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GEL ELECTROPHORESIS PATTERNS OF THE PROTEINS OF ORGANELLES ISOLATED FROM BOVINE LIVER

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SUMMARY

Mitochondria, smooth and rough microsomes, Golgi vesicles, and plasma membranes have been isolated from bovine liver and their protein components separated by polyacrylamide gel electrophoresis. Characteristic patterns were obtained for each organelle studied. The mobilities of the major protein bands relative to ribonuclease A were determined and comparisons made among the organelles. The major protein components of rough and smooth microsomes were indistinguishable. Golgi vesicles were similar to, but easily distinguishable from, endoplasmic reticulum (as represented by the rough microsome fraction). A major band in the Golgi preparation had the same mobility as the major protein component found in serum β-lipoproteins ($d < 1.063$). All the organelles except plasma membranes had a major protein component with mobility identical to that found for purified "structural protein" isolated from mitochondria.

INTRODUCTION

The isolation of purified organelles and membranes from organs such as liver has developed to the point where meaningful comparisons of the composition of most of the membranes from a single cell type are now possible. Due to the insoluble nature of the protein components of membranes, new methods of separation and analyses must be devised to study membrane proteins. One approach, that of TAKAYAMA *et al.*[1], makes use of a mixture of phenol, acetic acid and urea to dissolve membranes. The components can then be separated by electrophoresis in acrylamide gels containing acetic acid and urea. We have applied a modification of this method to the analysis of the protein components of mitochondria, nuclei, rough and smooth microsomes, plasma membranes and a Golgi fraction isolated from bovine liver. These preparations have been characterized thoroughly both enzymically and by electron microscopy. The present communication presents and compares characteristic protein profiles found for these cell fractions and derived membranes.

MATERIALS AND METHODS

Electrophoresis on polyacrylamide gels was based on the method of TAKAYAMA *et al.*[1], with modifications to improve sensitivity and to allow comparison of gels run

on different days. The following solutions were prepared fresh for each set of gels: acrylamide solution: 3 g acrylamide, 0.08 g *N,N'*-methylenebisacrylamide, 6 g urea, and 14 ml glacial acetic acid were dissolved in water to a total volume of 30 ml; catalyst: 0.15 g of ammonium persulfate was dissolved in 10 ml of 10 M urea. Gel solutions were prepared by mixing 30 ml of acrylamide solution, 10 ml of catalyst, and 0.1 ml of *N,N,N',N'*-tetramethylethylenediamine. After degassing, the solution was pipetted into twelve 5-inch gel tubes to within 3/4 inch of the top of the tube and overlayed with about 0.1 ml of water. Gels were allowed to polymerize for 3 h at room temperature in the dark. Pre-electrophoresis was carried out in a Canalco model 12 apparatus for 3 h at 5 mA per gel. Acetic acid (35 %) was used as the buffer for pre-electrophoresis and the top of each gel was overlayed with 0.1 ml of 5 M urea in 35 % acetic acid. Gels were stored at 4° until use, usually the next day.

Samples (0.1 ml) were dissolved in 0.9 ml of phenol–acetic acid–8 M urea (2:1:1, by vol.) and up to 0.25 ml layered on top of the gel. After adding the sample, containing 15–30 μg protein, 2 μg of crystalline ribonuclease A (beef pancreas, Worthington) in 0.05 ml of the phenol solution were usually layered on each gel to serve as a marker protein. In some cases it was necessary to use crystalline sperm whale myoglobin (Mann) in place of ribonuclease. Electrophoresis was carried out with 10 % acetic acid as buffer. The current was set at 1 mA per tube for the first 10 min to allow the sample to enter the gel, after which the current was increased to 2.5 mA per tube for 3–4 h. Gels were stained with Coomassie Brillant Blue (0.25 % in 7 % acetic acid) and destained by soaking for several days in several changes of 7 % acetic acid. A Photovolt Corporation densitometer, model 52-C modified according to PRIVETT *et al.*[2], was used to obtain densitometry tracings of the gels.

Cell fractions from liver were prepared and checked for purity as previously described. The fractions used were mitochondria[3], plasma membranes, rough and smooth microsomes[4], and Golgi vesicles[5], from bovine liver. A modification of the method of CHAUVEAU *et al.*[6] was used to prepare nuclei from bovine liver.

β-Lipoproteins were prepared from bovine serum as described previously[7] by a modification of the flotation procedure of DELALLA AND GOFMAN[8].

Protein was determined by the method of LOWRY *et al.*[9]. Glucose-6-phosphatase activities of the cell fractions were determined by the procedure of SWANSON[10].

RESULTS

Table I summarizes the mobilities of the major protein peaks observed after densitometer tracing of the electrophoresis patterns of the cell fractions indicated. Mobilities are expressed relative to the mobility of ribonuclease which is taken as 1.000. Rough and smooth microsomes give very similar protein profiles (Table I and Figs. 1A and 1B). The major bands have identical mobilities, and the only differences appear in minor components. Considerable overlap can be seen between Golgi membranes and microsomes as well (Table I and Fig. 1C). The major differences are: (1) Bands e and g are less prominent in Golgi than in microsomes; (2) Band n of microsomes is absent in Golgi; and (3) Band o appears to be very prominent in Golgi and is absent in microsomal membranes. Band n of both smooth and rough microsomes can sometimes be resolved into two components (Fig. 1A), but more often appears as a single diffuse band (Fig. 1B). In some preparations, Band d of microsomes

TABLE I

RELATIVE MOBILITIES OF MAJOR PROTEIN COMPONENTS OF BEEF LIVER ORGANELLES

Electrophoresis was run in either duplicate or triplicate on at least two different preparations of each organelle. Mobilities, given relative to that of added ribonuclease, are averages of at least four gels, ± S.E.

Band	*Microsomes*		*Golgi*	*Mitochondria*			*Nuclei*	*Plasma membranes*
	Smooth	*Rough*		*Whole*	*Vesicles*	*Supernatant*		
a								0.085 ± 0.003
b				0.112 ± 0.008				0.127 ± 0.008
c				0.138 ± 0.005	0.140 ± 0.016	0.137 ± 0.021		
d	0.183 ± 0.009	0.193 ± 0.005						
e	0.215 ± 0.008	0.217 ± 0.005	0.228 ± 0.007					
f				0.246 ± 0.008	0.260 ± 0.011		0.250 ± 0.002	
g	0.266 ± 0.009	0.269 ± 0.010	0.279 ± 0.009					0.276 ± 0.004
h	0.309 ± 0.006	0.312 ± 0.008	0.317 ± 0.008	0.320 ± 0.005		0.321 ± 0.021		
i	0.336 ± 0.008	0.337 ± 0.009	0.338 ± 0.009	0.336 ± 0.006	0.337 ± 0.008	0.341 ± 0.026	0.334 ± 0.004	
j	0.351 ± 0.007	0.351 ± 0.010	0.360 ± 0.010					0.351 ± 0.006
k	0.372 ± 0.007	0.373 ± 0.014	0.377 ± 0.009					
l				0.396 ± 0.007	0.416 ± 0.007	0.395 ± 0.025		0.388 ± 0.011
m								0.417 ± 0.009
n	0.445 ± 0.005	0.438 ± 0.009		0.446 ± 0.006	0.455 ± 0.016	0.451 ± 0.018	0.455 ± 0.004	0.437 ± 0.007
o			0.465 ± 0.009					
p				0.501 ± 0.004	0.506 ± 0.006	0.506 ± 0.016		
q					0.640 ± 0.001			
r					0.681 ± 0.001			
s							0.718 ± 0.006	
t							0.783 ± 0.008	
u							0.952 ± 0.009	
v							1.064 ± 0.001	

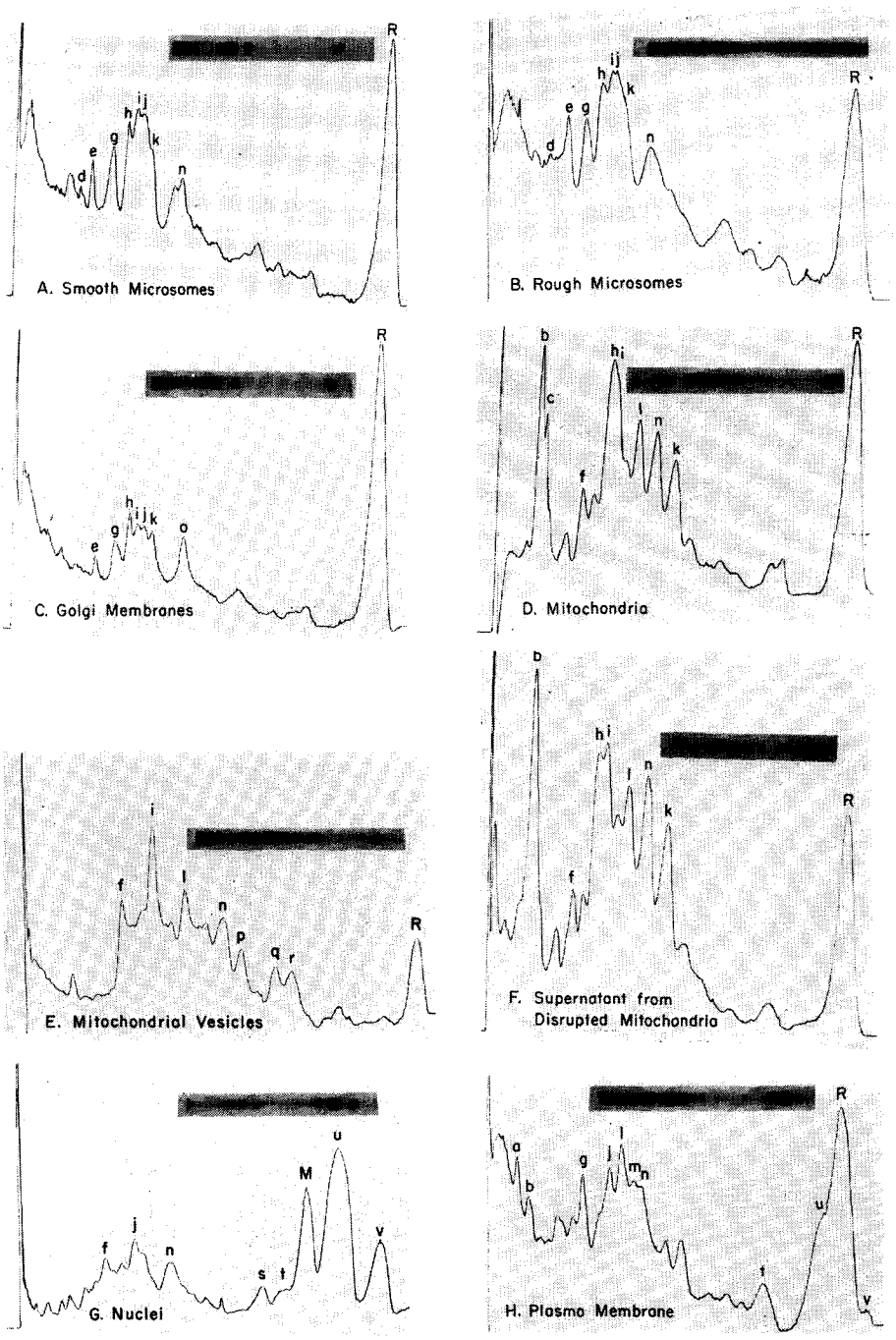

Fig. 1. A–H. Typical disc gel electrophoresis patterns and densitometer tracings of preparations of organelles from bovine liver. The major peaks are designated by letters. The mobilities of these peaks relative to ribonuclease are given in Table I. R = ribonuclease standard, M = sperm whale myoglobin standard. Ribonuclease and myoglobin were added as marker proteins in order to calculate relative mobilities.

is a prominent peak, while in others it is a minor component. The peak does not appear in the Golgi preparations.

Mitochondria, plasma membranes and nuclei have patterns which are characteristic of each cell fraction and are distinct from each other and from microsomes or Golgi preparations. Band i of mitochondria (Fig. 1D) has the mobility of "structural protein" as defined by ZAHLER *et al.*[11]. A prominent band of similar mobility is present in all the preparations in Table I and Fig. 1 except plasma membranes which have a prominent Band j with a slightly faster mobility.

Mitochondria were disrupted using a French pressure cell and the vesicles separated from the soluble proteins by centrifugation for 30 min at 30000 rev./min in a Spinco No. 50 rotor[12]. Electrophoretic patterns of the resulting fractions are shown in Figs. 1E and 1F, and the relative migrations of the major protein peaks are shown in Table I. It can be seen that the pattern for whole liver mitochondria (Fig. 1D) is strikingly similar to that found for the soluble proteins of the mitochondria. The vesicles on the other hand, are considerably different from whole mitochondria. The most prominent, Peak i, has a similar mobility as purified structural protein as isolated by ZAHLER *et al.*[11].

Some of the minor peaks in whole mitochondria appear more prominently in the vesicle pattern (*e.g.* Peaks q and r). It can also be seen that a peak with the mobility of structural protein (Peak i) is also present in the supernatant fraction.

Electrophoretic patterns for whole nuclei (Table I and Fig. 1G) are characterized by very prominent bands similar in mobility to ribonuclease (Bands u and v). For these fractions, myoglobin (relative mobility 0.844) was used as a reference protein rather than ribonuclease. Band u can sometimes be resolved into two bands. The fast-moving bands of nuclei are probably histones. Similar bands are found to a small extent in the plasma membranes prepared from bovine liver by our modification of the method of Neville[4] (Fig. 1H). When electrophoresis is carried out on plasma membranes using myoglobin as a standard, significant amounts of bands corresponding to Bands t, u and v of nuclei can be observed in the plasma membrane preparations. These bands are probably due to contamination of the plasma membrane preparations either with nuclei or with histones released from nuclei during the preparation of the plasma membranes.

In order to ascertain whether the differences observed in the electrophoretic patterns of microsomes and Golgi membranes were real, *in vitro* mixtures of rough microsomes and Golgi membranes were made and electrophoresis of the mixture carried out. As increasing amounts of rough microsomes were added to Golgi membranes, Bands e and g increased in relative amounts, as did Bands i and j. Band o, on the other hand, decreased in intensity and appeared more diffuse, with a shoulder appearing corresponding in relative migration to Band n of microsomes. A minor band of relative migration 0.635 also appeared whereas a minor Golgi band at 0.605 disappeared. An example of the pattern obtained with 50 % Golgi mixed with 50 % rough microsomes (on a protein basis) is shown in Fig. 2.

Since we have observed what appear to be lipoprotein particles in our Golgi preparations[5] we electrophoresed serum β-lipoprotein preparations in acrylamide gels as well. It can be seen in Fig. 3, that β-lipoproteins give a single major protein peak in this gel electrophoresis system. The relative mobility of this peak compared to ribonuclease is 0.453. This mobility corresponds within experimental error to Band o

of the Golgi preparations, the only major band found which is characteristic of the Golgi preparation. Artificial mixtures of β-lipoproteins with Golgi membranes showed that Band o was indistinguishable from the β-lipoprotein major band by this criterion.

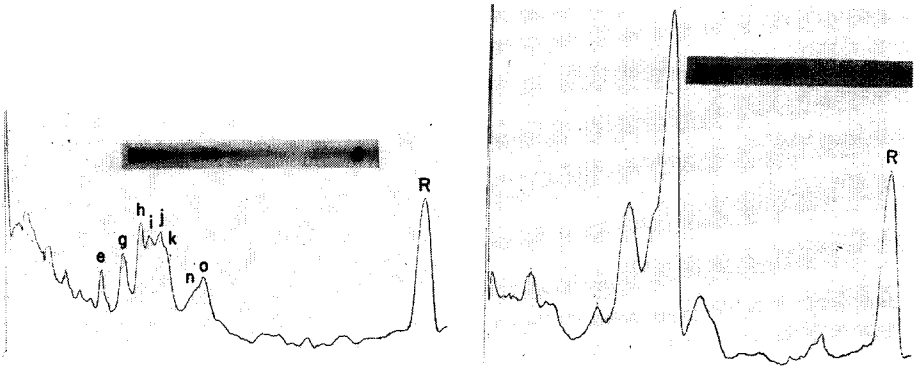

Fig. 2. Disc gel electrophoresis and densitometer tracing of a mixture of 50% Golgi membranes and 50% rough microsomes.

Fig. 3. Disc gel electrophoresis and densitometer tracing of bovine serum β-lipoproteins ($d < 1.063$). The mobility of the major peak relative to ribonuclease is 0.453.

A further test of identity was obtained using the split gel technique described by DUNKER AND REUCKERT[14]. In this method, a small polyethylene divider is inserted across the top of an individual acrylamide gel. Two samples can then be placed on one gel and run side by side. Figs. 4a and 4b illustrate this technique comparing Golgi vesicles with rough microsomes on the one hand (Fig. 4a) and with serum β-lipoproteins on the other hand (Fig. 4b). The differences found between Golgi vesicles and microsomes by densitometry tracings are confirmed by this technique. The identical mobility of the major band of β-lipoprotein with Band o of Golgi vesicles is also evident.

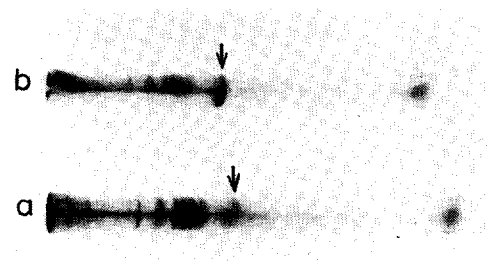

Fig. 4. Comparison of Golgi membranes with (a) rough microsomes and (b) bovine serum β-lipoproteins by the split gel technique[14]. Golgi membranes appear in the upper half of the gel in both (a) and (b). Band o of the Golgi preparation is indicated in each case by arrows.

DISCUSSION

Electrophoresis in polyacrylamide gels has proven to be a valuable tool for studying membrane-bound proteins. The use of phenol in the solvent mixture results in the dissociation of lipid and protein and probably gives nearly complete disaggregation of the membrane proteins. This results in good reproducibility of the protein profiles of the cell fractions. The largest variation in relative mobility occurred from

one set of gels to the next, resulting in an overall standard deviation of the order of $\pm$ 0.01. For this reason it is advisable to run mixtures or use the split gel technique when small differences are suspected, as in the case of Golgi *versus* microsomes.

Patterns obtained using purified organelles from a single organ are characteristic for the organelle used, with the exception of smooth and rough microsomes which are practically indistinguishable. Since smooth endoplasmic reticulum is probably derived from rough endoplasmic reticulum[15], this relationship is perhaps not surprising. Ribosomal proteins do not contribute heavily to the pattern observed for rough endoplasmic reticulum. Preparations of free ribosomes when analyzed in this system, give about ten diffuse bands in the lower half of the gel. In rough microsome fractions these bands are present but are barely discernable above the background staining of the gel.

Golgi membranes appear closely related to endoplasmic reticulum in their overall protein patterns although several significant differences are found. The presence of Bands e and g in a reduced amount as compared to microsomes is probably a measure of microsomal contamination of the preparation. The preparations used in this study varied between 10 and 20 % contamination with microsomes as judged by their level of glucose-6-phosphatase activity[5]. The height of Bands e and g appeared to be roughly related to the amount of contamination present. Some 10–15 % contamination with plasma membranes also was present in the Golgi membranes as judged by galactosidase activity[4]. Traces of Bands a and b of plasma membranes were also present in the electrophoretic patterns.

The only prominent band characteristic of Golgi membranes appears to be identical in electrophoretic properties to the major band found on electrophoresis of purified β-lipoproteins. This is consistent with the observation of MAHLEY *et al.*[13] and our own[5], that particles similar to very low density lipoproteins are present in Golgi membranes prepared from liver.

The electrophoretic pattern found for whole liver mitochondria is almost identical, in its major components, to that of the supernatant fraction after fragmentation of the mitochondria. This is understandable when one considers that more than two-thirds of the total protein of liver mitochondria is soluble after disruption using a French pressure cell. In heart mitochondria, where only 10–20 % of the total protein is soluble after disruption of the mitochondria, the patterns for whole mitochondria and vesicles are more closely related (unpublished studies).

The most prominent band found in vesicles derived from liver mitochondria or from heart mitochondria appears to be due to structural protein as defined by ZAHLER *et al.*[11]. This protein, which constitutes about 35 % of the total protein of the vesicles, appears to be associated with the membrane but not part of the trilaminar arrangement itself (H. OZAWA, W. ZAHLER AND S. FLEISCHER, in preparation).

Gel electrophoresis can be useful as a tool in cell fractionation studies, both as a confirmation of contamination estimates based on enzymic studies and to detect new contaminants. Contamination by both endoplasmic reticulum and plasma membranes can be detected in Golgi membrane preparations which help substantiate enzymic data in a qualitative way. It is difficult to use densitometer tracings as a quantitative estimation of contamination or of protein distribution. Most of the patterns have a large number of protein bands, which appear to stain to different extents, and destaining of the background is variable. Further, in most cases, some protein

remains at the origin of the gel. In the case of plasma membrane preparations, we have detected components which are probably due to contamination with nuclei and procedures designed to reduce this contaminant are now under study.

The most useful application of this procedure will be as a tool in the fractionation and identification of tightly-bound membrane proteins. The high resolution offered by this gel electrophoresis method, coupled with its high sensitivity should make it a most valuable tool in the study of membrane structure. Rapid analysis of the fractions obtained after a given treatment will indicate whether selective removal of protein has occurred, thus complementing enzymatic data. The removal of structural protein from mitochondria provides one example of this approach to membrane fractionation[11].

Recently, SCHNAITMAN has applied gel electrophoresis to the analysis of membrane proteins of the inner and outer membranes of mitochondria, and smooth and rough microsomes of rat liver[16]. The results obtained are not directly comparable to those described in this communication, since the source of the membranes was different. In addition, the proteins were treated extensively before analysis and were electrophoresed in the presence of urea and 1 % sodium dodecyl sulfate, a procedure which essentially separates only on the basis of molecular weight differences. This procedure is not as well suited to routine analysis of fractions due to the tedious preparation of samples which it requires*.

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* *Note added in proof* (Received April 6th, 1970):
After this paper was submitted, we found that bovine serum albumin has a relative mobility of 0.463 ± 0.007 compared to ribonuclease. It is therefore possible that bovine serum albumin also constitutes part of Band 0 in Golgi preparations.