CHARACTERIZATION OF THE HYDROPHOBIC PROPERTIES OF AMINO ACIDS. II. HOW HYDROPHOBIC, HYDROPHILIC AND LIPOPHILIC IS TRYPTOPHAN?

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The model for characterization of hydrophobicity, hydrophilicity and lipophilicity of compounds is presented. It is based on the vapour-to-solvent coefficients for hydrophilicity and lipophilicity and the partition coefficient for hydrophobicity. It is shown that some apparently contradictory facts can be understood on the basis of this model.

The quantitative assessment of hydrophobicity of amino acids and its role in protein folding and stability is one of the most discussed topics in protein chemistry[1]. However, more than 30 years after publishing of Kauzmann's seminal review[2] this problem is far from being elucidated completely. The discussion on hydrophobic properties of tryptophan between Fauchere[3,4] and Wolfenden[5,6] is a clear evidence of this fact.

There appear two fundamental problems in the determination of hydrophobicity of compounds. One is associated with the definition of hydrophobicity, hydrophilicity and lipophilicity, i.e., with the selection of a suitable quantity which would characterize the given property. Different indices (e.g., solubility, partition coefficient, surface tension and chromatographic retention) were used to describe these properties, however, a general consensus has not been achieved (for review see ref.[7]).

In the present work, a suitable model for characterization of hydrophobicity, hydrophilicity and lipophilicity is shown and their relationships are discussed. The subtitle of the paper is evoking the above mentioned discussion[3 – 6] as an example of complexity of this topic.

THEORETICAL

Characterization of affinity of the given compound to water or a nonpolar solvent in terms of solubility and vapour-to-solvent distribution coefficients seems to be a suitable way to express hydrophilicity and lipophilicity of this compound. In view of the character of the data discussed in the above cited polemic[3 – 6] the present paper is restricted to the vapour-to-sovent coefficients only. In the case of hydrophilicity the vapour-to-water coefficient K_W is defined by Eq. (*1*) and in the case of lipophilicity the vapour-to-nonpolar solvent coefficient K_N is defined by Eq. (*2*)

$$K_W = a_W / a_G \tag{1}$$

$$K_N = a_N / a_G , \tag{2}$$

where a_W is the activity of the compound in water, a_N is the activity of the compound in the nonpolar solvent and a_G is the activity of the compound in the gaseous phase.

The suitable expression of hydrophobicity is the partition coefficient $K_{H\Phi}$ of the compound in a suitable solvent system defined by Eq. (*3*)

$$K_{H\Phi} = a_O / a_A , \tag{3}$$

where a_O is the activity of the compound in the organic phase and a_A is the activity of the compound in the aqueous phase. With respect to the fact that the partition coefficients are studied in a water – finitely miscible organic solvent system, if hydrophobic properties are to be characterized, a_O and a_A express the activity of the compound in the organic and aqueous phases, respectively, and not in a pure organic solvent or in water alone. This means that the coefficient K_X (given by the ratio of coefficients K_N/K_W) is not identical with the partition coefficient $K_{H\Phi}$ since, as follows from Eq. (*1*) to Eq. (*3*),

$$a_N / a_W \neq a_O / a_A . \tag{4}$$

DISCUSSION

So that vapour-to-solvent coefficients could be used for characterization of hydrophobic properties of compounds (corresponding to the partition coefficient $K_{H\Phi}$), it is necessary to use as solvents such mixtures which correspond to the equilibrium composition of the organic and aqueous phases during the partitioning. If the coefficient K_X is used, considerable deviations from the value $K_{H\Phi}$ may occur (see Table I which compares the data for three hydrophobic amino acids – leucine, phenylalanine and tryptophan). These deviations are, first of all, due to the absence of water in a pure nonpolar solvent.

The other problem lies in the selection of a suitable system for the partition process. It is obvious that it is not possible to use generally only one solvent system because of the behaviour of compounds during the processes as different as, for instance, accumulation of compounds in tissues, adsorption on various surfaces, extraction from different commodities or protein folding. For this reason, it is necessary to select such a solvent system which would correspond best with the conditions of the process under study.

In the case of protein folding such a suitable system is 1-octanol–water and, moreover, the partitioning models real processes in the protein folding better than, e.g., dissolution of amino acids in different solvents. 1-octanol has relatively nonpolar character, however, at the same time, it contains a polar group capable of forming hydrogen bonds. Its permittivity approaches that of protein interiors. The presence of water in the octanol phase permits the formation of hydrated molecules of the dissolved compound in the organic phase. Hydration of polar and charged side chains of amino acids decreases unfavourable energetic contribution to the transfer of the side chains into a nonpolar medium and hence into both the nonpolar solvent and the protein interior. Hydrated side chains were found in the protein interiors.

The above-mentioned relationships show that both Fauchere and Wolfenden are right as far as tryptophan is concerned although they do not agree with each other. It follows from the measurement of the partition coefficients in 1-octanol–water system[9 – 11] that tryptophan is the most hydrophobic amino acid occurring in proteins. The finding of Wolfenden et al.[12] that the analogue of the side chain of tryptophan (3-methylindole) is more hydrophilic than the analogue of the side chain of phenylalanine (toluene) is also correct. How is it possible that tryptophan is both more hydrophobic and more hydrophilic than phenylalanine? Because it is also more lipophilic – see the coefficient K_N in Table I. If a nonaqueous nonpolar solvent such as cyclohexane is used, phenylalanine has a higher value K_X (see Table I). The same also holds for oil[13 – 15] but tryptophan has a higher affinity to other organic solvents[16]. The presence or absence of water in the organic phase is of great importance. Cyclohexane was chosen as a reference solvent because it contains only slightly higher amount of water at saturation than vapour phase over water at room temperature. Different character of the partitioning of amines in the aqueous and nonaqueous octanol was observed experimentally[17]. It is probable that hydration of the nitrogen containing part of indole[18,19] contributes to tryptophan transfer into the octanol phase.

TABLE I

Comparison of lipophilic (K_N), hydrophilic (K_W) and hydrophobic ($K_{H\Phi}$) properties of side chains of leucine, phenylalanine and tryptophan

Side chain	$\log K_N$[a]	$\log K_W$[a]	$\log K_X$[a]	$\log K_{H\Phi,A}$[a]	$\log K_{H\Phi,R}$[b]
Leu	1.94	–1.68	3.62	2.76	1.50
Phe	2.75	0.56	2.19	2.58	1.91
Trp	6.04	4.32	1.71	2.60	2.01

[a] Data from ref.[8] for volatile analogues (isobutane for leucine, toluene for phenylalanine and 3-methylindole for tryptophan): K_N is the vapour-to-cyclohexane coefficient, K_W is the vapour-to-water coefficient, $K_X = K_N/K_W$ and $K_{H\Phi,A}$ is the partition coefficient in the 1-octanol–water system. [b] Data from ref.[9] for amino acid side chains: $K_{H\Phi,R}$ is the partition coefficient in the 1-octanol–water system.

In characterizing hydrophobic properties of side chains of amino acids the third problem appears – the selection of a suitable type of model compounds. Amino acids are used most frequently, however, their character is too polar with regard to the polypeptide chain. The use of volatile analogues is suitable in some cases, however, it is not advantageous in others, such as hydrogen for glycine. Another possibility is the use of short peptides (e.g. Gly-X-Gly) and their less charged analogues (e.g. methyl esters or N-acetyl amides), however, no comprehensive study has so far been published on the partitioning of these compounds. For this reason, N-acetyl-amino acid amides used by Fauchere and Pliška[10] seem to be suitable model compounds. The different values of the partition coefficients for amino acid side chains $K_{\mathrm{H\Phi,R}}$ and their volatile analogues $\mathrm{K_{H\Phi,A}}$ (see Table I) can be explained by change of properties of side chains as a result of their attachment to the hydrophilic moiety of the amino acid molecule[20].

Tryptophan and phenylalanine are the most hydrophobic amino acids occurring in proteins and their side chains rank among the least accessible for solvent molecules in globular proteins. More polar character of the side chain of tryptophan may cause its somewhat higher accessibility for the solvent and a lower percentage of entirely unaccessible residues than in the case of phenylalanine[21].

Characterization of hydrophobic properties of compounds is a complex problem and the investigation of hydrophobic properties of amino acids and their role in protein folding has not been concluded yet[22 – 25]. Discussing hydrophobic properties we must be aware what the terms such as hydrophobicity, hydrophilicity and lipophilicity designate. The proposal mentioned above, based on the partition coefficients $K_{\mathrm{H\Phi}}$, K_{W} and K_{N}, provides a certain idea of the characterization of these concepts and shows the relationships among them on the basis of which some apparently contradictory facts may be understood.

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