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Protein Bindings. V.[1] Binding of N^4-Acetylsulfonamides to Bovine Serum Albumin

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Binding constant, K_{ac}, for 11 N^4-acetylsulfonamides with bovine serum albumin was evaluated spectrophotometrically utilizing the competion between 2-(4′-hydroxyphenylazo)benzoic acid and N^4-acetylsulfonamides at 37° and 5°, and was compared with K_f, binding constant for free sulfonamides with bovine serum albumin. Free energy and entropy changes for the binding were calculated. The correlations of log (K_{ac}/K_f) with the fraction of unchanged sulfonamides excreted in the urine after the administration, and with half-life for elimination of sulfonamides from human blood plasma were indicated to be significant.

Previously several papers[3-5] have been published on the metabolites of sulfonamides after the administration. Unchanged sulfonamides, N^4-acetylsulfonamides, sulfonamide-N^4-glucuronides, and sulfonamide-N^4-sulfonates were mainly found to be present in blood, urine, and other body fluids.[6] Within these compounds, the contents of unchanged sulfonamides and N^4-acetylsulfonamides are comparatively higher than sulfonamide-N^4-glucuronides and sulfonamide-N^4-sulfonates in the body.

In the present paper, binding constant for eleven N^4-acetylsulfonamides with bovine serum albumin was evaluated spectrophotometrically and correlations of the constant with some properties of sulfonamides concerning with the excretion were investigated.

Assuming that N^4-acetylsulfonamides and anionic dye 2-(4′-hydroxyphenylazo)benzoic acid (HABA) compete for the same binding sites on albumin molecules, log K_{ac}, logarithm of intrinsic binding constant for N^4-acetylsulfonamides with bovine serum albumin, may be written in the same form as Eqs. (7) and (9) of the previous paper:[7]

$$\log K_{ac}=\log K_A+\log\{(a-x)/(b-y)\}+(1/m)\log(y/x) \quad (1)$$

$$y=np-x\{1+1/K_A^m(a-x)^m\} \quad (2)$$

where K_A represents intrinsic binding constant for HABA to each site on albumin molecules, a and b denote the initial concentration of HABA and N^4-acetylsulfonamides, respectively, x is the concentration of bound HABA, y the concentration of bound N^4-acetylsulfonamide, m a parameter dependent on the experimental conditions, n the number of binding sites on a molecule of albumin, and p the total concentration of albumin. x is given in the form, $x=\Delta E/\Delta\varepsilon d$. ΔE is the difference between the absorbancies at 482 mμ of HABA in the presence and the absence of alubmin, and $\Delta\varepsilon$ the difference between the molar extinction coefficients at 482 mμ of bound HABA and unbound HABA.

1) Part IV: I. Moriguchi and S. Wada, *Chem. Pharm. Bull.* (Tokyo), **16**, 734 (1968).
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3) R.J. Schnitzer, F. Hawking, "Experimental Chemotherapy," Vol. 11, Academic Press, New York, 1963, p. 276.
4) H. Shibaki, *Chemotherapy*, **12**, 390 (1964).
5) T. Uno and M. Ueda, *Chem. Pharm. Bull.* (Tokyo), **11**, 709 (1963).
6) T. Uno and Y. Sekine, *Chem. Pharm. Bull.* (Tokyo), **14**, 687 (1966).
7) I. Moriguchi, *Chem. Pharm. Bull.* (Tokyo), **16**, 597 (1968).

Fig. 1 shows ΔE values *versus* the concentration of N^4–acetylsulfonamides added to a solution containing bovine serum albumin and HABA at 37°. The extent of the reversion of the albumin–induced spectral change of HABA increases with the increasing concentrations of the N^4–acetylsulfonamides added.

The values of log K_{ac} at 37° were obtained from the values of ΔE by Eqs. (1) and (2). $\Delta\varepsilon$ and m were evaluated as previously described.[8] The values of log K_{ac} are listed in Table I, together with the values of log K_f, logarithm of intrinsic binding constant for free sulfonamides with bovine serum albumin. Table I indicates that the values of log K_{ac} are nearly constant irrespective of the initial concentration of the N^4–acetylsulfonamides and are generally larger than log K_f. Correlation between log K_{ac} and log K_f is highly significant with the coefficient of 0.95 (11 samples).

The values of log K_{ac} at 5° were also obtained by the same method. They are listed in Table II.

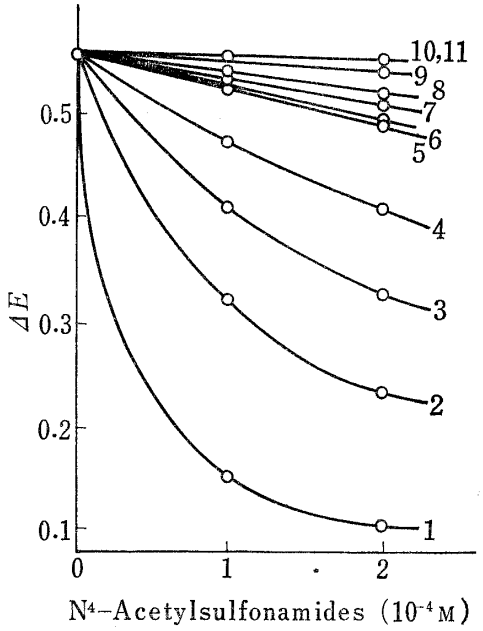

Fig. 1. ΔE of HABA–Albumin Solutions with varying Concentration of N^4–Acetylsulfonamides

1×10^{-4} M HABA and 5×10^{-5} M bovine serum albumin with 0—2×10^{-4} M N^4–acetylsulfonamides in 0.15 M tris buffer solution at pH 7.4 and 37°. For numbering, see Table I.

Table III shows free energy and entropy changes for the association of one mole N^4–acetylsulfonamide with one mole bovine serum albumin calculated from the log K_{ac}–values of Tables I and II.[8]

The value of the entropy change is positive for all the compounds, and this may suggest that the binding is hydrophobic as in the case of free sulfonamides.[9]

Table I. Intrinsic Binding Constant[a] for N^4–Acetylsulfonamides (K_{ac}) and for Sulfonamides (K_f) with Bovine Serum Albumin at 37°

No.	N^4–Acetylsulfonamides	log K_{ac}			log K_f[c]
		1×10^{-4} M[b]	2×10^{-4} M[b]	Average	
1	Sulfadimethoxine	5.62	5.43	5.5	5.4
2	Sulfisoxazole	5.12	5.39	5.3	5.0
3	Sulfamethoxypyridazine	4.74	4.66	4.7	4.8
4	Sulfamerazine	4.28	4.32	4.3	3.7
5	Sulfisomidine	4.03	3.90	4.0	3.1
6	Sulfisomezole	3.86	3.80	3.8	3.7
7	Sulfamonomethoxine	3.20	3.48	3.3	3.3
8	Sulfaphenazole	3.49	3.75	3.6	3.3
9	Sulfadiazine	3.19	3.41	3.3	2.9
10	Sulfathiazole	3.40	3.65	3.5	3.1
11	Sulfanilamide	3.50	3.54	3.5	2.7

a) in 0.15 M tris. buffer solution at pH 7.4
Unit of K_{ac} and K_f are liter/Avogadro number of binding sites on albumin.
b) initial concentration of N^4–acetylsulfonamides
c) I. Moriguchi, S. Wada, and T. Nishizawa, *Chem. Pharm. Bull.* (Tokyo), **16**, 601 (1968)

8) I. Moriguchi, S. Wada, and H. Sano, *Chem. Pharm. Bull.* (Tokyo), **16**, 592 (1968).
9) W. Scholtan, *Arzneimittel-Forsch.*, **14**, 469 (1964).

Table II. Intrinsic Binding Constant[a] for N⁴–Acetylfulfonamides (K_{ac}) with Bovine Serum Albumin at 5°

No.	N⁴–Acetylsulfonamides	log K_{ac}		
		1×10^{-4} M[b]	2×10^{-4} M[b]	Average
1	Sulfadimethoxine	5.64	5.58	5.6
2	Sulfisoxazole	5.71	5.33	5.5
3	Sulfamethoxypyridazine	4.68	4.95	4.8
4	Sulfamerazine	4.65	4.48	4.6
5	Sulfisomidine	4.16	4.02	4.1
6	Sulfisomezole	3.83	4.20	4.0
7	Sulfamonomethoxine	3.75	3.48	3.6
8	Sulfaphenazole	4.02	3.80	3.9
9	Sulfadiazine	3.66	3.37	3.5
10	Sulfathiazole	3.98	3.66	3.8
11	Sulfanilamide	4.19	3.88	4.0

a) in 0.15 M tris. buffer solution at pH 7.4
Unit of K_{ac} is liter/Avogadro number of binding sites on albumin.
b) initial concentration of N⁴–acetylsulfonamides

Table III. Free Energy and Entropy Changes for Binding of N⁴–Acetylsulfonamides with Bovine Serum Albumin

No.	N⁴–Acetylsulfonamides	$\Delta G_1^0(37°)$[a]	$\Delta G_1^0(5°)$[a]	ΔS_1^0[b]
1	Sulfadimethoxine	−8.23	−7.37	0.027
2	Sulfisoxazole	−7.94	−7.24	0.022
3	Sulfamethoxypyridazine	−7.09	−6.37	0.023
4	Sulfamerazine	−6.53	−6.12	0.013
5	Sulfisomidine	−5.96	−5.50	0.014
6	Sulfisomezole	−5.82	−5.37	0.014
7	Sulfamonomethoxine	−5.11	−4.87	0.008
8	Sulfaphenazole	−5.53	−5.27	0.009
9	Sulfadiazine	−5.11	−4.75	0.011
10	Sulfathiazole	−5.39	−5.12	0.008
11	Sulfanilamide	−5.39	−5.37	0.001

a) in 0.15 M tris. buffer solution at pH 7.4
Unit of ΔG_1^0 is k cal/mole.
b) Unit of ΔS_1^0 is k cal/mole deg.

Fig. 2 shows relation of log (K_{ac}/K_f) with f–value,[10] fraction of unchanged sulfonamide excreted in the urine after the administration. A good linear relationship is recognized with these sulfonamides except sulfadimethoxine, sulfanilamide, and sulfamerazine.

The larger is the value of log (K_{ac}/K_f), the larger amount of N⁴–acetylated sulfonamide may replace free sulfonamide to be bound to serum albumin, and consequently the larger amount of free sulfonamide may be excreted in the urine, assuming that there is almost no difference in the clearance of the kidney and the rate and mechanism of the metabolism of these sulfonamides. Of the above three exceptional sulfonamides, sulfadimethoxine is known to be excreted as glucuronide form in considerably high amount,[11] and to give very low velocity of *in vitro* acetylation,[10] while sulfanilamide is known to give very high velocity.[10] This may be the reason why sulfadimethoxine and sulfanilamide do not fall around the line in Fig. 2, but the reason is unclear in the case of sulfamerazine.

10) K. Kakemi, T. Arita, and T. Koizumi, *Yakuzaigaku*, **25**, 22 (1965).
11) B.A. Koechlin, W. Kern, and R. Engelberg, *Antibiot. Med. Clin. Therapy*, **6**, No. 2 (Suppl. 1), 22 (1959).

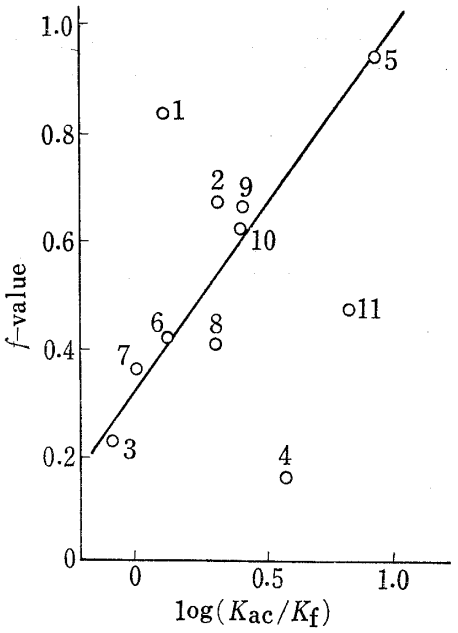

Fig. 2. Correlation of log (K_{ac}/K_f) with f-value[a]
For numbering, see Table I.
a) See ref. 10.

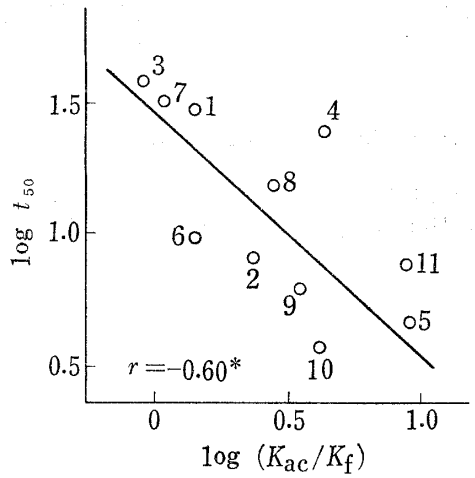

Fig. 3. Correlation of log (K_{ac}/K_f) with Half-Life[a] (t_{50}, in hr) for Elimination of Sulfonamides from Human Blood Plasma
For numbering, see Table I.
a) J. Rieder, *Arzneimittel-Forsch.*, **13**, 81 (1963)

Long-activity of sulfonamides is known to be mainly owing to their high reabsorption rate in the renal tubules,[12] but it was supposed[13] that binding to serum protein might have some connection with long-activity of sulfonamides. In the previous paper,[14] it was indicated that the correlation of log K_f with log t_{50}, half-life for elimination of sulfonamides from human blood plasma, was not significant. The correlation of log K_{ac} with log t_{50} was also indicated to be insignificant in the present work. But the correlation of log (K_{ac}/K_f) with log t_{50} is significant at the 5-percent level with the coefficient of −0.60 (11 samples) as shown in Fig. 3. The correlation is not so highly significant, but the following explanation for the correlation may not be unreasonable. The smaller is the value of log (K_{ac}/K_f), the larger amount of free sulfonamide may be bound to serum albumin and stored, and the larger amount of acetylated sulfonamide may be released and excreted. And the clearance of free sulfonamides is known to be much smaller,[15] about a tenth or so,[16] than that of acetylated salfonamides. But this explanation for the correlation of long-activity of sulfonamides with log (K_{ac}/K_f) seems to require the same assumption as that described in the explanation for the correlation of f-value with log (K_{ac}/K_f).

Experimental

Materials——Eleven N^4-acetylsulfonamides were obtained by acetylation[17] of the following sulfonamides: sulfadiazine and sulfadimethoxine (Chugai Seiyaku Co.), sulfisoxazole (Yamanouchi Seiyaku Co.), sulfamethoxypyridazine (Japan Lederle Co.), sulfamerazine (Tanabe Seiyaku Co.), sulfaphenazole and sulfisomidine (Dainihon Seiyaku Co.), sulfamonomethoxine (Daiichi Seiyaku Co.), sulfisomezole (Shionogi Seiyaku Co.), and sulfathiazole and sulfanilamide (Tokyo Kasei Kogyo Co.). All other materials were used as previously described.[8] All the test solutions for photometry were prepared with 0.15 M tris. buffer solution of pH 7.4.

12) For example, K. Yamamoto, *Saishin Igaku*, **14**, 3147 (1959).
13) A.R. Torres, *Muench. Med. Wochschr.*, **100**, 1611 (1958).
14) I. Moriguchi, S. Wada, and T. Nishizawa, *Chem. Pharm. Bull.* (Tokyo), **16**, 601 (1968).
15) H. Röttger, *Arzneimittel-Forsch.*, **1**, 225 (1951).
16) R.L. Nichols, W.F. Jones, Jr., and M. Finland, *Proc. Soc. Exp. Biol. Med.*, **92**, 637 (1956).
17) K. Enoki, *Yakugaku Zasshi*, **81**, 116 (1961).

Measurement of ΔE——Optical absorption (E') of the solutions containing 1×10^{-4} M HABA and 5×10^{-5} M bovine serum albumin with 0, 1×10^{-4} M, and 2×10^{-4} M sulfonamides, absorption (E) of the solution of 1×10^{-4} M HABA, and absorption (E'') of 5×10^{-5} M bovine serum albumin solution were measured at 482 mμ at 37° and 5° as previously described.[8] The values of ΔE were calculated as

$$\Delta E=E'-(E+E'')$$

$\Delta\varepsilon$ was also evaluated as previously described.[8]

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