SYNTHESIS AND ALBUMIN BINDING PROPERTIES OF THREE SULFUR CONTAINING ORGANIC COMPOUNDS

by

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INTRODUCTION

Purpose

Protein binding is involved in: governing the effective concentration of a drug or metabolite in the presence of serum proteins, transporting substances from one body part to another, and influencing the excretion and retention of small molecules and ions in the tissues (Klotz, et al., 15).

Some types of compounds such as penicillin K and fatty acids (including oleic acid, which is used in the treatment of tuberculosis) are bound so strongly to serum proteins that they are ineffective (Davis and MacLeod, 8). In fact simple long chain fatty acids have been disqualified as chemotherapeutics because of their great affinity for albumin.

Many sulfones, sulfonic acids, and other sulfur containing compounds are used as medicines--or at least have biological properties (Jenkins and Hartung, 11). For example, sulfhydryl group compounds inactivate many antibiotics; several members of the disulfone series possess hypnotic properties; the sulfonic acid salt, zinc phenolsulfonate, is used as an astringent both internally and externally; ichthammol (which is a mixture of sulfides, sulfones, and sulfonates) is used as an emollient and demulcent; and potassium guaiacol sulfonate is used as a sedative expectorant in treatment of colds. Some sulfur containing anticonvulsants include ethylphenyl sulfone, ethylphenyl sulfide, and propylphenyl sulfone (Burger, 4). Finally,

perhaps the best known sulfur containing medicinals are the sulfa drugs.

Since many sulfur containing compounds are used as medicinals and since reversible interaction (binding) with proteins is probably involved in the action, a study of binding of various sulfur compounds with a protein should be beneficial. It was decided to compare compounds which were identical except with respect to the oxidation state of the sulfur. All had a nitro group so as to facilitate quantitative determination on the Beckman spectrophotometer.

Past Work on Protein Binding

Similarly Charged Groups. Klotz and coworkers (17,18) have found that serum albumin shows roughly equal binding affinities toward each of the following anions: methyl orange and compounds having CO_2^-, PO_3H^-, or AsO_3^- instead of the SO_3^- group.

Positional Isomers. It has been found also by Klotz and coworkers (17,18) that serum albumin forms complexes of roughly comparable stability with azo dyes in which the carboxyl group is switched from ortho to meta to para. However, in a compound where a second substituent can intramolecularly hydrogen bond, the binding varies when that substituent is shifted. For example Klotz (14) has found that o-aminobenzoate ion is bound more strongly than p-aminobenzoate. Similarly, it has been found by Luck and Schmit (22) that o-hydroxyphenyl acetate shows

a greater affinity for albumin than does the corresponding para compound.

Sterioisomers. Karush (12) detected small differences in affinity for dextro and levo forms of certain compounds.

Size of Molecule. In interactions with albumin a smooth increase in binding ability generally occurs as chain length is increased (Klotz, et al., 15). This is attributed to stronger van der Waals' forces which accompany increased molecular size. Boyer, et al. (2) observed increased affinity in the order: butyrate, caproate, caprylate, and caprate.

Favoring of Certain Groups and Effect of a Second Group. Teresi and Luck (24) found that added nitro groups in phenols tend to increase binding affinities, but according to Klotz (14) and Rideal (23), added polar groups such as SO_3^- lower the affinity generally because of interaction with water. Similarly it was found by Luck (21) and confirmed by Burkhard, et al. (5) that the introduction of polar groups such as hydroxyl or amino groups into an anion decreases the ability of the anion to form complexes with bovine serum albumin.

Comparison of Ionic with Nonionic Compounds and Factors Causing the Binding. It was shown in one case by Burkhard and Moore (6) that in uncharged molecules the solubility may affect binding (less soluble molecules binding more strongly).

Klotz and Ayers (16) postulated that binding of aminoazobenzene involves a hydrogen bond between a side chain of the protein and an electron pair of the uncharged molecule. They

found that bovine serum albumin binds the neutral dye aminoazobenzene less strongly than it does the methyl orange anion. Boyer, et. al. (3) and Klotz (14) found that protein affinity for small molecules of nearly equal size and structure but with negative charge absent in one shows a marked reduction with loss of charge.

Carsten and Eisen (7) studied a 2,4-dinitrobenzene series with the following groups at the number one position: hydroxyl, ϵ amino caproic acid, Br, NH_2, CH_3, H, and ϵ-N lysine. The binding affinities decreased in the order listed (-OH binding the most and -ϵ-N lysine the least). The authors state that both ionic and non-ionic interactions are involved. Probably van der Waals' interaction with the aromatic ring, hydrogen bonding through the strongly polar nitro groups, and specific effects of substituent groups cause the interaction. They further state that stronger binding of dinitrophenol than of ϵ amino caproic acid may be due to steric effects (the negative group of the former being able to approach closer to the positive sites of the protein). The authors also found evidence that some of the sites may preferentially bind dinitrophenol while others may preferentially bind dinitrotoluene.

EXPERIMENTAL

Synthesis of Compounds

p-Nitrophenyl Methyl Sulfide. Sodium p-nitrothiopheno-

late was first prepared by reaction of p-nitrochlorobenzene with sodium disulfide according to the procedure of Waldron and Reid (26). Twice as much ethyl alcohol as called for was used in order to dissolve the p-nitrochlorobenzene. The p-nitrophenyl methyl sulfide was then prepared by reaction of p-nitrothiophenolate with methyl sulfate according to the method of Gilman and Beaber (9). The product was recrystallized twice from 75 per cent acetic acid giving a product melting at 71 to 71.5° (literature value 72°, Waldron and Reid, 26).

p-Nitrophenyl Methyl Sulfone. This compound was prepared from p-nitrophenyl methyl sulfide by oxidation with hydrogen peroxide according to the procedure of Hinsberg (10) except that acetone was used as the solvent instead of glacial acetic acid. The product was recrystallized twice from methanol giving crystals melting at 143-144° (literature value 142.5°, Waldron and Reid, 26).

Ammonium p-Nitrobenzene Sulfonate. This compound was prepared from p-nitrochlorobenzene by formation of the disulfide with sodium disulfide followed by oxidation with fuming nitric acid and neutralization with ammonium hydroxide according to the method of Bell (1). The product was recrystallized from a solution of four parts ethyl alcohol to one part water, and it was dried over phosphorus pentoxide.

A nitrogen determination by micro Kjeldahl was tried (unsuccessfully) as a means of determining purity of the compound since a melting point could not be obtained. Purity was finally

demonstrated by recrystallizing and scanning on the Beckman spectrophotometer until two successive spectra were identical.

Preparation of Buffer

A buffer having a pH of 6.8 was obtained by dissolving 6.00 grams of potassium dihydrogen phosphate and 7.87 grams of disodium hydrogen phosphate in enough distilled water to make one liter of solution.

Properties of Compounds

Spectra. A buffer solution of each compound was scanned on the Beckman model D. U. spectrophotometer, and the wavelength of maximum absorption was determined for each compound.

Molar Absorbtivity. A buffer solution of each compound of known concentration was prepared. Weighings were made on a micro balance. (The sulfide and sulfone solutions were agitated for 48 hours in order to put all of the solid into solution.) The absorbance of each solution at its wavelength of maximum absorption was then read on the Beckman spectrophotometer using buffer as a blank. The molar absorbtivity was calculated from the molar concentration and the absorbance.

Application of Beer's Law. All three compounds were checked to see that their buffer solutions obeyed Beer's Law over the concentration range to be studied (the absorbance being read on the Beckman). The absorbances of all compounds showed a straight line relationship with concentration.

Solubilities. The sulfide and the sulfone were each placed in buffer solution at 0° C. and allowed to stand or shake (on a Burrell shaker) for a period of about 20 days during which time the concentrations of aliquots were determined. After this length of time the concentrations no longer seemed to be increasing. A correction was made for the fact that aliquots were taken at 0° C.

Absorption Spectra of Compound-Protein Mixture

Buffer solutions of each compound with bovine serum albumin were prepared at room temperature so as to have the three compounds all at the same molar concentration (7.94×10^{-6} M/L) and the protein at a concentration of 0.2 per cent. The spectra were taken on the Beckman using 0.2 per cent protein solution as the blank.

Preparation of Casing

It was noticed especially at shorter wavelengths(264.5 millimicrons for the sulfonate and 254 millimicrons for the sulfone) that some compound (or compounds) from the cellulose casing, which absorbed strongly, was being extracted by the buffer and compound solutions. It was therefore necessary to wash the casing very thoroughly before using it. Various organic solvents (including toluene, ethanol, methanol, benzene, Skelly B, carbon tetrachloride, and acetone) were tried on pieces of casing six inches in length. Water, sodium bicar-

bonate, "Tide" detergent, and phosphate buffer solutions were also tried. A washing time of 18 hours was used after which the casing was soaked in water. Each piece was then placed in a test tube containing 20 milliliters of buffer. The tubes were shaken for 12 hours. The absorbances of the buffer solutions were then read on the Beckman to determine the amount of contamination.

It was found that of the organic solvents tried methanol was best. (Heating the casing in methanol did not seem to clean it any better than did soaking it in methanol at room temperature.) Buffer solution was found to be better than methanol. Various combinations of the liquids were then tried.

The washing technique finally decided upon was to give the casing two six or seven-hour buffer washes using fresh buffer. This was followed by three half-hour hot distilled water washes as used by Teresi and Luck (24) after which the casing was soaked in distilled water until needed. (The casing should be soaked at least 10 or 12 hours, but longer soaking seems unnecessary.

Determination of Dry Weight of Protein

About 50 milligrams of the bovine serum albumin--hereafter to be referred to as BSA or "the protein"--Lot No. P67403 from Armour Laboratories, Chicago, Illinois) were weighed and placed in a weighed crucible. It was allowed to remain in an oven at 105 to 110° for at least 24 hours. The

protein was cooled and weighed a number of times during the drying until the weight no longer decreased. The loss in weight was assumed to be due to moisture, and the per cent proteinaceous material could then be calculated. It was found to average a little over 95 per cent.

Binding Technique

The equilibrium dialysis of each compound was performed in a manner similar to that of Klotz, et al. (13,20). The desired amount of compound was dissolved in 6.8 buffer solution, and 20 milliliters of this solution was placed in each of six eight-inch test tubes. Twenty milliliters of buffer solution was placed in each of two other test tubes. In three of the compound solutions and one buffer solution, cellulose bags containing five milliliters of buffer solution were immersed. A 0.2 per cent solution of BSA was prepared by dissolving 50 milligrams in enough 6.8 buffer to make 25 milliliters of solution. Bags containing five milliliters each of this BSA solution were then placed in the other four test tubes. Strips of cellulose casing about seven inches in length were used for the bags. After having been washed and soaked (as already described), a piece of casing was removed from the water and stroked three or four times to remove excess water. Then two knots were tied (one on top of the other) at the bottom of the casing, and five milliliters of the solution was pipetted into the bag. Air bubbles were removed, and a knot was tied above

the liquid. The casing was cut off one-eighth of an inch from the knot at each end after which a piece of white thread was tied to the bag to facilitate removal after the dialysis. The bag was placed in a test tube, and the free end of the thread was laid over the lip of the test tube. A stopper was then inserted.

The test tubes were attached to a Burrell shaker in a manner so as to be immersed in an ice-water bath and were shaken for 12 hours. At the end of this time the bags were removed, and the test tubes were taken from the ice bath and allowed to come to room temperature. Each solution was then read at the wavelength of maximum absorption on the Beckman (the blanks, of course, being read at the same wavelength as the compound). One centimeter cells were used. A hydrogen discharge tube served as the light source.

The test tube containing buffer inside and outside the bag served as a blank for the three tubes containing buffer inside the bag and compound outside. The test tube containing protein inside the bag and buffer outside served as a blank for the three tubes containing protein inside the bag and the compound outside. Using molar absorbtivities the concentration of compound was calculated from its absorbance.

Treatment of Data

The difference in concentration between a tube which had a buffer bag and one which had a protein bag (after adjustment

was made for the blanks) was designated as Δconcentration. When Δconcentration (moles per liter) is determined for the quantity of solution in a single test tube, it indicates the amount of compound bound. The amount bound (moles) was divided by the number of moles of protein per bag. This value (moles of compound bound per mole of protein) was designated as "r". Unbound compound concentration (concentration in the tube which contained the protein bags) was designated by "A". 1/r was plotted against 1/A, and r was plotted against log A. A least squares determination was used to find the best line on the 1/r versus 1/A plots and to calculate the intercepts and slopes for the equation $1/r = 1/n + 1/nkA$ (13,20). The size of nk indicates the binding affinity. Ninetyfive per cent confidence intervals were determined for calculated 1/r values at given values of 1/A. The t-test was used to determine the statistical significances of the differences of the slopes and intercepts.[1]

RESULTS AND DISCUSSION

Properties of Compounds

The Wavelengths of Maximum Absorbance and Molar Absorbtivities of the Compounds. These are shown in Table 1.

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[1]Statistical analyses were done with the aid of Dr. R. K. Burkhard.

The maximum absorbance for the sulfide is at 349 millimicrons, but some of the earlier readings were taken at 341; so the molar absorbtivity at that wavelength is included.

Table 1. Molar absorbtivities.

Compound	Wavelength of Maximum Absorbance (millimicrons)	ϵ (molar absorbtivity)
Ammonium p-nitrobenzene sulfonate	264.5	1.11×10^4
p-Nitrophenyl methyl sulfone	254	1.15×10^4
p-Nitrophenyl methyl sulfide	349	1.18×10^4
	341	1.14×10^4

Solubilities. The sulfone was found to be about five times as soluble as the sulfide at 0° C. Concentration of a saturated solution of the sulfone was 11.94×10^{-4} M. That of the sulfide was 2.53×10^{-4} M.

Protein-Compound Spectra. The spectra of the individual compounds and of the compound-protein mixtures are compared in Figs. 1, 2, and 3. For the sulfone and sulfonate-protein mixtures the wavelengths of maximum absorbance were near the wavelength of complete absorbance of the protein; consequently readings were not obtainable at wavelengths much shorter than that of the peak.

Binding Results

The interaction with BSA is compared for the three com-

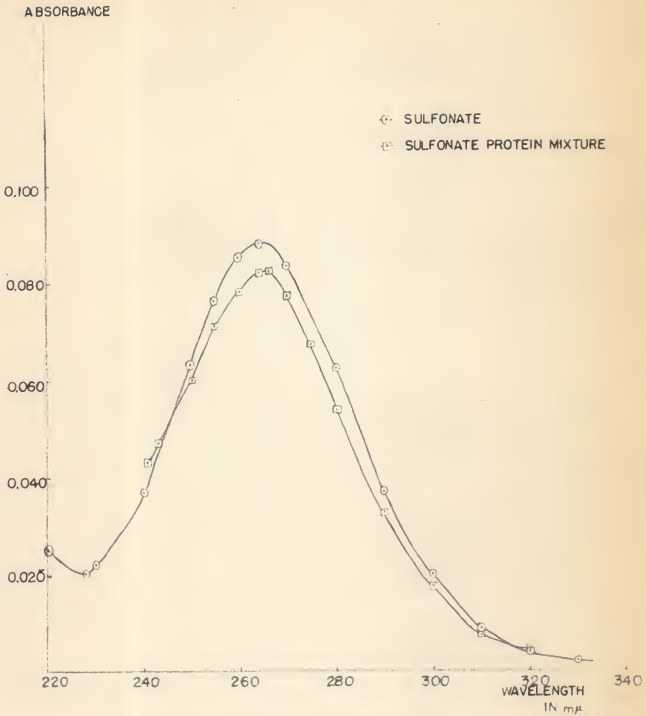

Fig. 1. Free sulfonate compared with sulfonate-protein mixture.

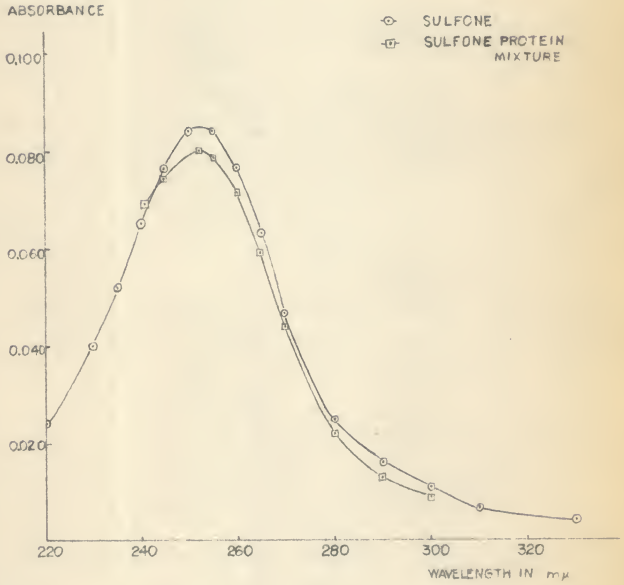

Fig. 2. Free sulfone compared with sulfone-protein mixture.

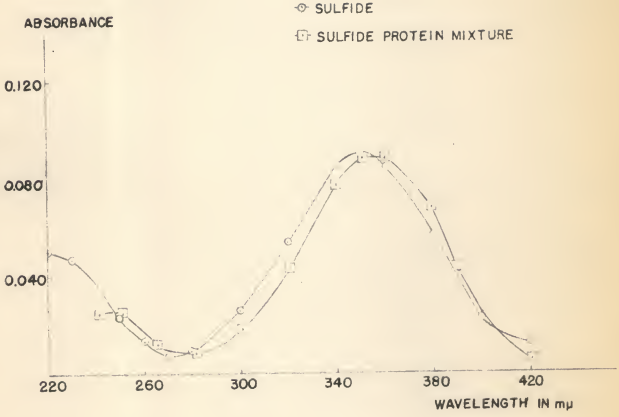

Fig. 3. Free sulfide compared with sulfide-protein mixture.

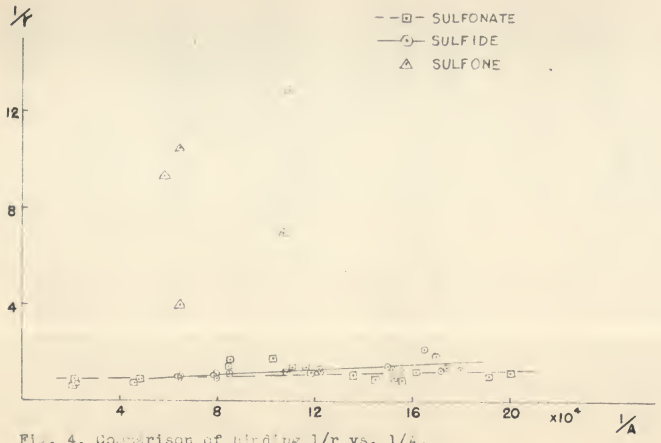

Fig. 4. Comparison of binding 1/r vs. 1/A.

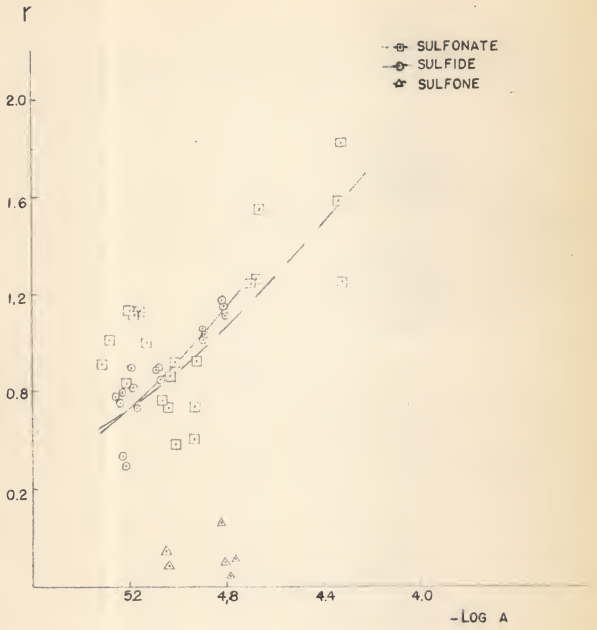

Fig. 5. Comparison of binding.

pounds in Figs. 4 and 5.

The first determinations were done using a protein the supply of which was nearly exhausted, and it was found that this protein gave significantly smaller binding with the sulfide than did Lot No. P67403. Accordingly all subsequent binding results were obtained using this latter batch of protein so that comparison between compounds would be possible. The 1/nk and 1/n values obtained with the sulfonate and sulfide are listed in Table 2. The values appearing at the bottom of the table are those obtained using the first batch of protein.

Table 2. Values of 1/n and 1/nk.

System	Number of determinations	1/n	1/nk
Sulfonate-BSA	21	0.728	3.16×10^{-6}
Sulfide-BSA	18	0.544	5.44×10^{-6}
Sulfide-First batch BSA	18	0.479	12.6×10^{-6}

All binding results are shown in the appendix.

The results for the sulfone were scattered enough that it appeared that a least squares determination would not have much significance. The main reason for the scattering was that the binding (r value) was very small (approximately 0.00 to 0.26); Thus small differences in binding will become large differences when the reciprocal (1/r) is taken. Also, the low binding, together with large absorbance for the blanks, could cause a relatively large error to be produced by a small error in the

blanks. The blanks, as has been pointed out, had a higher absorbance at low wavelengths due to impurities in the casing. Although absorbance was greatly reduced by the washing technique, it was still high enough to cause some trouble.

Table 3 shows the nk values of the sulfide and sulfonate. Statistical analysis showed that the slopes (1/nk values) and the intercepts (1/n) of these lines were not significantly different at the five per cent level (t=1.13, t=0.794, respectively, D. F.=35).

Reference to Table 4 leads to similar conclusions.

Table 3. nk values for sulfide and sulfonate. (pH 6.8, 0° C., ionic strength 0.1)

Compound	nk
Sulfonate	3.16×10^5
Sulfide	1.84×10^5

Table 4. Calculated values of 1/r and 95% confidence intervals for given values of 1/A.

Compound	1/A	1/r	95% confidence interval
Sulfonate	1.0×10^5	1.04	0.90-1.18
	1.5×10^5	1.20	1.02-1.38
Sulfide	1.0×10^5	1.09	0.96-1.22
	1.5×10^5	1.36	1.23-1.49

The above data shows that the binding of these two compounds is of the same order of magnitude for the concentration range studied.

Interpretation

Spectra. Little can be concluded from comparison of spectra of the compounds with that of their corresponding compound-protein mixtures except that some change apparently does occur when each compound is mixed with the protein.

Binding Influence of the Nitro Group. The binding in all three compounds must be influenced to some extent by the presence of the nitro group. Teresi and Luck (24) have shown that a nitro group added to a phenol increases binding. Reference to Table 5 will show this to be true.

Table 5. Increase in binding of phenol due to added nitro groups. (pH 7.6, 1° C., ionic strength 0.2, Teresi and Luck 24 and 25)

Compound	nk
p-nitrophenol	0.073×10^5
2,4-dinitrophenol	1.88
picrate	1.89

In these cases the nitro group increases the ionization of the phenol group; consequently, the ionic binding would be increased. Hydrogen bonding might also be increased due to additional nitro groups.

Table 6 shows that the nitro group by itself does not cause a high binding affinity. For the compounds listed it is only with those having ionic groups that strong binding occurs.

Variation in the binding of the three compounds studied

could then be due to some interaction of the nitro group with the sulfur containing group as well as to the change in the sulfur containing group.

Table 6. Change of binding of dinitrobenzene with addition of ionic and nonionic groups to the ring. (pH 7.4, 5° C., ionic strength 0.05, Carsten and Eisen 7)

Compound	nk
m-dinitrobenzene	0.045×10^5
2,4-dinitrotoluene	0.20
ε-(2,4-dinitrophenylamino) caproic acid	1.84
2,4-dinitrophenol	7.0×10^5

Comparison of Sulfide and Sulfone. Until further work is done, the only significant factor to which greater sulfide binding can be attributed is the solubility effect (the sulfide being less soluble than the sulfone). Steric effects may play a small part in that the sulfone group is a little larger than the sulfide group.

Hydrogen bonding to the nitro group of the sulfone should be less than it is to the nitro group of the sulfide (due to attraction of electrons from the nitro group by the sulfone group). However, the sulfone group itself should hydrogen bond with the protein.

Perhaps the fact that there are two negative groups in the sulfone to interact with solvent whereas there is only one in the sulfide could decrease the binding of the sulfone.

Possibly there are factors which have not been considered or which are not known as yet that could explain the low sulfone binding as compared with the sulfide.

Comparison of Sulfonate and Sulfide. The principal factor which would cause the sulfonate to bind more strongly is that it is an anion and hence will undergo ionic interaction. It should also hydrogen bond more.

Factors which might cause the sulfide to bind more strongly than the sulfonate are that the sulfide is less soluble, and it should also undergo less solvent interaction.

Comparison of Sulfonate and Sulfone. Ionic interaction is the main factor favoring the sulfonate, although it might hydrogen bond to a slightly greater extent.

The only factor favoring the sulfone would be the solubility effect.

SUMMARY

The binding of p-nitrophenyl methyl sulfide, p-nitrophenyl methyl sulfone, and ammonium p-nitrobenzene sulfonate to bovine serum albumin was studied by means of equilibrium dialysis. It was found that the sulfide and sulfonate bound to about the same extent whereas the sulfone bound much less.

Binding in all three compounds may be due, in part, to hydrogen bonding with the nitro groups and perhaps some due to van der Waals' forces. The ionic interactions of the anionic sulfonate is probably an important factor in its binding. The

sulfide binds more strongly than the sulfone perhaps because of lower solubility and less interaction with solvent. Steric effects could be involved.

Further study will be necessary to determine all the factors involved in the binding differences of compounds of the type studied.

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APPENDIX

Spectra of sulfonate-BSA mixture.
7.94×10^{-6} sulfonate 0.2% BSA solution

Wave length (m. microns)	Absorbance	Wave length (m. microns)	Absorbance
241	0.043	270	0.077
250	0.060	280	0.054
260	0.078	290	0.033
264	0.082	300	0.018
266	0.082		

Spectra of sulfonate 7.94×10^{-6} M solution.

Wave length (m. microns)	Absorbance	Wave length (m. microns)	Absorbance
240	0.037	270	0.083
250	0.063	280	0.062
260	0.085	290	0.037
264	0.088	300	0.020
265	0.088		

Spectra of sulfone-BSA mixture.
7.94×10^{-6} sulfone 0.2% BSA solution

Wave length (m. microns)	Absorbance	Wave length (m. microns)	Absorbance
241	0.069	255	0.078
245	0.074	260	0.071
252	0.080	270	0.044
253	0.080	280	0.022
250	0.080	290	0.013

Spectra of sulfone 7.94×10^{-6} M solution.

Wave length (m. microns)	Absorbance	Wave length (m. microns)	Absorbance
230	0.040	255	0.084
240	0.065	260	0.076
250	0.084	270	0.047
253	0.084	280	0.025
254	0.084	290	0.016

Spectra of sulfide-BSA mixture.
7.94×10^{-6} M sulfide 0.2% BSA solution

Wave length (m. microns)	Absorbance	Wave length (m. microns)	Absorbance
250	0.023	330	0.060
260	0.014	340	0.076
270	0.008	349	0.086
280	0.008	350	0.087
290	0.011	360	0.087
300	0.017	370	0.078
310	0.027	380	0.068
320	0.042	390	0.043

Spectra of sulfide 7.94×10^{-6} M solution.

Wave length (m. microns)	Absorbance	Wave length (m. microns)	Absorbance
250	0.023	340	0.084
260	0.013	348	0.088
270	0.007	349	0.089
280	0.009	350	0.089
290	0.016	360	0.084
300	0.026	370	0.072
310	0.038	380	0.056
320	0.054	390	0.039
330	0.071		

Binding of ammonium p-nitrobenzene sulfonate-BSA.

Tube	Conc. $\times 10^{-6}$ M/L	Δ Conc. $\times 10^{-6}$ M/L	Amt. bound $\times 10^{-8}$ M	Protein $\times 10^{-8}$ M	r
1	54.8				
1a	48.0	6.8	17.0	13.7	1.24
2	55.7				
2a	47.1	8.6	21.5	13.7	1.57
3	57.9				
3a	48.0	9.9	24.7	13.7	1.81
4	30.4				
4a	22.0	8.4	21.0	13.6	1.54
5	27.4				
5a	20.6	6.8	17.0	13.6	1.25
6	27.5				
6a	20.7	6.8	17.0	13.6	1.25
7	13.50				
7a	8.48	5.02	12.6	13.8	0.912
8	12.88				
8a	7.39	5.49	13.7	13.8	0.995
9	12.80				
9a	8.65	4.15	10.4	13.8	0.753
10	15.86				
10a	11.80	4.06	10.2	13.8	0.739
11	16.87				
11a	11.80	5.07	12.7	13.8	0.919
12	15.13				
12a	11.80	3.33	8.33	13.8	0.604
13	12.50				
13a	6.40	6.10	15.3	13.5	1.13
14	12.98				
14a	6.94	6.04	15.1	13.5	1.12
15	12.60				
15a	6.58	6.02	15.1	13.5	1.12
16	10.72				
16a	6.22	4.50	11.25	13.7	0.821
17	9.83				
17a	4.87	4.96	12.4	13.7	0.905
18	10.72				
18a	5.23	5.49	13.7	13.7	1.00

Binding of ammonium p-nitrobenzene sulfonate-BSA (cont.)

Tube	Conc. $M/L \times 10^{-6}$	Δ Conc. $M/L \times 10^{-6}$	Amt. bound $M \times 10^{-8}$	Protein $M \times 10^{-8}$	r
19	13.05				
19a	9.10	3.95	9.87	13.7	0.720
20	13.96				
20a	9.29	4.67	11.7	13.7	0.853
21	12.88				
21a	9.74	3.14	7.85	13.7	0.573

Binding of p-nitrophenyl methyl sulfone-BSA.
Average values*

Tubes	Conc. $M/L \times 10^{-6}$	Δ Conc. $M/L \times 10^{-6}$	Amt. bound $M \times 10^{-8}$	Protein $M \times 10^{-8}$	r
1,2,3	16.35				
1a,2a, 3a	16.20	0.15	0.50	13.5	0.037
4,5,6	16.10				
4a,5a, 6a	15.58	0.52	1.3	13.5	0.096
7,8,9	16.89				
7a,8a, 9a	15.49	1.40	3.50	13.7	0.255
10,11, 12,13, 14	17.90				
10a,11a, 12a,13a, 14a	17.30	0.60	1.5	13.8	0.11
15,16, 17	9.65				
15a,16a, 17a	9.22	0.43	1.1	13.7	0.078
18,19, 20	10.18				

Binding of p-nitrophenyl methyl sulfone-BSA (cont.).

Tubes	Conc.$^{-6}$ M/L x 10^{-6}	Δ Conc.$^{-6}$ M/L x 10^{-6}	Amt. bound: M x 10^{-8}	Protein$_8$ M x 10^{-8}	r
18a,19a, 20a	9.39	0.79	2.0	13.7	0.14

*Three tubes having compound outside the bag with protein inside and three with buffer inside were used each time (five in one case). Since some tubes showed no binding whatsoever, the concentrations for the three similar tubes (five in one case) were averaged.

Binding of p-nitrophenyl methyl sulfide-BSA.

Tube	Conc.$^{-6}$ M/L x 10^{-6}	Δ Conc.$^{-6}$ M/L x 10^{-6}	Amt. bound: M x 10^{-8}	Protein$_8$ M x 10^{-8}	r
1	8.82				
1a	5.93	2.89	7.23	13.7	0.528
2	8.73				
2a	6.10	2.63	6.59	13.7	0.481
3	11.36				
3a	6.44	4.92	12.3	13.7	0.899
4	10.18				
4a	5.85	4.33	10.8	13.7	0.790
5	9.83				
5a	5.60	4.23	10.6	13.7	0.773
6	9.83				
6a	5.76	4.07	10.2	13.7	0.744
7	11.00				
7a	6.61	4.39	11.0	13.7	0.802
8	10.69				
8a	6.70	3.99	9.98	13.7	0.728
9	10.69				
9a	6.70	3.99	9.98	13.7	0.728
10	13.13				
10a	8.48	4.65	11.6	13.8	0.840

Binding of p-nitrophenyl methyl sulfide-BSA (cont.).

Tube	Conc. $\times 10^{-6}$ M/L	Δ Conc. $\times 10^{-6}$ M/L	Amt. bound $\times 10^{-8}$ M	Protein $\times 10^{-8}$ M	r
11	13.22				
11a	8.30	4.92	12.3	13.8	0.891
12	13.13				
12a	8.22	4.91	12.3	13.8	0.891
13	18.30				
13a	12.70	5.60	14.0	13.6	1.03
14	18.40				
14a	12.70	5.70	14.2	13.6	1.05
15	18.30				
15a	12.80	5.50	13.7	13.6	1.01
16	21.70				
16a	15.70	6.00	15.0	13.7	1.10
17	21.90				
17a	15.60	6.30	15.8	13.7	1.15
18	21.90				
18a	15.50	6.40	16.0	13.7	1.17

Binding of p-nitrophenyl methyl sulfide-BSA(separate batch of BSA).

Tube	Conc. $\times 10^{-6}$ M/L	Δ Conc. $\times 10^{-6}$ M/L	Amt. bound $\times 10^{-8}$ M	Protein $\times 10^{-8}$ M	r
1	18.1				
1a	14.2	3.9	9.75	13.5	0.722
2	17.3				
2a	13.8	3.5	8.75	13.5	0.648
3	17.5				
3a	13.7	3.8	9.50	13.5	0.703
4	18.4				
4a	14.0	4.4	11.0	13.8	0.798
5	18.5				
5a	14.0	4.5	11.2	13.8	0.813
6	18.5				
6a	14.4	4.1	10.3	13.8	0.747
7	13.15				
7a	9.74	3.41	8.53	13.5	0.631

Binding of p-nitrophenyl methyl sulfide-BSA(separate batch of BSA)(cont.).

Tube	Conc. M/L x 10^{-6}	Δ Conc. M/L x 10^{-6}	Amt. bound M x 10^{-8}	Protein M x 10^{-8}	r
8	13.25				
8a	9.65	3.60	9.00	13.5	0.666
9	13.42				
9a	9.83	3.59	8.97	13.5	0.664
10	10.00				
10a	7.02	2.98	7.46	13.7	0.545
11	9.83				
11a	6.93	2.90	7.25	13.7	0.529
12	10.00				
12a	7.98	2.02	5.06	13.7	0.369
13	10.85				
13a	8.55	2.30	5.76	13.7	0.420
14	10.50				
14a	8.30	2.20	5.50	13.7	0.402
15	10.85				
15a	8.39	2.46	6.16	13.7	0.450
16	10.77				
16a	8.05	2.72	6.80	13.8	0.493
17	11.10				
17a	8.14	2.96	7.41	13.8	0.537
18	10.69				
18a	8.05	2.64	6.60	13.8	0.478

SYNTHESIS AND ALBUMIN BINDING PROPERTIES OF THREE SULFUR CONTAINING ORGANIC COMPOUNDS

by

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The activity of many medicinal compounds is influenced by binding with serum albumin. Since many of these compounds contain a sulfur functional group, it was decided to compare binding properties of compounds whose structure differed only at the sulfur containing group. Thus it was hoped that factors involved in protein binding to such groups could be determined.

Previous work has shown that nitro groups added to an aromatic ring of a compound tend to increase its binding. Polar groups such as SO_3^- or OH added to an anionic compound tend to decrease the binding.

Previous work has further shown that ionic compounds bind more strongly than neutral ones and that of two neutral compounds the less soluble one may bind more strongly.

The three compounds, ammonium p-nitrobenzene sulfonate, p-nitrophenyl methyl sulfide and p-nitrophenyl methyl sulfone, were synthesized and purified. Spectra and molar absorbtivities in 6.8 buffer solutions were determined for all three (using the Beckman model D.U. spectrophotometer). Solubilities in buffer solution were determined for the two relatively insoluble compounds (the sulfide and sulfone). Spectra were also determined for mixtures of each of the three compounds with bovine serum albumin (BSA).

Binding with BSA was studied by means of the equilibrium dialysis technique. It was found to be necessary to wash out the dialysis casing before using it since it contained impurities which absorbed light at the lower wavelengths used.

Some buffer solution of the compound being studied was placed in each of six test tubes. Dialysis bags containing buffer were placed in three of the test tubes, and bags containing BSA dissolved in buffer were placed in the other three. (Blanks were also set up--one with buffer inside and outside the bag and another with BSA inside and buffer outside). The dialysis was run for 12 hours at 0° C.

It was found that the sulfide and sulfonate compounds were bound about the same amount. The sulfone was bound much less. The binding with all three compounds might be due in part to hydrogen bonding of the protein with the nitro group. The principal cause of the binding of the sulfonate should be ionic interaction. The sulfide is the least soluble of the three compounds, and hence, if solubility is a factor in its binding, the binding should be increased over that of more soluble compounds (if other factors are equal). The sulfide also has only one group which can interact appreciably with solvent whereas the other compounds have two such groups.

Further work needs to be done before all the factors involved in the binding can be determined.