ELECTROPHORETIC FRACTIONATION OF NUCLEAR MEMBRANE PROTEINS OF THE RAT LIVER IN POLYACRYLAMIDE GEL IN THE PRESENCE OF SODIUM DODECYLSULFATE

K. A. Perevoshchikova and I. V. Nochvina

UDC 612.35.014.22:612.398.7-087.4:543.545

Proteins of nuclear membranes and of membranes of erythrocytes and liver mitochondria of rats were subjected to electrophoretic fractionation by molecular weight in 5% polyacrylamide gel (PAG) in the presence of low (0.1%) and high (1%) concentrations of sodium dodecylsulfate (DDS). In the presence of 0.1% DDS, besides high-molecular-weight fractions, the proteins of all the membranes investigated contained a protein fraction with low molecular weight (about 6000). The low-molecular-weight fraction was absent in the membrane proteins on electrophoresis in PAG with 1% DDS. The possibility of proteolytic degradation of membrane proteins in the presence of low DDS concentrations is discussed.

Besides their characteristic differences, the various cell membranes also share a number of common features concerning their structure and composition [1, 3, 10]. For example, the presence of structural proteins with evidently similar structure and properties has frequently been reported. The results of electrophoresis of membranes of the mitochondria, erythrocytes, and outer segments of the retinal rods of the bovine eye, treated with sodium dodecylsulfate, in polyacrylamide gel (PAG) have recently been described [7], and analogous components were found in all these three types of membranes. This method separates proteins by their molecular weight and records them in the monomer state [9]. In all membranes investigated, besides certain characteristic components with a high molecular weight, a number of very similar low-molecular-weight proteins were found; these proteins were evidently cyclic in structure and showed a well-marked capacity for aggregation. The workers cited assume that these "miniproteins" with a molecular weight of about 6000 form the structural basis of different types of membranes [7]. Investigation of miniproteins in other types of membranes is important both to verify this concept and also to study different types of biological membranes, especially nuclear membranes. An earlier electrophoretic study of the proteins of isolated nuclear membranes in PAG in an acid medium [8] revealed the heterogeneity of their proteins and showed that they contain protein components corresponding in their mobility to histone [2].

This paper describes the electrophoretic fractionation of proteins of nuclear membranes and also of liver mitochondria and erythrocytes of rats in PAG.

EXPERIMENTAL METHOD

Rat liver nuclear membranes were obtained by a modification of the writers' earlier method [4]. Rat erythrocyte membranes were isolated by osmotic shock [6]. Rat liver mitochondrial membranes were obtained by Parsons' method [11].

Laboratory of Biochemistry, Institute of Biology of Development, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 75, No. 2, pp. 34-37, February, 1973. Original article submitted April 10, 1972.

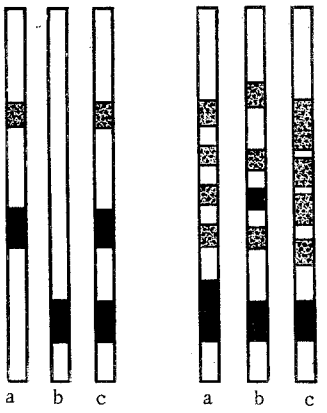

Fig. 1

Fig. 2

Fig. 1. Scheme of electrophoretic fractionation of membrane proteins in polyacrylamide gel in the presence of 0.1% sodium dodecylsulfate; a) serum albumin; b) insulin; c) mixture of proteins (a, b); d) erythrocyte membranes; e) mitochondrial membranes; f) nuclear membranes. Here and in Fig. 2, dark shading represents intensely stained fractions; light shading corresponds to weakly stained fraction.

Fig. 2. Scheme of electrophoretic fractionation of membrane proteins in polyacrylamide gel in the presence of 1% sodium dodecylsulfate (by Fairbanks' method): a) nuclear membranes; b) mitochondrial membranes; c) insulin.

Electrophoresis was carried out initially by the original method of Shapiro and Maizel [9]. Before the specimen was prepared for electrophoresis the isolated nuclear membranes were incubated in 0.1 M phosphate buffer, pH 7.2, with 2.5% DDS and 2.5% mercaptoethanol for 3 h at 37°C.

The specimen was then dialyzed for 16 h against 0.01 M phosphate buffer, pH 7.2, containing 0.1% DDS and applied to a gel consisting of 5% acrylamide (England) and 0.1 M phosphate buffer, pH 7.2, with 0.1% DDS. Polymerization was catalyzed with 0.05% NNN'N'-tetramethylenediamine and 0.075% ammonium persulfate.

Electrophoresis was carried out to the anode at 30°C, with the tube placed in 0.1 M phosphate buffer (pH 7.2) containing 0.1% DDS with a current of 1 mA applied to the tube for 4 h. The gel was then expressed from the tube, fixed for 36 h in 20% sulfosalicylic acid, and stained for 5 h with 0.5% Coomassie dye dissolved in 7% acetic acid. The excess of dye was removed with 7% acetic acid.

In the second part of the work electrophoresis was carried out by Fairbanks' method [5] at room temperature in 5% polyacrylamide in the presence of 1% DDS to the anode with a current of 8 mA applied to the tube. The top and bottom electrophoretic chambers contained 0.1 M tris-HCl (pH 7.4) with 1% DDS. During preparation of the specimen for electrophoresis, the membranes were treated with the following substances in the concentrations specified: DDS 1%, sucrose 5-10%, 10 mM tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0), 40 mM dithiotritol, and pyronine as reference substance. The specimen was incubated for 15-30 min at 37°C and then applied to the tubes. In the case of the marker protein, which was insulin, the specimen was incubated under the same conditions at 45-50°C for 20 min. After electrophoresis the gel was fixed for 18 h in a mixture of 15% trichloroacetic acid and 25% isopropanol. Staining was then carried out with 0.025% Coomassie blue dye.

EXPERIMENTAL RESULTS AND DISCUSSION

To begin with, the electrophoretic mobility of proteins whose molecular weight was known, and which could be used as the standard for estimating the mobility of the test nuclear membrane proteins, was investigated. The proteins used were bovine serum albumin (England) and insulin (Holland).

During electrophoresis of the albumin two bands (light and heavy fractions) with different intensities of staining were discovered. The molecular weight of the clearly distinguishable, faster moving albumin fraction was 50,000; the single insulin fraction corresponded to a molecular weight of 5800.

Electrophoresis of the erythrocyte membrane proteins showed that the greater part of them consisted of a fraction with the mobility equal to that of insulin. The remaining four fractions were less marked and the mobility of two of them coincided with that of the serum albumin fractions (Fig. 1). During investigation of the mitochondrial membranes a low-molecular-weight fraction with mobility similar to that of insulin also was found. In addition, five other fractions were detected, of which the fourth was present in a greater amount than the rest (Fig. 1). During electrophoresis of the nuclear membrane proteins after fixation with 20% sulfosalycilic acid, a low-molecular-weight protein fraction was discovered similar to that observed during electrophoresis of insulin. Besides the low-molecular-weight fraction,a further four fractions with lower electrical mobility, i.e., with higher molecular weight, were found, one of which corresponded to serum albumin in its molecular weight.

During electrophoresis by the method of Shapiro and Maizel, besides high-molecular-weight fractions a protein fraction with low molecular weight (about 6000), corresponding to insulin, was found in all the membranes investigated (erythrocytes, mitochondria, and nuclei of the rat liver).

After this investigation of the fractions of the various membrane proteins had been completed a paper [5] was published which described electrophoresis of erythrocyte membrane proteins in the presence of 1% DDS, a concentration 10 times higher than that used by Laico [7] and ourselves. During electrophoresis under these conditions no peptides with low molecular weight (about 6000) could be detected in the erythrocyte membrane proteins. After comparing his own experimental observations with those reported in the literature, the author of the paper cited concludes that proteolytic degradation of erythrocyte membrane proteins may take place in the presence of low concentrations of DDS.

In connection with these observations, electrophoretic fractionation of mitochondrial and nuclear membranes from rat liver was later carried out by Fairbanks' method. Insulin was used as the protein with known molecular weight. The results obtained are shown schematically in Fig. 2. The mitochondrial membrane proteins were separated into five fractions by molecular weight. No fraction with a low molecular weight, corresponding to insulin, was found. During electrophoresis of the rat liver nuclear membrane proteins eight fractions were obtained (Fig. 2). Just as in the case of the mitochondria, no proteins with a molecular weight of about 6000 were found in the nuclear membranes.

Under these conditions of electrophoresis no low-molecular-weight proteins with the mobility of insulin could thus be found in the nuclear and mitochondrial membranes of rat liver. Under the same conditions Fairbanks found no such proteins in erythrocyte membranes either. After a full analysis of the conditions of radial electrophoresis with DDS Fairbanks considers that treatment of the specimen of membrane proteins in the presence of DDS produces denaturation, with an increase in the sensitivity of the peptide bonds to the action of DDS-resistant proteinase. In that case the cleavage products formed dissociate and migrate more rapidly during electrophoresis than the original polypeptides, and for this reason to prevent proteolytic degradation of proteins during electrophoresis in the presence of DDS by Fairbanks' method it is necessary to use high concentrations of DDS (1%), both during preparation of the specimen and during electrophoresis, and to include EDTA both in the specimen and in the electrophoretic buffer. If these conditions are observed, proteolytic degradation of the proteins of the erythrocyte membranes is excluded. In the present experiment by Fairbanks' method, no fraction with molecular weight of about 6000 likewise was detected. However, the question of whether proteolytic degradation takes place in the case of the proteins of nuclear or mitochondrial membranes during preparation of the specimens and electrophoresis is not yet finally solved and requires special investigation.

LITERATURE CITED

1. V. A. Borovyagin, Tsitologiya, 9, 505 (1967).
2. I. B. Zbarskii, K. A. Perevoshchikova, and L. N. Delektorskaya, Byull. Éksperim. Biol. i Med., 9, 49 (1969).
3. E. A. Lehninger, The Mitochondrion, Benjamin (1964).
4. K. A. Perevoshchikova, L. N. Delektorskaya, V. V. Delektorskii, et al., Tsitologiya, 10, 573 (1968).
5. G. Fairbanks, T. L. Steeck, and D. F. H. Wallach, Biochemistry (Washington), 10, 2606 (1971).

6. D. E. Green, E. Murer, H. O. Hultrin, et al., Arch. Biochem., 112, 635 (1965).
7. M. T. Laico, E. I. Rouslahti, D. S. Papermaster, et al., Proc. Nat. Acad. Sci. (Washington), 67, 120 (1970).
8. D. M. Nevielle, Biochim. Biophys. Acta, 133, 168 (1967).
9. A. L. Shapiro, E. Venuela and J. V. Maizel, Biochem. Biophys. Res. Commun., 23, 815 (1967).
10. F. S. Sjostrand, Biochim. Biophys. Acta, 11, 1 (1954).
11. D. F. Parsons, Methods Enzymol., 10, 443 (1967).