ON THE PREDICTION OF PARTITION COEFFICIENTS AND R_F VALUES OF PEPTIDES

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Summary

The partition coefficients of 17 amino acids and 25 peptides have been determined in the 1-butanol–0·5% trichloroacetic acid–water system. Partition coefficients for β-corticotrophin and some large peptides derived from it are then calculated (Martin 1950) and compared with reported values.

R_F values reported for paper chromatography of β-corticotrophin fragments with 1-butanol–acetic acid–water agree reasonably with those predicted from R_F values of the constituent amino acids but, due to adsorption, are of little value in predicting partition coefficients in liquid–liquid systems.

I. Introduction

Interest in the present problem arose when counter-current distribution proved useful in the purification of secretin (Legge *et al.* 1957) and the factors governing choice of solvent systems were considered.

In this procedure, a solute is partitioned between two immiscible phases, generally at concentrations far below saturation. Solute–solute interactions are initially disregarded, and the movement of a molecule from one environment to another is considered to be primarily dependent on solvent–solute interactions.

Martin (1950), in considering the partition in ideal cases (where $\ln K_A = \Delta\mu_A/RT$), suggested that as a first approximation $\Delta\mu_A$, the change in chemical potential, may be regarded as being made up of

$$d\Delta\mu_{-CH_2-} + e\Delta\mu_{-COO-} + f\Delta\mu_{-NH_3^+} + g\Delta\mu_{-OH} + \ldots \text{ etc.},$$

the sum of the potential differences of the various groups of which molecule A is composed. The free energy required to transport a given group, e.g. $-CH_2-$, from one solvent to another would thus be independent of that needed for the transport of the rest of the molecule.

If one considers the partition coefficients K_A and K_B of two substances A and B, which differ in that B contains an additional group X, we have

$$\ln K_A = \Delta\mu_A/RT; \ \ln K_B = \Delta\mu_A/RT + \Delta\mu_X/RT;$$

and

$$\ln (K_B/K_A) = \Delta\mu_X/RT.$$

Thus the addition of X changes the partition coefficient by a given factor depending on the nature of the group, and on the pair of phases employed, but not the rest of the molecule.

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For a polypeptide the hypothesis may be expressed in the form

$$\ln K_P - \Sigma \ln K_{AA} = (n-1)C + D,$$

where K_P and K_{AA} are the partition coefficients of the peptide and the amino acid respectively. C is a constant which includes the μ values of the amino, carboxyl, and –CONH– groups, and D is a correction term for the difference between the terminal amino and carboxyl groups of a peptide and the corresponding amino acids. C and D include the factor RT.

Pardee (1951) applied this hypothesis to the R_F values of a number of amino acids and peptides, reported by Knight (1951), using the form

$$RT \ln(1/R_F - 1)_P - \Sigma RT \ln(1/R_F - 1)_{AA} = (n-1)A + B.$$

Glycine and leucine peptides predominated, the largest being a hexapeptide. Predicted R_F values agreed well with those found.

The equation was also applied by Moore and Baker (1958) to 33 dipeptides, 11 tripeptides, a tetrapeptide, and a pentapeptide in nine solvent systems. For the majority of compounds the agreement between calculated and found R_F values was within 0·05. Glycine and alanine residues predominated in the series. It has recently been used by Milstein and Sanger (1961).

Shepherd *et al.* (1956) and Bell *et al.* (1956) reported R_F values and partition coefficients for β-corticotrophin and a number of the peptides derived from it. It was therefore possible to assess the theoretical approach on data which included a greater variety of residues and peptides of larger size. The latter point is important since tertiary structure in solutions would hardly be significant in the case of the small peptides on which calculations have hitherto been based.

In addition, the newer data permitted a comparison of partition coefficients determined directly with those derived from R_F values. The prospect of being able to do this with confidence is tempting, in view of the greater ease of assessing the resolving power of a system by partition chromatography than by counter-current distribution. It would depend, however, on controlling adsorption—including the influence of a solid phase on transient tertiary structures—and any effect the paper might have on the phase compositions of the mixtures employed.

In many cases, however, amphipathic adjuvants such as *p*-toluenesulphonic acid, chloroacetic acids, etc. are needed for the counter-current distributions of the larger peptides. These are non-volatile and well adsorbed on paper. In such cases more elaborate forms of paper chromatography are needed in which the sample is spotted on a wet region where analysis has shown a constant phase composition. The convenience of the method is thereby lost and trial and error counter-current distribution must be used. Even this, however, might be assisted if Martin's theoretical approach were shown to be applicable.

The partition coefficients of amino acids and of a number of peptides were therefore determined in a 1-butanol–trichloroacetic acid–water system and the approach tested on the abovementioned data of Shepherd *et al.* (1956) and Bell *et al.* (1956).

II. Experimental

Samples of amino acids and peptides, many of the latter being gifts from the Division of Protein Chemistry, CSIRO, were examined by paper chromatography and those which were homogeneous were used for the determination of the partition coefficient. The peptides were DL-forms; Moore and Baker (1958) reported no differences in D-, L-, or DL-stereoisomers with the exception of certain cysteinyl peptides. These are absent from the series considered here. The 1-butanol was redistilled before use, the purity of the trichloroacetic acid (British Drug Houses, Ltd.) was checked analytically by alkalimetry.

The two phases in which the solutes were distributed were freshly prepared and used immediately so that esterification would be minimal (cf. Legge and Morieson 1964).

The concentration of the amino acid or peptide was approximately 2 mM. Vessels containing the amino acid solutions were equilibrated in a water-bath at 18°C. After reaching constant temperature they were shaken vigorously to attain equilibrium and allowed to settle in the bath. They were again shaken and, after phase separation, the concentration of amino acid in each phase was determined. The ninhydrin method of Connell, Dixon, and Hanes (1955) was used for all the estimations of amino acids and dipeptides except proline, which was estimated by the method of Chinard (1952). The solvent mixture did not interfere with the estimation of the amino acids.

The R_F values for the peptide fragments from β-corticotrophin had been obtained with the system 1-butanol–acetic acid–water in the ratio 1 : 5 : 4 by volume. Since the composition of this sytem is virtually the same as that used by Block (1952)—1 : 4 : 5 by volume, his data were used for the computations of the expected R_F values. Those for proline and glutamine were estimated from the chromatograms of Levy and Chung (1953) and Proom and Woiwood (1949).

The values used were as follows: Ala, 0·39; Arg, 0·19; Asp, 0·33; Glu, 0·39; $GluNH_2$, 0·27; Gly, 0·33; His, 0·19; Leu, 0·72; Lys, 0·18; Meth, 0·57; Phe, 0·66; Pro, 0·43; Ser, 0·31; Try, 0·53; Val, 0·56.

III. Computation of R_F Values from Pardee's Equation

There is no way of evaluating the constants A and B on purely theoretical grounds so they have to be derived from the empirical data.

The value of the function $\ln(1/R_F-1)_P-\Sigma \ln(1/R_F-1)_{AA}$ was calculated from these values for the peptides whose structures are set out in Table 1 (Shepherd *et al.* 1956) and plotted in Figure 1 against the number of peptide bonds in the peptide.*

The line drawn was that which minimizes deviations from linearity for the compounds up to nonapeptides. The two observations for compounds with 17 and 20 peptide bonds lie within the calculated limits. The values for A and B derived from the graph are −185 and −47 calories per mole respectively. Using these values R_F

* Note that these results must be multiplied by RT to give the left-hand side of Pardee's equation.

values for the peptides were computed from those of their constituent residues and are set out in Table 1, together with the deviation from the values found.

TABLE 1

DEDUCED STRUCTURE, AND OBSERVED AND CALCULATED R_F VALUES OF SOME PEPTIDES DERIVED FROM β-CORTICOTROPHIN

Symbol*	Deduced Structure of Peptide	Experimental R_F	Calculated R_F	ΔR_F
(3)	H.Asp.Glu.OH	0·22	0·27	+0·05
T_{19}	H.Lys.Arg.OH	0·05	0·07	+0·02
IV	H.Ala.Glu.OH	0·31	0·35	+0·04
C_4	H.Ser.Tyr.OH	0·39	0·42	+0·03
(1)	H.Glu.Leu.OH	0·71	0·68	−0·03
C_2	H.Glu.Phe.OH	0·66	0·62	−0·04
C_5	H.Arg.Tyr.OH	0·39	0·27	−0·12
(2)	H.Asp.Glu.Leu.OH	0·55	0·59	+0·04
VI	H.Gly.Ala.Glu.OH	0·25	0·25	0
II	H.Val.Tyr.Pro.OH	0·70	0·68	−0·02
PA_2	H.Pro.Leu.Glu.Phe.OH	0·75	0·86	+0·11
T_{15}	H.Arg.Pro.Val.Lys.OH	0·08	0·12	+0·04
I	H.Val.Tyr.Pro.Asp.OH	0·59	0·60	+0·01
a	H.Try.Gly.Lys.Pro.OH	0·36	0·25	−0·11
C_7	H.Ser.Met.Glu.His.Phe.OH	0·39	0·61	+0·22
PA_4	H.Leu.Ala.Glu.Ala.Phe.OH	0·67	0·82	+0·15
b	H.Try.Gly.Lys.Pro.Val.OH	0·45	0·38	−0·07
T_{18}	H.Arg.Arg.Pro.Val.Lys.OH	0·10	0·04	−0·06
C_{16}	H.Gly.Lys.Pro.Val.Gly.Lys.OH	0·02	0·05	+0·03
T_{17}	H.Lys.Arg.Arg.Pro.Val.Lys.OH	0·02	0·01	−0·01
c	H.Try.Gly.Lys.Pro.Val.Gly.OH	0·61	0·27	−0·32
PA_3	H.Ala.Glu.Ala.Phe.Pro.Leu.Glu.OH	0·67	0·79	+0·12
P_4T_1	H.Val.Tyr.Pro.Asp.Gly.Ala.Glu.OH	0·46	0·41	−0·05
T_{14}	H.Try.Gly.Lys.Pro.Val.Gly.Lys.OH	0·20	0·10	−0·10
PA_1	H.Ala.Glu.Ala.Phe.Pro.Leu.Glu.Phe.OH	0·75	0·91	+0·16
T_{10}	H.Ser.Tyr.Ser.Met.Glu.His.Phe.Arg.OH	0·36	0·33	−0·03
T_{16}	H.Try.Gly.Lys.Pro.Val.Gly.Lys.Lys.OH	0·13	0·02	−0·11
PA_5	H.Leu.Ala.Glu.Ala.Phe.Pro.Leu.Glu.Phe.OH	0·83	0·97	+0·14
P_3T_1	H.Val.Tyr.Pro.Asp.Gly.Ala.Glu.Asp.GluNH_2.OH	0·36	0·19	−0·17
T_1	H.Val.Tyr.Pro.Asp.Gly.Ala.Glu.Asp.GluNH_2.Leu.Ala.-Glu.Ala.Phe.Pro.Leu.Glu.Phe.OH	0·72	0·97	+0·25
C_{10}	H.Lys.Arg.Arg.Pro.Val.Lys.Val.Tyr.Pro.Asp.Gly.Ala.-Glu.Asp.Glu.Leu.Ala.Glu.Ala.Phe.Pro.Leu.OH	0·11	0·11	0

* See Shepherd *et al.* (1956).

The deviation of points from the line of best fit in Figure 1 corresponds to a free energy change of ±340 calories. This comprises both experimental error and theoretical defect. The change in $\Delta\mu$ due to an error in R_F is large when the R_F value approaches 0 or 1, and is a minimum when $R_F = 0{\cdot}50$, e.g. when $R_F = 0{\cdot}95$ and $0{\cdot}50$ an error of $0{\cdot}02$ causes a change in $\Delta\mu$ of 250 and 50 calories respectively.

Duplicate determinations of R_F generally agree by no more than 0·04, and in 17 of the 31 peptides, with a wide range of R_F values, the deviations between observed and calculated values is less than 0·06.

If the ratio of the volumes of the stationary and mobile phase in a particular chromatogram is known, it is possible to relate the R_F to the partition coefficient according to the equation

$$A_L/A_S = 1/\mathrm{K}\{(1/R_F - 1)\},$$

where A_L/A_S is equal to the ratio of the volume of organic and aqueous phase in the chromatograms. This ratio is generally assumed constant for a given temperature.

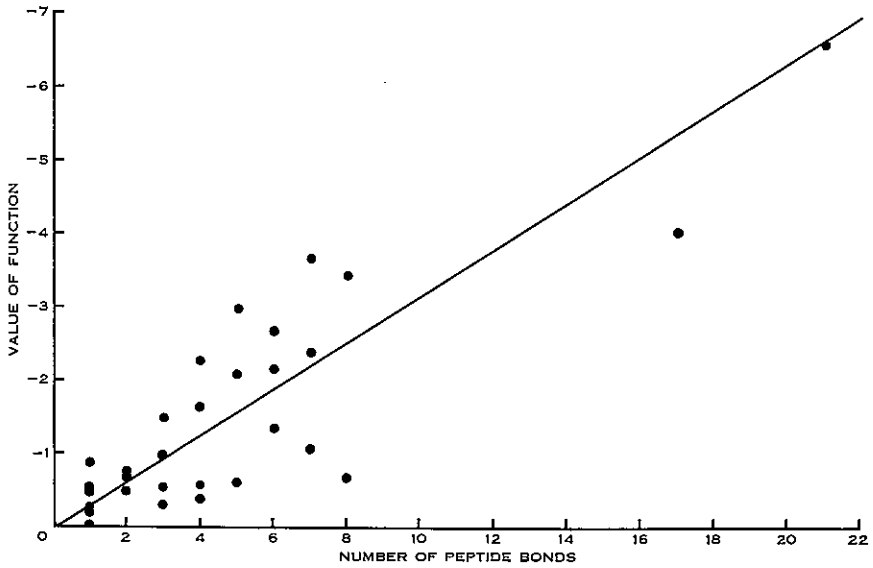

Fig. 1.—Value of function $\{\ln(1/R_F-1)_P-\Sigma\ln(1/R_F-1)_{AA}\}$ plotted against number of peptide bonds for peptides in Table 1.

Independent determinations are available (Bell *et al.* 1956; Shepherd *et al.* 1956) for the partition coefficients of 16 of the peptides in Table 1. From these, and from the R_F values determined from partition coefficients the ratio A_L/A_S can be determined. The results are recorded in Table 2.

The peptides are arranged in order of increasing values of A_L/A_S. This may be seen to vary eightfold. Possible causes for this variation are discussed later but it may be noted here that if an "average" value for A_L/A_S is taken and used to calculate a partition coefficient from an R_F value the result may differ from that found experimentally by a factor of four.

IV. Computation of Partition Coefficients from Martin's Equation

In the case of liquid systems any effects of adsorption on a solid phase is neglected. Table 3 sets out the experimentally determined partition coefficients

of 17 amino acids between organic and aqueous phases of the 1-butanol–0·5% trichloroacetic acid–water system.

Table 4 sets out the experimentally determined partition coefficients for 25 peptides in this system together with derived data.

As in the previous case the constants C and D must be determined graphically. The function $\ln K_P - \Sigma \ln K_{AA}$ was evaluated (cf. Table 4). According to Martin's hypothesis this should be constant for a given dipeptide, but this is not so and possible reasons are considered later. Average values for di- and tripeptides together with the individual values for the four larger peptides (Bell *et al.* 1956) are graphed in Figure 2 against the number of peptide bonds present.

TABLE 2
CALCULATION OF A_L/A_S

Symbol*	Deduced Structure of Peptide	Experimental R_F	Partition Coefficient	A_L/A_S
T_{17}	H.Lys.Arg.Arg.Pro.Val.Lys.OH	0·02	0·04	0·5
T_1	H.Val.Tyr.Pro.Asp.Gly.Ala.Glu.Asp.GluNH$_2$-Leu.Ala.Glu.Ala.Phe.Pro.Leu.Glu.Phe.OH	0·72	5	0·5
T_{19}	H.Lys.Arg.OH	0·05	*c.*0·05	1·0
P_3T_1	H.Val.Tyr.Pro.Asp.Gly.Ala.Glu.Asp.GluNH$_2$.OH	0·36	0·39	1·4
C_4	H.Ser.Tyr.OH	0·39	0·4	1·6
T_{15}	H.Arg.Pro.Val.Lys.OH	0·08	*c.*0·05	1·7
C_2	H.Glu.Phe.OH	0·66	0·8	2·0
C_{16}	H.Gly.Lys.Pro.Val.Gly.Lys.OH	0·02	*c.*0·01	2·0
T_{18}	H.Arg.Arg.Pro.Val.Lys.OH	0·10	*c.*0·05	2·1
C_5	H.Arg.Tyr.OH	0·39	0·3	2·1
P_4T_1	H.Val.Tyr.Pro.Asp.Gly.Ala.Glu.OH	0·46	0·39	2·2
C_{10}	H.Lys.Arg.Arg.Pro.Val.Lys.Val.Tyr.Pro.Asp.-Gly.Ala.Glu.Asp.Glu.Leu.Ala.Glu.Ala.Phe.Pro.Leu.OH	0·11	*c.*0·5	2·5
T_{14}	H.Try.Gly.Lys.Pro.Val.Gly.Lys.OH	0·20	0·09	2·8
T_{16}	H.Try.Gly.Lys.Pro.Val.Gly.Lys.Lys.OH	0·13	*c.*0·05	3·0
C_7	H.Ser.Met.Glu.His.Phe.OH	0·39	0·2	3·2
T_{10}	H.Ser.Tyr.Ser.Met.Glu.His.Phe.Arg.OH	0·36	0·13	4·3

* See Shepherd *et al.* (1956).

Values of C and D derived from the graph were used to calculate the partition coefficients which are recorded in Table 4.

With the exceptions of Leu.Glu, the agreement between found and calculated partition constants for di- and tripeptides is within a factor of 2. With P_4, P_3, P_2, and β-corticotrophin, however, the predicted value may differ from that found by a factor of 10.

V. DISCUSSION

The data in Table 2 represent an attempt to compare R_F values and partition coefficients for the same peptides in the same system. If adsorption can be neglected then A_L/A_S, the ratio of the volumes of the mobile to the stationary phase, should be constant (cf. Martin 1948). This was not the case. A small value for A_L/A_S

TABLE 3

PARTITION OF AMINO ACIDS BETWEEN 0·5% TRICHLOROACETIC ACID–1-BUTANOL–WATER

Amino Acid	K	ln K	Amino Acid	K	ln K
Alanine	0·172	−1·760	Lysine	0·036	−3·324
Arginine	0·108	−2·226	Methionine	0·591	−0·521
Aspartic acid	0·131	−2·033	Phenylalanine	1·44	0·365
Asparagine	0·059	−2·837	Proline	0·145	−1·931
Glutamic acid	0·168	−1·784	Serine	0·098	−2·323
Glutamine	0·085	−2·465	Tryptophan	3·50	1·253
Glycine	0·101	−2·273	Tyrosine	0·794	−0·231
Histidine	0·048	−3·037	Valine	0·529	−0·637
Leucine	1·28	0·247			

TABLE 4

PARTITION OF PEPTIDES BETWEEN 0·5% TRICHLOROACETIC ACID–1-BUTANOL–WATER

Peptide	K	ln K	Calc. K	$\ln K_P - \Sigma \ln K_{AA}$
Ala.Ala	0·300	−1·204	0·20	2·31
Ala.Gly	0·159	−1·839	0·12	2·19
Ala.Val	0·954	−0·047	0·62	2·35
Asp.Gly	0·089	−2·419	0·09	1·89
Glu.Phe	2·51	0·920	1·65	2·24
Gly.Ala	0·103	−2·273	0·12	1·76
Gly.Asp	0·117	−2·146	0·09	1·16
Gly.AspNH_2	0·049	−3·008	0·04	2·10
Gly.Glu	0·074	−2·602	0·12	1·45
Gly.Gly	0·075	−2·590	0·07	1·95
Gly.Leu	1·68	0·519	0·90	2·54
Gly.Phe	1·77	0·571	1·01	2·48
Gly.Pro	0·101	−2·323	0·10	1·88
Gly.Try	3·28	1·188	2·46	2·21
Gly.Tyr	0·859	−0·152	0·55	2·35
Gly.Val	0·602	−0·507	0·37	2·40
Leu.AspNH_2	0·405	−0·904	0·51	1·69
Leu.Glu	0·515	−0·664	1·46	0·87
Leu.GluNH_2	0·534	−0·627	0·74	1·59
Leu.Gly	0·826	−0·191	0·90	1·84
Leu.Tyr	4·41	1·484	6·91	1·47
Leu.Val	5·01	1·611	4·61	2·00
Tyr.Leu	9·91	2·294	6·91	2·28
Val.Gly	0·340	−1·079	0·37	1·83
Ala.Gly.Gly	0·090	−2·412	0·06	3·89
Leu.Gly.Gly	0·587	−0·533	0·70	3·77
P_4*	0·32	−1·140	0·06	47·76
P_3*	0·23	−1·470	0·02	53·56
P_2*	1·5	0·405	0·14	51·93
β-Corticotrophin	7	1·946	40·5	63·14

* See Bell *et al.* (1956).

would be consistent with adsorption on the swollen paper. However, inspection of the data fails to show any correlation between A_L/A_S and size of fragment, proportion of cationic residues in the peptide present in the 1-butanol–acetic acid–water system, or the proportion of potential H-bonding groups. In 11 of the 16 cases the calculated R_F values lie within 0·06 of the value found; these cases, however, show just as great a scatter in the value for A_L/A_S as do those where the R_F value has not been so adequately predicted. This suggests that, in 11 cases, the effect of the paper is reflected equally in the R_F values of the free and combined amino acids.

The data cannot be readily accommodated by any simple adsorption or molecular sieve theory and it appears more likely that the presence of swollen paper has significantly altered the composition of the phases, thus invalidating the basis of the

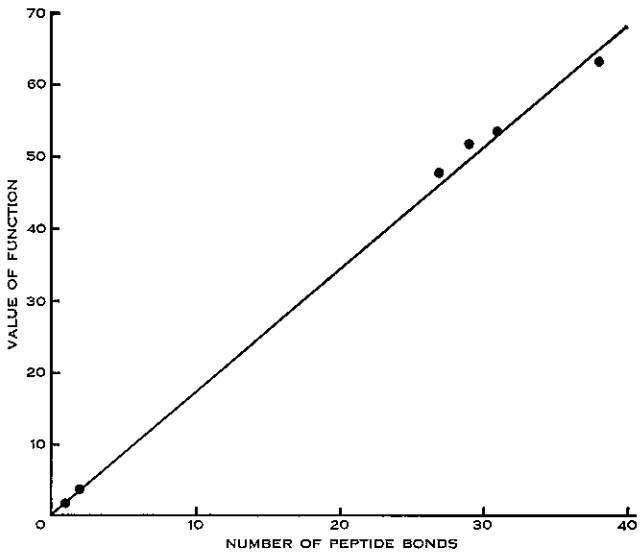

Fig. 2.—Value of function $\{\ln K_P - \Sigma \ln K_{AA}\}$ plotted against number of peptide bonds for peptides in Table 4.

comparison. In any event it must be concluded that, in the case examined, R_F values are quite inadequate for predicting partition coefficients; indeed, the relative order in which some components would travel would be quite different in the two systems.

The uncertainty on this point makes it difficult to decide whether the theory outlined in the Introduction applies better to liquid–liquid systems or to partition chromatography. Since both the peptides examined and the systems used differ in each case they cannot be directly compared with one another. Nor could much confidence be placed in an estimate of the variance as a measure of adequacy.

In Table 1 the greatest deviation between found and predicted R_F values is in the case of peptide c— found 0·61, predicted 0·29, deviation −0·32. In Table 4 the greatest discrepancy between found and predicted partition coefficients is represented by peptide P_2, found 1·5, predicted 0·14. If a value for A_L/A_S of 3 is assumed, purely in order to convert the data to the same unit, then the "found" R_F value is

0·33, and that "predicted" 0·05, a deviation of −0·28. In these two extreme cases the theory can be said to fail equally badly, no doubt due to the fact that it specifically excluded consideration of residue–residue interactions within a given molecule as well as intermolecular interactions. The function $\ln K_P - \Sigma \ln K_{AA}$, which would reflect these interactions, is not even constant for dipeptides (Table 4). Even in relatively simple molecules, substitution with lyophilic groups can have apparently paradoxical effects on solubility (Albert 1951). Interactions between residues in all but the simplest proteins is a far more complicated problem (cf. Perutz 1962, p. 59). Its solution may be of greater assistance in designing distribution systems than the approach considered here.

TABLE 5

INFLUENCE OF GROUPS ON PARTITION COEFFICIENT IN 0·5% TRICHLOROACETIC ACID–1-BUTANOL–WATER

Influence of:	Substance *A*, *B*	$\ln K_A$	$\ln K_B$	$\ln K_B - \ln K_A$
$-CH_2-$	Gly, Ala	−2·273	−1·760	+0·51
	Val, Leu	−0·637	0·247	+0·88
	Asp, Glu	−2·033	−1·784	+0·25
	$AspNH_2$, $GluNH_2$	−2·837	−2·465	+0·37
–OH–	Ala, Ser	−1·760	−2·323	−0·56
$-C_6H_5$	Ala, Phe	−1·760	0·365	+2·13
$-NH_3^+$	Leu, Lys	0·247	−3·324	−3·57
–COOH	Ala, Asp	−1·760	−2·033	−0·27
$-CONH_2$	Asp, $AspNH_2$	−2·033	−2·837	−0·80
	Glu, $GluNH_2$	−1·784	−2·465	−0·68
$-CH_2CONH-$	X, Gly.X (10 peptides)	—	—	−0·18 (+0·27 to −0·82)
$-CONHCH_2-$	X, X.Gly (5 peptides)	—	—	−0·36 (−0·08 to −0·44)
–CONH–	$-CH_2-$, $-CH_2CONH-$	—	—	−0·68
	$-CH_2-$, $-CONHCH_2-$	—	—	−0·86

The data for amino acids and smaller peptides in Tables 3 and 4 may be used, as a first approximation, to estimate the influence of various chemical groupings in the structure on the partition coefficient. This may be used to judge the possible effect of substituents on the partition coefficient of a peptide or protein.

Pairs of substances, differing in a particular grouping, such as $-CH_2-$, –OH, etc. are selected from Tables 3 and 4 and the differences in the logarithms of their partition coefficients recorded in Table 5.

The actual effect is much as one might expect in the solvent system. Thus each $-CH_2-$ group in a side chain terminating in a methyl group may be thought of as

increasing the solubility in the organic phase relative to that in water by a factor of 1·7. The change in relative solubility due to $-CH_2-$ as one goes from valine to leucine is greater (2·4-fold) than the change from glycine to alanine. The influence of $-CH_2-$ groups between polar groups is much less. Thus the differences in relative solubility for aspartic acid and glutamic acid is only 1·3-fold and for asparagine and glutamine 1·5-fold. The –OH group increases the solubility of serine relative to alanine in the aqueous phase by a factor of 1·8. The effect of substituting an un-ionized carboxyl group for hydrogen in enhancing solubility in the aqueous phase is less than is the effect of an –OH group. The influence of a benzene ring in increasing relative solubility in the organic phase is equivalent to between four and five $-CH_2-$ groups (cf. Langmuir and Waugh 1940). Amide formation increases the relative solubility in the aqueous phase by a factor of approximately 2. In this system cationic groups will carry a full positive charge, and this has a marked effect if one compares the $-NH_3^+$ group with a methyl group as between lysine and leucine, the former is some 35 times more soluble in the aqueous phase. The effect of the peptide bond is to increase the solubility in the aqueous phase relative to that in the organic phase.

In view of the uncertainty as to the species present in the two phases (Legge and Morieson 1964) and of difficulties in the interpretation of the influence of non-ionic and amphipathic substances on the structure of aqueous solutions [cf. Everett (and others) 1957] it would seem unprofitable to theorize on these empirical results. They do, however, agree reasonably with some reported by Harfenist (1953) on the partition of substituted insulins in 2-butanol–1% dichloroacetic acid–water. Methylation of one carboxyl group alters the K by a factor of 2. This is of the same order that is expected for a change of one $-CH_2-$ group in the system 1-butanol–0·5% trichloroacetic acid–water. The fivefold change when one amino group has been reacted with fluorodinitrobenzene is approximately the same order as that expected for a benzene ring in the systems examined here. The di-DNP derivative shows a further three- to fourfold change in K.

VI. Acknowledgments

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