CHROM. 8104

Note

Chromatographic substituent constants and their use in quantitative structure activity relationships

The binding of acetanilides to serum albumin

ERIC TOMLINSON[*,**] and J. C. DEARDEN

School of Pharmacy, Liverpool Polytechnic, Liverpool L3 3AF (Great Britain)

(First received November 8th, 1974; revised manuscript received December 2nd, 1974)

Increasing use of multiple regression analysis for studying linear free-energy relationships (LFERs) existing between physicochemical parameters and biochemical and biological systems, has shown the necessity for accurate and quick measurement of the partitioning of drugs between polar and non-polar solvents (Leo *et al.*[1]). Because the direct measurement of partition coefficients is normally tedious, and may often present practical difficulties, there has been some movement[2,3] towards the use of chromatographic R_M values to describe the hydrophobic nature of compounds. This paper reports relationships existing between the extrathermodynamic hydrophobic substituent parameters π (ref. 4) and ΔR_M, and the binding of some simple acetanilides to bovine serum albumin (BSA).

EXPERIMENTAL AND RESULTS

The chromatographic R_M values were determined in two systems: 1-octanol/acetone–water (1:9) (R^{Ma}), and liquid paraffin/acetone–water (2:8) (R^{Ma}), using a reversedphase TLC method, details of which have been described previously[3]. Partition coefficients (P) were measured at 25° by a shake method using 1-octanol and phosphate buffer, pH 7.2, as the solvent pair. The measurements of the binding of the acetanilides to BSA has already been reported[5]; this involved the use of a non-equilibrium dialysis technique. Results are shown in Table I, BSA binding being characterized by the intrinsic association constant (K) for the primary binding site. Regression analysis of the data is shown in Table II.

For purposes of analysis, three data point sets have been employed: $n = 13$ comprises all the simple p-substituted acetanilides, $n = 16$ includes the N-methylated compounds, and $n = 18$ includes the two o-substituted compounds. Eqns. 1–9 show the relationships existing between log K and the derived substituent constants and are found to be significant at 99.9% confidence limit using variance-ratio tests.

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[*] Present address: Pharmaceutics Research Group, Department of Pharmacy, University of Aston, Birmingham B4 7ET, Great Britain.
[**] To whom communications should be sent.

TABLE I

BSA BINDING AND HYDROPHOBIC SUBSTITUENT CONSTANTS

Functional group	K (liter mol^{-1})	Hydrophobic parameter and relative standard deviations		
		log P	$R_M{}^a$	$R_M{}^b$
p-H	21,500	1.16 ± 2.0	-0.19 ± 1.3	-0.18 ± 1.5
-CH$_3$	29,800	1.39 ± 2.0	-0.05 ± 1.9	-0.11 ± 0.7
-OH	17,000	0.80 ± 2.0	-0.50 ± 1.1	-0.40 ± 0.3
-OCH$_3$	16,100	1.02 ± 2.0	-0.31 ± 1.5	-0.30 ± 1.3
-OC$_2$H$_5$	20,200	1.06 ± 2.0	-0.30 ± 2.1	-0.20 ± 0.5
-NH$_2$	7,000	0.08 ± 2.0	-0.95 ± 1.2	-0.68 ± 1.0
-F	29,900	1.46 ± 1.0	0.11 ± 2.3	-0.02 ± 0.3
-Cl	62,500	1.87 ± 2.0	0.50 ± 3.0	0.26 ± 0.6
-Br	105,500	2.29 ± 2.0	0.75 ± 1.3	0.37 ± 0.8
-I	142,000	2.46 ± 3.0	0.95 ± 1.2	0.54 ± 0.2
-CHO	26,000	1.25 ± 2.0	0.12 ± 2.4	0.17 ± 0.6
-COOH	13,400	0.55 ± 2.0	0.87 ± 2.0	0.88 ± 0.6
-NO$_2$	48,600	1.65 ± 2.0	0.26 ± 1.4	0.16 ± 0.6
=N-CH$_3$, -H	18,800	0.97 ± 2.0	0.41 ± 1.9	0.34 ± 0.2
=N-CH$_3$, -OH	16,000	0.61 ± 2.0	0.57 ± 3.6	0.39 ± 0.1
=N-CH$_3$, -OCH$_3$	16,900	0.72 ± 1.0	0.36 ± 1.2	0.38 ± 0.8
o-OH	3,700	0.74 ± 1.0	0.69 ± 1.3	0.40 ± 0.1
-OC$_2$H$_5$	7,200	1.04 ± 1.0	0.01 ± 0.9	0.04 ± 0.2

As has been shown previously[5,6], the facts that such LFERs are well correlated, and that the slope regression coefficients are positive in all cases, indicate that binding between such non-polar molecules and BSA is hydrophobic (non-specific) in nature, *i.e.* two "partitioning-like" processes are correlated and that further factoring of the shown physicochemical description of binding is not necessary. (For example, the addition of the Hammett electronic σ term to eqns. 1–9 does not statistically improve these correlations.) The derived LFERs show that N-methylation produces no significant effect on the binding model, suggesting that hydrogen bonding in this system is unimportant.

TABLE II

LINEAR FREE-ENERGY RELATIONSHIPS BETWEEN BSA BINDING AND HYDRO-PHOBIC SUBSTITUENT CONSTANTS FOR A SERIES OF p-SUBSTITUTED ACETANILIDES

Values in parentheses are the standard errors of the regression coefficients; n is the number of data points used for any particular regression; r is the correlation coefficient; F is the variance-ratio value for the correlation.

No.	Equation	n	r	F
1	$\log K = 4.38\ (0.02) + 0.55\ (0.03)\ \pi$	13	0.989	489.0
2	$\log K = 4.40\ (0.02) + 0.53\ (0.03)\ \pi$	16	0.985	452.8
3	$\log K = 4.34\ (0.05) + 0.58\ (0.08)\ \pi$	18	0.887	59.0
4	$\log K = 4.38\ (0.02) + 0.63\ (0.04)\ \Delta R_M{}^a$	13	0.981	281.6
5	$\log K = 4.38\ (0.02) + 0.62\ (0.03)\ \Delta R_M{}^a$	16	0.981	358.3
6	$\log K = 4.33\ (0.04) + 0.70\ (0.03)\ \Delta R_M{}^a$	18	0.919	87.0
7	$\log K = 4.42\ (0.04) + 0.86\ (0.09)\ \Delta R_M{}^b$	13	0.943	88.3
8	$\log K = 4.41\ (0.03) + 0.87\ (0.08)\ \Delta R_M{}^b$	16	0.947	121.7
9	$\log K = 4.34\ (0.06) + 0.89\ (0.02)\ \Delta R_M{}^b$	18	0.798	28.1

Table II shows that correlations of reduced significance are obtained between log K and $\Delta R_M{}^b$ values compared with those between log K and $\Delta R_M{}^a$ and π. Thus, the 1-octanol/acetone–water system serves as a better model of transfer of a non-polar solute molecule from an aqueous environment onto a protein macromolecule than does the liquid paraffin/acetone–water system. (Eqns. 7–9, using $\Delta R_M{}^b$, show that between 10 and 36% of the variance in the experimental data is "unexplained" by the correlations, compared with between 1 and 21% when using π and $\Delta R_M{}^a$ values.)

The better correlations found using the 1-octanol systems over the liquid paraffin system show that the free-energy change in binding of the acetanilides to BSA parallels quite closely their free-energy change in transfer from an aqueous phase to 1-octanol. The slope regression coefficients as presented in Table II are shown to be <1.0, indicating that for a given increase in hydrophobicity more molecules move from water to the organic phase (especially when this is 1-octanol) than are found moving from water to the protein phase. Thus, solute molecules bound to BSA can be envisaged as being placed under more constraint than those in the organic phases, as is to be expected from the binding phenomenon. Conversely, it appears that when the lipophilicity of the organic phase is decreased then the energy required to transfer a drug molecule from the non-aqueous to the aqueous phase also decreases.

Relative standard deviations of the determined hydrophobic parameters π and $\Delta R_M{}^a$ are seen to be similar (Table I), but the liquid paraffin chromatographic system gives improved replication of the determined values. Gandolfi et al.[6] have pointed out some advantages of the use of R_M values in quantitative structure activity relationships (QSAR). It is now suggested practice[7] in QSAR to examine various substituent π values derived in differing solute/solvent pair systems, and to use σ values from that system which are found to fit correlated data most closely. This is because π depends to some extend on σ. However, the measurement of chromatographic hydrophobic substituent parameters in such a way may be severely limited, owing to the adsorption process which can affect distribution of the solute between the polar and non-polar phases (compare eqn. 6 with eqn. 9, where o-hydroxyacetanilide, which is capable of intramolecular H-bonding, is included in the regression analysis). If chromatographic techniques are the method of choice for determination of hydrophobic parameters, then it is preferable to adopt a technique in which partition is the sole or dominant process. Methods involving adsorption processes are more susceptible to experimental variables such as temperature, and it should be remembered that the theory relating R_M values to partition coefficients is strictly valid only for methods were partition processes are occurring. The fact that π and ΔR_M constants vary from system to system, depending on σ (ref. 1) (especially when amino, carboxy, hydroxy or methoxy groups are involved), which gives rise to some non-additivity, indicates that direct measurement of the partitioning of each compound is preferable.

If thermodynamic parameters, other than the free-energy term, are required, then a method more suitable to temperature studies, such as liquid–liquid chromatography, should be used.

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