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An Index of Protein Hydrophobicity. Its Application to Membrane Proteins

J. C. Gómez *, M. A. Ventura **, F. M. Goñi *** and E. Santiago

Departamento de Bioquímica
Universidad de Navarra
Pamplona (España)

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A hydrophobicity index is proposed for proteins. The calculation of this index is made, assuming a direct relationship between the hydrophobicity of each aminoacid and its Rf in a partition chromatography. This index is applied to membrane proteins and offers statistically significant differences between integral and peripheral proteins.

Biological membrane proteins may be divided into two groups, namely *peripheral* and *integral* proteins (26). Integral proteins are tsrongly and, perhaps, specifically bound to membrane lipids, whereas peripheral proteins are more loosely bound to the membrane core.

It is not known what makes a protein to behave as integral or peripheral, or else as a *soluble* protein, unrelated to membranes. It has been proposed that integral proteins would possess hydrophobic and hydrophilic peptides in contact with the lipid and aqueous phase respectively. Aminoacid sequence determination of cytochrome b_5 has actually shown the existence of these hydrophilic and hydrophobic peptides (21, 31). It is also possible that, although these two types of zones would not be identified by the study of the primary structure, a given protein could show an amphipathic character because of its tertiary structure.

Attempts have been made (2, 7) to relate the more or less hydrophobic character of a protein, and therefore its integral or peripheral nature, to its aminoacid composition. These attempts have not been proved completely satisfactory. In this report an index for measuring protein hydrophobicity is proposed, assuming a direct relatioship between the hydrophobicity of each animoacid and its Rf in a partition chromatography. Statistically significant differences can be observed

* Present address: Dpt. of Biochemistry, University of Oxford, Oxford (U.K.).

** Present address: Laboratoire d'Endocrinologie, Université de Paris XI, Orsay (France).

*** Present address: Dpto. de Bioquímica, Universidad de Bilbao, Bilbao (Spain).

between hydrophobicity indexes (H.I.) of integral and peripheral proteins.

Materials and Methods

Calculation of the H.I. This index is based on the assumption that the hydrophobicity of a given protein may be estimated by adding the hydrophobicities due to each aminoacid. The hydrophobicities of the different aminoacids are assimilated to their respective Rf × 100 values in a chromatographic system based mainly on partition processes.

The Rf × 100 values chosen have been those cited by SMITH (27) for paper chromatography using butanol-pyridine-water (1:1:1) as solvent. These values are reproduced in table 1. The H.I. is computed from the percent composition in aminoacids of the protein. Percent molar values of each aminoacid are multiplied by the respective Rf's × 100, and added. The total sum is the so-called «hydrophobicity index» (H.I.).

Table I. *Rf × 100 values of the 20 common aminoacids*
These Rf values correspond to an ascending partition chromatography, using butanol-pyridine-water (1:1:1) as solvent (27).

Name	*Rf × 100*
Lysine	13
Cysteine	14
Arginine	15
Aspartic acid	20
Glutamic acid	20
Histidine	24
Glycine	29
Hidroxyproline	30
Serine	33
Proline	34
Threonine	36
Alanine	37
Valine	48
Methionine	53
Isoleucine	56
Leucine	60
Tyrosine	60
Tryptophan	62
Phenylalanine	63

Aminoacid composition of ten integral, ten peripheral and ten soluble proteins was taken from the literature. The validity of our results was tested by means of a Model 1 single classification analysis of the variance, according to SOKAL and ROHLF (28).

Results and Discussion

Table II shows the H.I. of the thirty proteins mentioned above.

The analysis of the variance was significant ($p < 0.01$). Thus, comparisons among means were established using an *a posteriori* SS-STP test (28).

Integral proteins had an H.I. significantly higher than peripheral and soluble proteins. No significant difference was found between peripheral and soluble proteins.

The H.I. was also calculated for the different subunits of proteins showing complex quaternary structure, such as the oligomycin-sensitive mitochondrial ATPase. This enzyme has a spherical «head», which protrudes out of the membrane into the water phase, and is linked through a «stalk» to a «base piece». Interestingly enough, the «head» polypeptides (24) have an average value for H.I. of 3266, which accounts for a protein of extrinsic nature, while one of the main polypeptides of the base piece, described by TZAGOLOFF *et al.* (34), which is supposed to be deeply buried into the lipid core, has the extremely high H.I. value of 4109, which corresponds to a protein of a highly integral character.

The validity of the H.I. was tested by calculating the corresponding values for the 30 proteins on different data: namely, Rf values cited by SMITH (27) for paper chromatography using butanol-acetic acid-water (12:3:5) as solvent. This mobile phase is acid, in contrast with butanol-

Table II. *Hydrophobicity indexes of ten inte-gral, ten peripheral and ten soluble proteins.* For details see text.

	H.I.	Ref.
Integral proteins		
C_{55} Isoprenyl alcohol phosphokinase	4261	22
Carotenoid glycoprotein, *Sarcina flava*	4070	33
Folch-Lees proteolipid protein, sciatic myelin	4064	6
Cytochrome b, mitochondrial	4019	19
Proteolipid, sarcoplasmic reticulum	3936	13
Cytochrome a, mitochondrial beef heart	3880	15
Purple membrane protein of *Halobacterium halobium*	3878	30
Rhodopsin, bovine	3791	25
Sarcoplasmic reticulum ATPase	3611	11
Cytochrome b_5, microsomal	3503	29
Mean H.I. value±S.D.	3901±71	
Peripheral proteins		
D-glyceraldehyde-3-phospate dehydrogenase	3601	32
ATPase, *Bacillus megaterium*	3591	18
ATPase, mitochondrial	3567	3
ATPase, *Streptococcus foecalis*	3544	23
Monoamine oxidase, mitochondrial	3530	20
Calsequestrin, sarcoplasmic reticulum	3365	12
Cytochrome c, human	3297	16
Basic protein, sciatic myelin	3203	6
Basic protein, brain myelin	3154	6
54 K protein, sarcoplasmic reticulum	3107	13
Mean H.I. value±S.D.	3396±91	
Soluble proteins (unrelated to membranes)		
Acetoacetate decarboxilase	3662	10
Alanine aminotransferase	3599	17
Ceruloplasmin	3598	9
Adenylate kinase	3595	14
Fumarate hydratase	3579	8
Molate dehydrogenase	3546	5
Phospoenolpyruvate carboxykinase	3530	4
D-Amylase	3524	35
K-Casein (component A-1)	3480	36
Chorionic gonadotropin	3288	1
Mean H.I. value±S.D.	3540±32	

pyridine-water, which is alkaline, and thus the relative mobilities of the ionizable aminoacids are different. However, similar significant differences were found between the indexes of peripheral and integral proteins.

These results suggest that a direct relationship exists between the aminoacid composition of a membrane protein and its relation to membrane structure. The hydrophobicity index permits in this way to predict with reasonable accuracy the integral or peripheral character of a protein whose aminoacid composition has been investigated after polyacrylamide gel electrophosis of a complex mixture.

Resumen

Se propone un índice de hidrofobicidad aplicable a las proteínas. El cálculo de este índice se realiza suponiendo que existe una relación

directa entre el índice de hidrofobicidad de cada aminoácido y su Rf en una cromatografía de partición. Al aplicar este índice a proteínas de membranas se observa la existencia de diferencias estadísticamente significativas entre proteínas integrales y periféricas.

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