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Aminoacid Composition and Hydrophobicity Index of Mitochondrial Polypeptides

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The aminoacid composition of protein stained bands in polyacrylamide gels, after electrophoresis of proteins from inner mitochondrial membranes, was investigated hydrolyzing directly the gel slices. The Hydrophobicity Index of 18 prominent polypeptide bands was calculated after their aminoacid analysis. The polypeptides less related to the membrane have low hydrophobicity as inferred from their Hydrophobicity Indexes.

Inner mitochondrial membranes involve a great variety of peripheral and integral proteins. The study of each individual protein presents some difficults because of the problems of separating the strongly linked integral proteins. However, the polyacrylamide gel electrophoresis technique allows a good resolution of a considerable number of polypeptide fractions. It is also possible to carry out direct hydrolysis of the stained proteins in the gel using the procedure described by HOUSTON (4). This technique has been used to study the aminoacid composition of eighteen polypeptide fractions which appear particularly abundant in mitochondrial inner membranes.

The aminoacid composition of a given protein may have a strong relation to the more or less polar character of the protein and therefore to its localization with respect to membranes. The integral or peripheral character of a protein may actually be related to its aminoacid composition. In that sense the «Hydrophobicity Index» (H.I.) of these polypeptide fractions from inner membranes, was calculated as already described (2).

Materials and Methods

Male Wister rats weighing approximately 200 g were used. Liver mitochondria were prepared according to the method of HOGEBOOM (3). Inner mitochondrial

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membranes were prepared by the method of PARSONS *et al.* (5) with the modifications already described (6). These inner membranes were contaminated with matrix. In order to separate the protein related to membranes from the unrelated ones, a procedure of osmotic lysis followed by freezing and thawing was used. Membranes, containing matrix, were lysed by osmotic shock in 0.05 M Tris-HCl, 0.01 M $MgCl_2$ (pH 7-4), for 12 hours at 0-4°, following by two freeze-thawing steps. Membranes were sedimented by centrifugation at 150,000 × *g* for 90 min.

Sodium dodecylsulphate polyacrylamide gel electrophoresis was performed according to FAIRBANKS *et al.* (1). 150 μg of protein were layered on each 80×5 mm gel. The gels were fixed and stained for protein with Coomassie brilliant blue, also according to the method of FAIRBANKS *et al.* (1).

For aminoacid analysis of the proteins from the inner membranes, containing matrix, prepared as already described (6), 40 slices of each gel band were used. The slices were hydrolyzed with 6N HCl in vacuum-sealed glass ampoules, at 115° for 22 hours; 2-mercaptoethanol was added to each ampoule to give a final concentration of 2 %. Concentrated NaOH solution was added to the hydrolyzate until pH 12, in order to liberate the ammonia formed in the hydrolysis of polyacrylamide. The samples were brought to a final volume of 1.5 ml with the same acetate buffer, pH 4.10, to be used later in the chromatographic analysis. This was performed in a Hitachi KLA-5 aminoacid autoanalyzer.

TZAGOLOFF *et al.* (7) have reported the loss of several aminoacids when using this procedure. In order to establish the extent of these losses, standard mixtures of the 20 more common aminoacids were treated as described, together with fragments of gel.

After subjecting the standards to the whole procedure, they were analyzed and the results compared with those of non-processed standards.

Results and Discussion

Figure 1 shows the electrophoretic pattern of mitochondrial inner membrane proteins and the 18 bands selected for aminoacid analysis.

Table I shows the aminoacid composition of each of the 18 fractions. Tryptophan, tyrosine and methionine were near completely destroyed and 60 % of histidine was also lost; this figure was considered in all subsequent calculations.

In the Hydrophobicity Indexes (H.I.) calculated as previously described (3) (table I), the degree of polarity of each aminoacid is taken into account, assuming a direct relationship between polarity and behaviour in partition chromatography.

Figure 2 shows the electrophoretograms corresponding to the proteins of sediment and supernatant of the centrifugation at 150,000 × *g* for 90 min, after the osmotic and freeze-thawing treatment of the inner membranes containing matrix, as described in Materials and Methods. In each fraction, sediment or supernatant,

Fig. 1. *Electrophoretic analysis of rat liver mitochondrial inner membrane proteins.*
The preparation (3 mg/ml) was solubilized with sodium dodecyl sulfate (1 %) and 2-mercaptoethanol (1 %), subjected to electrophoresis in 5.6 % polyacrylamide gels, and stained as described by FAIRBANKS *et al.* (1). The gel contained 150 μg protein. The 18 prominent bands selected for aminoacid analysis are numbered.

Table I. *Aminoacid composition in mole per cent and Hydrophobicity Index of proteins from inner mitochondrial membrane.*

		Mole %													
Band number[1]	H.I.[2]	$CySO_3H$	His	Lys	Arg	Ser	Asp	Gly	Thr	Glu	Ala	Val	Phe	Ile	Leu
1	3,384	0.94	4.73	3.44	4.42	6.14	14.80	7.64	3.44	14.37	7.84	8.23	4.69	6.51	11.67
2	2,883	0.87	1.34	0.34	9.35	12.07	18.48	6.09	5.65	18.15	9.02	3.80	0.11	6.09	5.76
3	2,684	11.37	5.62	1.07	12.23	10.30	20.75	4.22	5.08	13.02	5.29	5.28	3.01	2.15	3.22
4	2,647	14.63	0.91	4.07	16.26	7.32	8.13	15.45	4.07	10.57	6.50	4.07	2.44	3.25	3.26
5	2,533	16.12	3.71	7.10	16.11	7.12	10.41	7.15	4.37	10.69	5.39	3.58	5.42	0.91	1.82
6	3,428	6.85	1.55	5.84	3.65	13.24	5.94	5.86	6.85	9.13	9.11	4.70	8.51	9.42	5.73
7	3,272	4.70	2.84	2.84	14.12	6.42	9.48	3.72	5.62	11.22	8.42	4.63	8.51	9.42	5.52
8	3,188	9.61	2.30	2.30	7.51	8.36	10.60	6.43	4.51	11.23	5.80	5.81	4.53	10.02	11.51
9	2,689	14.91	5.01	5.01	7.39	6.71	9.30	12.61	4.10	10.79	6.30	2.34	1.51	6.69	6.02
10	3,066	12.01	1.33	1.33	7.10	9.02	11.23	8.01	5.03	12.15	12.13	8.01	2.80	4.71	5.33
11	2,509	7.77	6.83	6.83	16.53	12.65	7.78	2.33	11.65	13.59	1.94	2.91	5.83	1.36	1.17
12	2,752	18.63	9.32	9.32	3.11	12.42	6.21	7.76	3.11	15.53	6.21	6.83	1.55	3.73	4.04
13	2,646	10.96	4.39	6.58	10.96	6.80	13.16	4.82	13.60	6.58	7.24	4.61	2.19	2.63	5.48
14	2,923	2.44	4.88	7.32	8.05	15.35	11.86	3.26	12.56	14.19	3.02	1.16	2.33	2.79	1.63
15	3,373	5.23	4.77	4.55	2.50	12.95	6.82	6.59	10.45	11.82	7.50	12.73	3.41	5.23	5.45
16	3,251	5.24	1.01	8.06	6.45	15.73	13.31	9.48	4.93	13.51	6.45	9.78	2.22	6.25	4.03
17	2,981	8.83	0.85	3.42	6.55	9.12	8.83	4.84	3.70	10.26	7.12	9.40	1.42	7.69	8.26
18	3,101	7.98	0.25	3.99	3.74	7.48	20.45	3.74	6.23	11.47	9.48	5.49	3.24	9.23	7.23

1 Band numbers correspond to those of figure 1.
2 Hydrophobicity Index, calculated as previously described (2).

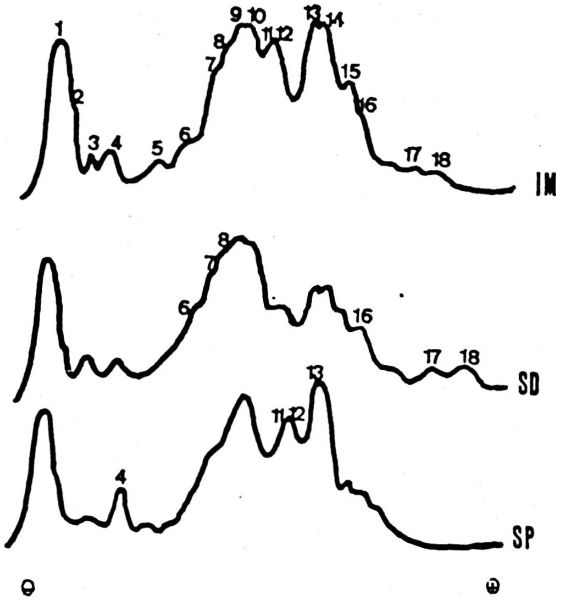

Fig. 2. *Densitometer tracing of polyacrylamide gel electrophoresis of the polypeptides of the inner membranes (containing matrix) and the fractions obtained by centrifugation after osmotic lysis and freeze-thawing.* The 18 polypeptides from inner membranes analyzed for aminoacids are numbered. The polypeptides clearly predominant in the sediment (the most firmly related to membranes) and in the supernatant (less related or unrelated to membranes) after fractionation of the inner membranes are numbered in the corresponding fraction. (See text for details.) IM: inner membranes; SD: sediment; SP: supernatant.

only the predominant bands are numbered.

If the bands are classified in decreasing order of H.I., i.e., in increasing order of polarity (table II) it could be observed that a good correlation exists between hydrophobicity, as indicated by their H.I. and the expected character according to their origin, more or less related to membranes. Other bands of mixed origin, such as numbers 2 and 9, occupy in the H.I. scale an intermediate position.

These results suggest that the Hydrophobicity Index could be used as a satisfactory measure of the polar or apolar character of a protein. It can also be inferred from the present data that a direct relationship exists between the aminoacid composition of a membrane protein and its localization within the membrane.

Table II. *Decreasing order of H.I. of 18 polypeptide bands from inner mitochondrial membranes.*

Band	Predominant in fraction
6	Sediment
1	
15	Sediment
7	Sediment
16	Sediment
8	Sediment
18	Sediment
10	
17	Sediment
14	
2	
12	Supernatant
9	
3	
4	Supernatant
13	Supernatant
5	
11	Supernantant

Resumen

Se estudia la composición en aminoácidos de bandas teñidas en geles de poliacrilamida, tras realizar electroforesis de las proteínas de la membrana interna mitocondrial, hidrolizando directamente los trozos de gel. Se calculó el Indice de Hidrofobicidad de 18 bandas polipeptídicas prominentes a partir de sus composiciones en aminoácidos. De estos 18 polipéptidos, los menos relacionados con membranas tienen hidrofobicidad baja según se puede deducir de sus Indices de Hidrofobicidad.

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