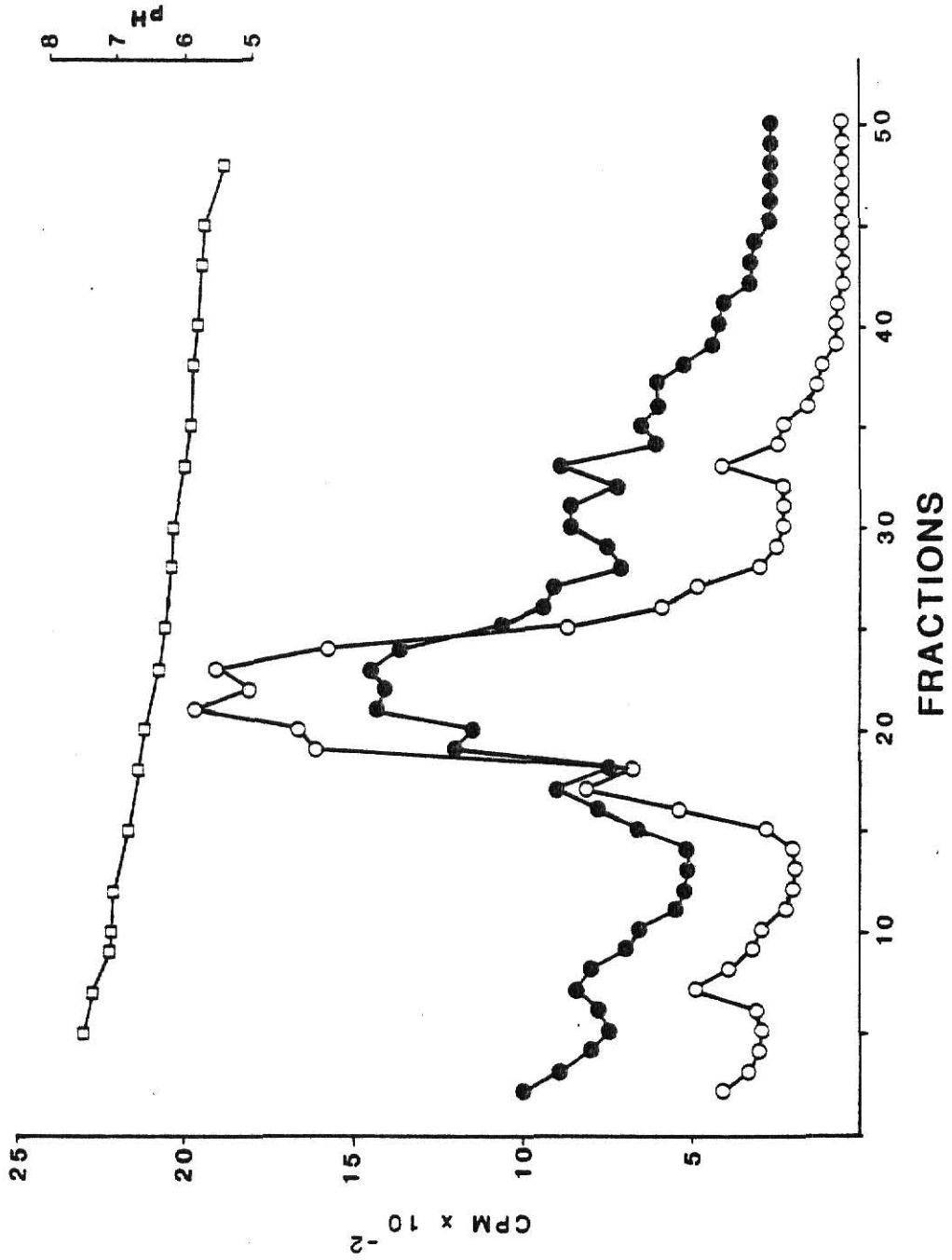
pH
8
7
6
5
CPM x 10 -2
25
20
15
10
5
10
20
30
40
50
FRACTIONS

Table 1. Distribution of percentage of radioactivity in the capsomere species of EGTA-ME dissociated virions.[1]

	% Dissociated							
	25°C				40°C			
	WT	tsA	ts3	ts10	WT	tsA	ts3	ts10
18s	21	17	20	13	19	14	18	8
12s	38	53	55	50	33	46	44	27
5s	41	30	25	37	48	40	38	65

[1]Percentages were calculated by determining the counts per minute present in each peak compared to the total count per minute in the entire gradient.

Table 2. Distribution of the percentages of the ^{125}I-labeled ts10 VP1 species and the ^{131}I-labeled wild type VP1 species after IEF.[1]

Subspecies	^{131}I wild type VP1	^{125}I ts10 VP1
A	13.8	14.5
B	35.7	23.5
C	36.0	25
D	5.0	14
E	6.0	13
F	3.5	10

[1]Percentages were calculated by determining the counts per minute present in each peak compared to the total counts per minute in the entire gel.

IV. ABSTRACT

DISSOCIATION STUDIES OF POLYOMA TEMPERATURE SENSITIVE MUTANT VIRIONS

by

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B. S., Kansas State University, 1978
D. V. M., Kansas State University, 1983

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ABSTRACT

The virion structure of three temperature sensitive (ts) mutants of polyoma virus; tsA, ts3, and ts10 were compared to wild type virus. By comparing structural susceptibility to β-mercaptoethanol (ME) in hemagglutination assays ts10 was found to be highly sensitive to the reducing agent followed respectively by ts3, tsA, and wild type polyoma. Treatment of each mutant with ethyleneglycol-bis-N,N'-tetracetic acid (EGTA) and ME at pH 8.5 resulted in dissociation of the virions into a DNA-protein complex and capsomere subunits. Velocity sedimentation analysis could detect no differences in the DNA-protein complexes of the mutants compared to the wild type DNA-protein complex. Velocity sedimentation analysis of the 18s, 12s, and 5s capsomere subunits revealed that the ts10, 18s and 12s species were highly susceptible to the experimental dissociation conditions followed respectively in sensitivity by ts3, tsA, and wild type polyoma. Raising the temperature of dissociation from 25°C to 40°C resulted in a relative decrease in the 18s and 12s species and a concomitant increase in the 5s species. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of capsomere species dissociated at 25°C and 40°C revealed a decrease of VP1 in the 18s and 12s species and an increase of VP1 in the 5s species when dissociation occurred at 40°C. Isoelectricfocusing analysis of the ts10 VP1 could detect no differences in the pI's of the six ts10 VP1 species as compared to the wild type VP1 species. It was found that the ts10 VP1 contained a relatively greater proportion of the phosphorylated species D, E, and F and a lesser proportion of the nonphosphorylated species B and C as compared to the wild type VP1 species.