THE NICOTINIC CHOLINERGIC RECEPTOR : DIFFERENT COMPOSITIONS EVIDENCED BY STATISTICAL ANALYSIS

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Summary:The available acetylcholine receptor proteins from the electric organs of Electrophoridae and Torpedinidae are analysed in respect of quantitative statistical parameters. These parameters take into account the hydrophilic and hydrophobic moieties of the membrane proteins. The statistical analysis, based on the use of discriminant functions, brings out clear differences between the cholinergic receptor proteins, on the one hand, and non-membrane and loosely bound, peripheral membrane proteins, on the other. Subtle differences between the receptor proteins are also observed. The significance of the findings is discussed in the light of the physico-chemical properties of these proteins.

Several physico-chemical criteria were outlined by Singer and Nicolson to distinguish between "integral" and "peripheral" membrane proteins (1). Unfortunately, none of these criteria has been worked to a point enabling a rigorous quantitative estimate of the differences between the two classes of proteins. In fact, the only properties of these proteins amenable to both i) quantification and ii) subsequent statistical treatment are, to date, those derived from their amino acid composition. With this in mind, the average hydrophobicity was introduced in the study of membrane proteins (2). Similarly, the polarity index (3) was used to draw a general distinction between "soluble" i.e. non-membrane and some membrane proteins (4). These two approaches (2,4) put emphasis respectively on the hydrophobic and hydrophilic moieties of the proteins, thus ignoring their relative proportion. This latter relationship was taken into account in a subsequent work in which ratios of amino acids were considered in the analysis of several membrane proteins from the nervous system (5).

More recently, however, Moore et al. (7) using the polarity

index (3) and hydrophobicity calculations conclude that the cholinergic receptor from Torpedo nobiliana has the characteristics of soluble proteins. This is in contradiction to the physico-chemical properties of this and all other nicotinic receptor proteins so far studied.

This paper describes the application of a statistical technique i.e. discriminant function analysis, which simultaneously takes into account the optimum combination of values derived from the hydrophilic and hydrophobic moieties of membrane proteins to separate the integral from the peripheral ones. These values were obtained from a comparative study of more than 240 membrane and non-membrane proteins, thus enabling a rigorous statistical treatment of the data. The analysis is applied to the amino acid parameters of all the available acetylcholine receptor proteins, resulting in their categorisation as per the physico-chemical criteria, i.e. as integral membrane proteins.

Material and Methods

The definition of amino acid sets and their ratios was given in a previous paper (5). In the present work only one ratio is used:

Ratio 3 = Lys+Arg+His+Asx+Glx / Ile+Tyr+Phe+Leu+Val+Met

The normalised average hydrophobicity (8) used in the present study is essentially that defined by Bigelow (9) as $H\Phi_{ave}$, based on the free energy of transfer of amino acid residues, ΔF_t , introduced by Tanford (10): $\bar{H}\Phi_{ave} = \Sigma \Delta F_t / n$

where the summation includes all amino acids, n, with the exception of Trp. This omission does not alter the distinction between integral and peripheral or between integral and non-membrane proteins (8). The discriminant functions are linear combinations of variables in the form:

$$Z = (aV_1) + (bV_2) + \ldots (nV_j)$$

where a,b,...n are the discriminant function coefficients and $V_1 \ldots V_j$ are the values of a given variable for each individual protein.

The mean value, standard deviation, and statistical significance

of the discrimination achieved, p_Z, was calculated for these functions. The probability of misclassification was evaluated by the t-test. Programmes for Elementary Population Statistics and Discriminant Function Analysis were written at the U.C.L.A. Health Sciences Computing Facilities and at the Centro de Cómputos, Faculty of Medicine, University of Buenos Aires, using Fortran IV language.

RESULTS AND DISCUSSION

As is shown in Table I, the differentiation between non-

TABLE I

MEAN VALUE ± S.D. OF THE INDIVIDUAL VARIABLES FOR MEMBRANE AND NON-MEMBRANE PROTEINS EMPLOYED IN THE DISCRIMINANT FUNCTION

VARIABLE	NON-MEMBRANE(205)[¶]	MEMBRANE(24)	
		INTEGRAL	PERIPHERAL
Lys+Arg+His+Asx+Glx	34.3±4.3	22.5±4.6	35.8±3.5
Ile+Tyr+Phe+Leu+Val+Met	28.6±4.5	39.4±5.2	27.2±4.7
Ratio 3 [▽]	1.26±0.42	0.59±0.18	1.37±0.35
Average hydrophobicity	995.7±98.2	1197.4±97.1	985.9±75.0

[¶]The figure in parentheses indicates the number of proteins analysed.

[▽]Ratio 3 is the ratio of the two above amino acid sets and Average hydrophobicity is the normalised $\bar{H}\Sigma\Delta f_t$ / n (see Material and Methods).

membrane and membrane proteins (total n=229) and between integral and peripheral proteins (n=24) is maximised when the ratio of hydrophilic and hydrophobic amino acids is considered. The mere comparison of the "polarity index" (3,4,9) and the average hydrophobicity of only a few non-membrane (n=4) and membrane (n=1) proteins lacks statistical significance and can lead to erroneous conclusions (see ref. 7).

The application of discriminant function analysis markedly improved the categorisation process, since the differentiation capacity of the individual variables shown in Table I is optimised. In the particular case of the Z function used in this study, the linear combination takes the form:

$$Z = -0.34544\ (\text{Ratio 3}) + 0.00060\ (\overline{H\Phi}_{ave})$$

The corresponding mean values and standard deviations of the Z functions for integral and peripheral membrane proteins are:

$$Z_I = 0.52 \pm 0.11 \quad \text{and} \quad Z_p = 0.12 \pm 0.16$$

The statistical level of significance of the discrimination between both types of proteins is $p << 0.001$ and the resulting misclassification probability is 8.5%.

Table II lists the acetycholine receptor proteins and a few other membrane proteins with respect to the parameters derived from the amino acid composition. Those proteins having low proportion of hydrophobic amino acids, low $\overline{H\Phi}_{ave}$, and low values for the Z function, correspond to the "peripheral" membrane proteins (1). Calsequestrin (11) for instance an extremely acidic protein from the sarcoplasmic reticulum, shows strict correspondence between solubility properties and amino acid data; its loose association with the membrane is now well documented (12). The extremely basic protein of myelin is another example of accord between its assignement by the Z

TABLE II

RATIO 3, $\overline{H}\Phi_{ave}$ AND DISCRIMINANT FUNCTION VALUES FOR SOME MEMBRANE PROTEINS

PROTEIN, Ref.	RATIO 3	$\overline{H}\Phi_{ave}$	Z^{Ψ}	Categorisation
Basic protein, central white matter (13)	2.03	861.76	-0.184	Peripheral
Calsequestrin, sarcoplasmic reticulum (11)	1.65	1030.44	0.048	Peripheral
Cytochrome c, human (19)	1.46	1096.00	0.153	Peripheral
ATPase, Streptococcus faecalis (20)	1.14	1007.25	0.210	Peripheral
Monoamino oxidase, liver mitochondria (21)	1.10	1054.36	0.253	Peripheral
Acetylcholine receptor, Torpedo marmorata (17)	1.15	1083.80	0.253	Peripheral (?)
Chlorophyll complex II, chloroplasts (22)	0.90	1087.30	0.341	Integral
Acetylcholine receptor, Torpedo nobiliana (7)	1.00	1157.72	0.349	Integral
Acetylcholine receptor, Torpedo marmorata (18)	0.90	1167.50	0.390	Integral
Rhodopsin, frog (23)	0.78	1107.45	0.395	Integral
Acetylcholine receptor, Electrophorus electricus (6)	0.88	1177.15	0.402	Integral
ATPase, sarcoplasmic reticulum (16)	1.00	1247.45	0.403	Integral
Carotenoid glycoprotein, Sarcina flava (24)	0.52	1100.88	0.481	Integral
Rhodopsin, bovine (19)	0.62	1208.29	0.511	Integral
Acetylcholine receptor, Electrophorus electricus (15)	0.84	1285.50	0.481	Integral

[¶] See Materials and Methods for a description of the variables Ratio 3, Normalised average hydrophobicity - $\overline{H}\Phi_{ave}$ - and the Z function.

[Ψ] The cutpoint between integral and peripheral proteins is 0.317.

function and its biochemical (13) and physical (14) properties.

The other extreme of the distribution of membrane proteins in Table II is represented by those proteins requiring energic extraction procedures (1) and which are often associated with lipids; these characteristics suggest a different kind of association of the "integral" proteins with the rest of the membrane components possibly involving a substantial degree of penetration into the membrane core. All the cholinergic receptor proteins from Electrophorus or Torpedo so far studied have the characteristics of integral membrane proteins, and most of them have high proportions of hydrophobic residues, high $\overline{H\Phi}_{ave}$ and Z function values, and therefore can be ascribed to the integral category (1) using such criteria. It is interesting that one of the preparations from Electrophorus (6) has almost identical values for the Z function as the sarcoplasmic reticulum ATPase (16), a typical integral protein comprising 80% of the membrane. The other preparation from Electrophorus (15), having the highest hydrophobicity levels, is identical in its Z value to the carotenoid glycoprotein from Sarcina flava (24), another typical integral protein. It is noteworthy that the nicotinic receptor in question (15) reacts with concanavalin A, indicating that it possesses a sugar moiety.

In the case of the nicotinic receptors isolated from Torpedinidae, the one from T. nobiliana (7) resembles the chlorophyll complex II (22), and one preparation from T. marmorata (18) has values similar to rhodopsin (23), all these proteins clearly categorised as integral. Curiously, another preparation from T. marmorata (18) has values simila to those of peripheral proteins. Variations in the preparative procedur not apparent in examining crude amino acid compositions, may account for such differences. The reported presence (15) or absence (6) of Trp

in the receptor from Electrophorus, and the isolation of electrophoretically homogeneous preparations (6) as opposed to the complex subunit structure reported by others (15) for the receptor from the same source, all support this interpretation. The existence (15) or non-existence (17) of carbohydrate moieties also points in this direction.

In conclusion, neither estimation of the raw amino acid data, as stressed by Bigelow (9) in the case of non-membrane proteins, nor comparison of the so-called "polarity" index (4) in the case of membrane proteins (8), are reliable ways to assess differences between proteins. The discriminant function analysis, on the other hand, permits accurate evaluations of statistical parameters, useful in considering subtle differences among closely related membrane proteins.

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