Biochimica et Biophysica Acta, 426 (1976) 88–100

BBA 77191

ANTIGENIC PROPERTIES OF RAT LIVER NUCLEAR MEMBRANE

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(Received August 1st, 1975)

SUMMARY

Antigenic properties of purified rat liver nuclear membrane were compared with those of other cellular membranes and nuclear components using the quantitative complement fixation assay. Rat liver nuclear membrane was prepared by heparin treatment and sucrose gradient centrifugation and found to be essentially free of plasma membrane, inner mitochondrial membrane and chromatin components as judged by biochemical analysis and electron microscopy.

Antisera to rat liver nuclear membrane were produced in rabbits. These antisera fix complement not only in the presence of rat liver nuclear membrane but also in the presence of rat liver rough or smooth endoplasmic reticulum, plasma membrane or mitochondria or rat muscle sarcoplasmic reticulum, indicating common antigenic sites in these membranes. Species specificity of these antigenic sites is suggested, since the antisera did not show complement fixation when rabbit skeletal muscle sarcoplasmic reticulum was tested. Rat liver chromatin or liver nonhistone protein · DNA complex also did not fix complement in the presence of antibodies to the nuclear membrane.

When the anti-rat liver nuclear membrane sera were absorbed with rat liver rough endoplasmic reticulum or mitochondria, antibodies specific for the rat liver nuclear membrane were obtained. It is proposed that specific antigens can be used as a marker for the nuclear membrane.

Furthermore it was found that sera from certain non-immunized rabbits contained antibodies which fixed complement in the presence of various membrane preparations.

INTRODUCTION

The nuclear envelope maintains the environment for the genetic material by selectively excluding cytoplasmic components during prophase and by regulating transport through pores which are dispersed throughout the double membrane structure. The outer membrane appears to be continuous with [1–3] and to have an

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enzyme composition similar to [4] endoplasmic reticulum. The inner membrane is thought to be involved in regulation of chromosome function through its association with DNA during replication [5–9]. A more detailed understanding of the function of nuclear membrane has been impeded by difficulties in its separation from nucleoplasmic proteins, DNA and other membranes and in its detection, owing to the lack of a marker.

In this study we have raised antibodies against highly purified rat liver nuclear membrane and have employed the quantitative microcomplement fixation assay to demonstrate the presence of antigenic sites which are unique to the nuclear membrane. In addition, we have found a group of antigens which are shared by several other subcellular membranes derived from the same species.

MATERIALS AND METHODS

Source of tissue. Male or female rats of 200 g were obtained from Holzman, Madison, Wisconsin (Sprague-Dawley albino strain) or Charles River, Wilmington, Massachusetts (CD strain). New Zealand white rabbits (Pel Freez, Rogers, Arkansas) were used for immunization and for preparation of skeletal muscle sarcoplasmic reticulum. Calf thymus was obtained fresh from a local slaughterhouse and stored at −20 °C until used. Sheep red blood cells were purchased in 50 % solution from Gibco, Madison, Wisconsin.

Nuclei. Rat liver and calf thymus nuclei were isolated by the procedure of Chaveau et al. [10] with modifications recently described by Spelsberg et al. [11], except that 2.2 M sucrose was used. Purified nuclei were resuspended in 1 M sucrose containing 0.005 M $MgCl_2$, 0.025 M KCl and 0.05 M Tris · HCl (pH 7.5) frozen in liquid N_2 and stored at −70 °C. Phase microscopy revealed intact nuclei after quick thawing. Nuclei were centrifuged at $20\,000 \times g_{max}$ for 10 min and resuspended in either 0.25 M sucrose/0.002 M sodium phosphate (pH 8) for nuclear membrane preparation or in 0.5 M sucrose containing 0.005 M $MgCl_2$, 0.025 M KCl, 0.2 % (v/v) Triton X-100, and 0.05 M Tris · HCl (pH 7.5) for chromatin isolation.

Nuclear membrane. The method of Bornens [12] was used to isolate nuclear membrane from Sprague-Dawley albino rat liver and from calf thymus nuclei. A sucrose gradient centrifugation step was added to the purification scheme. Briefly, sodium salt of heparin was added to purified nuclei to make the heparin to DNA weight ratio 2 : 1 at a concentration of about 1.5 mg protein/ml. Following 1 h incubation at 4 °C, the solubilized nuclei were centrifuged for 1 h at $70\,000 \times g_{max}$. The pellet of crude nuclear membrane was resuspended in 0.25 M sucrose/0.002 M sodium phosphate (pH 8). Initially the material was layered on a linear 27–47 % (w/w) sucrose gradient in 0.002 M sodium phosphate (pH 8) and centrifuged for 4 h at 38 000 rev./min in a Beckman SW 41 rotor. A band formed at 33 % sucrose. It was collected by aspiration, diluted with an equal volume of water, and centrifuged for 1 h at $80\,000 \times g$. Later, a discontinuous gradient was routinely used consisting of 2 ml of 45 % (w/w), 3 ml of 37 % (w/w), and 5 ml of 28 % (w/w) sucrose. The membranes were collected at the 28–37 % sucrose interface after centrifugation. The purified membrane pellet was resuspended in 0.25 M sucrose, frozen with liquid N_2 and stored at −70 °C.

Preparation of chromatin and nonhistone protein · DNA complex. Chromatin

was isolated according to Spelsberg and Hnilica [13]. Nonhistone protein · DNA complex was prepared by treating chromatin with 2 M NaCl and 5 M urea [14].

Preparation of other membranes. Rough and smooth endoplasmic reticulum, mitochondria and plasma membrane were isolated from rat liver according to Fleischer and Kervina [15]. The plasma membrane was the generous gift of Dr. F. Zambrano (Department of Molecular Biology, Vanderbilt University). Red blood cell membranes were isolated from sheep cells using an osmotic shock technique [16]. Rat and rabbit muscle sarcoplasmic reticulum kindly donated by Dr. G. Meissner (Departments of Biochemistry and Physiology, University of North Carolina, Chapel Hill) were isolated as previously described [17]. All purified membranes were resuspended in 0.25 M sucrose, quick frozen with liquid N_2 and stored at −70 °C.

Isolation of a microsomal fraction. Rat livers were homogenized in 5 vols of 0.3 M sucrose containing 0.005 M $MgCl_2$, 0.025 M KCl and 0.05 M Tris · HCl (pH 7.5). The homogenate was passed through cheese cloth and centrifuged for 30 min at $12000 \times g_{max}$. The supernatant was centrifuged for 1 h at $113000 \times g$ in a Beckman Ti 60 rotor. The resulting pellet was resuspended in 0.25 M sucrose, analyzed for protein, bound phosphorus and enzyme activities and stored at −70 °C.

Chemical analysis. DNA [18], protein [19] and bound phosphorus [20, 21] content were determined using calf thymus DNA, bovine serum albumin and inorganic phosphate as standards, respectively. Chromatin proteins were determined as previously described [22].

Immunization. To induce antibodies to rat liver nuclear membrane, 3-month-old male New Zealand white rabbits (Pel-Freez, Rogers, Ark.) received weekly injections of an equivalent of 250 μg membrane protein in 0.3 ml sterile saline and 0.3 ml of complete Freund's adjuvant. The first two injections were made in the hind feet toe pads and the next two intramuscularly, followed by a booster into the ear vein of an equivalent of 60 μg membrane protein in 0.4 ml sterile saline. About 30 ml of blood were taken from the ear vein before immunization and 6 days after each injection to monitor the presence of antibodies. Control serum was also obtained from a rabbit, which received identical injections of Freund's adjuvant and sterile saline but containing no immunogen. The collected blood samples were allowed to stand 1 h at room temperature and then overnight at 4 °C. Sera recovered after centrifugation were incubated at 56 °C for 20 min and stored at −20 °C.

Immunochemical analysis. The quantitative microcomplement fixation assay was carried out as described by Levine [23], except that a total assay volume of 1.4 ml was used. The incubation mixture contained 0.2 ml of an appropriate dilution of control or immune sera diluted with a dilution buffer (0.1 % (w/v) bovine serum albumin/0.14 M NaCl/0.5 mM $MgSO_4$/0.15 mM $CaCl_2$/0.01 M Tris · HCl (pH 7.4), 0.2 ml guinea pig complement diluted 150-fold with the dilution buffer and 0.2 ml of antigen in the appropriate concentration. After 18–20 h of incubation at 4 °C, 0.2 ml of sensitized sheep red blood cells was added. Controls for nonspecific binding or inactivation of the hemolytic ability of complement (anticomplementarity) were routinely carried out for all antigens and antisera.

Complement fixation (percent of complement fixed) is expressed as a function of the membrane or chromatin protein content in 1.4 ml assay volume over a range between 0.01 and 20 μg protein. Usually anti-nuclear membrane sera were diluted 1 : 400 before the assay.

Electron microscopy. Purified nuclear membrane was fixed with 2.5 % (w/v) glutaraldehyde at 4 °C for 1.5 h, then embedded and thin-sectioned [24]. The electron micrographs were kindly prepared by Mr. Akitsugu Saito (Department of Molecular Biology, Vanderbilt University).

Determination of enzyme activities. Glucose-6-phosphatase and 5'-nucleotidase activities were determined according to Swanson [25] and Michell and Hawthorne [26], respectively. Succinate-cytochrome *c* reductase was measured by the method of Fleischer and Fleischer [27].

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The method of Laemmli [28] was used, with the exception that the gels contained 4 M urea and 1 mM EDTA and the running buffer contained 0.096 M glycine. All samples were suspended in 1 % sodium dodecyl sulfate/2.5 mM EDTA/2.75 M urea/50 mM Tris · HCl (pH 6.7) containing 0.05 % bromophenol blue and 3 % 2-mercaptoethanol, and solubilized by heating 10 min at 60 °C. Samples were applied to a slab gel consisting of 5 % and 11 % acrylamide stacking and separating gel, respectively. Gels were stained with 0.25 % Coomassie blue in 50 % methanol/10 % acetic acid and destained in 20 % methanol/20 % acetic acid.

Materials. Heparin (sodium salt) and calf thymus DNA were purchased from Sigma, St. Louis, Missouri, and guinea pig complement and sheep red blood cells from Gibco, Madison, Wisconsin. Hemolysin and Freund's complete adjuvant were products of Difco, Detroit, Michigan.

RESULTS

Characteristics of purified rat liver nuclear membrane

As seen in Fig. 1, purified nuclear membranes display a characteristic trilaminar arrangement in the electron micrograph. Pore structures and contiguous double membranes were seldom seen. Approximately 3–6 % of total nuclear protein was recovered as nuclear membrane protein. The nuclear membrane preparations averaged 28–34 μg bound phosphorus per mg protein. The nuclei used for the preparation of nuclear membrane had a protein to DNA weight ratio of about 2.6. Small amounts of DNA were detectable in the membrane preparation as indicated by a protein to DNA weight ratio of about 32.

To assess the purity of the nuclear membrane preparations with respect to other subcellular organelles, certain enzyme activities of rat liver nuclei, nuclear membrane, endoplasmic reticulum, plasma membrane and mitochondria were determined as summarized in Table I. Glucose-6-phosphatase and 5'-nucleotidase activities of freshly purified nuclear membrane were found to be from 0.33 to 0.45 and 0.05 μmol of inorganic phosphorus released/mg protein per min, respectively. Both activities have been reported to be endogenous to nuclear membrane [29–33]. The glucose-6-phosphatase activity was similar to that reported by Kashnig and Kasper [29] and significantly higher than previously reported by others [30, 32]. It was found that storage of the isolated nuclear membrane at −70 °C results in lower activities of these enzymes. The microsomal, rough and smooth endoplasmic reticulum fractions were repeatedly found to have lower glucose-6-phosphatase activity than the nuclear membrane. When rough endoplasmic reticulum was treated with heparin under the conditions used for the isolation of nuclear membranes, no significant change in

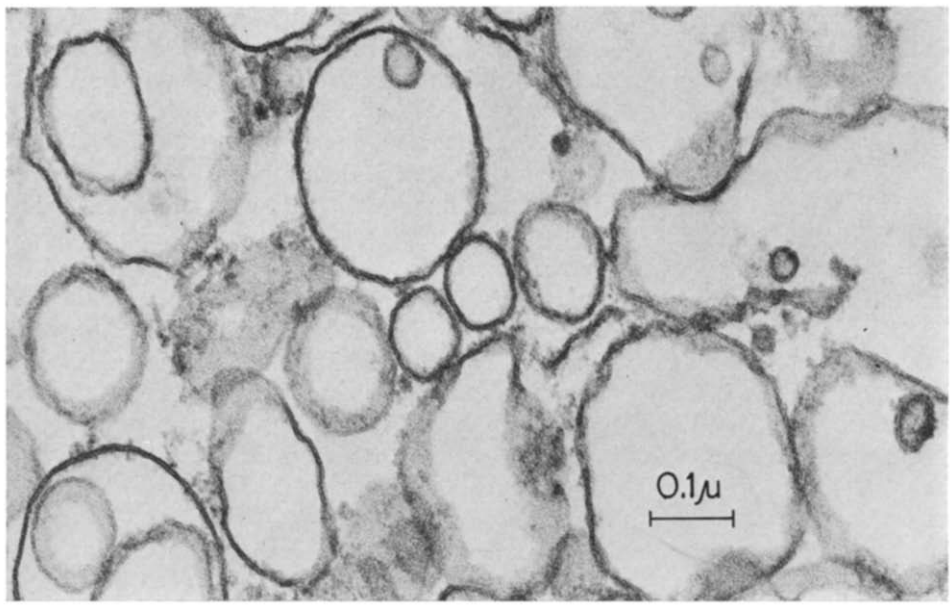

Fig. 1. Electron micrograph of rat liver nuclear membrane purified by sucrose gradient centrifugation (for details see Materials and Methods).

TABLE I

ENZYME ACTIVITIES OF SUBCELLULAR ORGANELLES OF RAT LIVER

Glucose-6-phosphatase and 5′-nucleotidase assays were carried out at 37 °C and the succinate-cytochrome *c* reductase assay at 32 °C. Organelles were prepared and enzymic assay performed as described in Materials and Methods. Results are expressed as μmol substrate hydrolyzed/mg protein per min (glucose-6-phosphatase and 5′-nucleotidase) and μmol substrate reduced/mg protein per min (succinate-cytochrome *c* reductase).

Fraction	Glucose-6-phosphatase	5′-Nucleotidase	Succinate-cytochrome-*c* reductase
Nuclei	0.04	0.006	–
Nuclear membrane	0.33–0.45	0.05	0.0012
Rough endoplasmic reticulum	0.16–0.26	0.08	0.004
Smooth endoplasmic reticulum	0.07–0.12	0.21	0.006
Total microsomes	0.12	0.10	–
Mitochondria	0.05	0.02	0.44
Plasma membrane	0.03	1.45	0.04

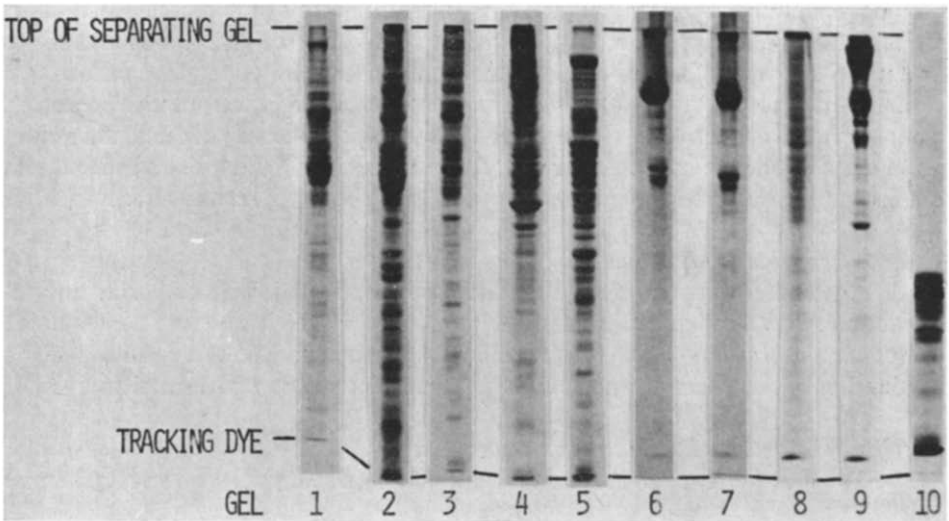

Fig. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of proteins of various purified membrane preparations. The gels represent (1) rat liver nuclear membrane, (2) rat liver rough endoplasmic reticulum, (3) rat liver smooth endoplasmic reticulum, (4) rat liver plasma membrane, (5) rat liver mitochondria, (6) rat skeletal muscle sarcoplasmic reticulum, (7) rabbit skeletal muscle sarcoplasmic reticulum, (8) calf thymus nuclear membrane, (9) sheep red blood cell membrane and (10) calf thymus histones.

glucose-6-phosphatase activity was observed. The nuclear membrane preparation was essentially free of inner mitochondrial membrane as judged by low succinate-cytochrome *c* reductase activity (Table I) and by electron microscopy (Fig. 1). It appeared also that nuclear membrane preparations were free of inverted mitochondrial vesicles, since addition of low concentrations (0.1–0.25 %) of cholate did not enhance succinate-cytochrome *c* reductase activity.

A comparison of the proteins of rat liver nuclear membrane with various other membrane preparations by sodium dodecyl sulfate-polyacrylamide gel electrophoresis shows that nuclear membrane has a protein profile (gel 1 of Fig. 2) distinct from that of other membranes. Most of the polypeptides of the nuclear membrane range in molecular weight between 50 000 and 100 000, with a dominant band around 50 000. The sparsity of low molecular weight components indicates the absence of histones and ribosomal proteins in the nuclear membrane preparation. Similarities can be observed between nuclear membrane and rough endoplasmic reticulum (gel 2 of Fig. 2) in the region corresponding to a molecular weight of 50 000.

Presence of anti-membrane antibody in sera of nonimmunized rabbits

It was found that sera from three out of four rabbits supplied by Hilltop Rabbit Ranch (Scotsdale, Pennsylvania) fixed complement when tested in a 1/400 dilution in the presence of various membrane preparations, indicating that the sera of these nonimmunized animals contained anti-membrane antibody. For example, calf thymus nuclear membrane reacted with 50 % fixation at 15 ng membrane protein. Other membranes including rat liver nuclear membrane, rough and smooth endo-

plasmic reticulum, plasma membrane and rat and rabbit skeletal muscle sarcoplasmic reticulum showed 50 % complement fixation between 0.2 and 3.0 μg membrane protein. Sheep red blood cell membrane and rat liver mitochondria showed little complement fixation below 30 μg protein. These results demonstrate the presence of complement-fixing antibodies for different membranes in the sera of certain nonimmunized rabbits. These antibodies were not further characterized. However, the presence of these antibodies necessitated the use of rabbits from another supplier.

Time course of antibody induction

Antibodies were detected in rabbits 2 weeks after injection of purified nuclear membrane. The antibody titer increased somewhat by the fifth week. Sera obtained prior to immunization or 1 week after injection of nuclear membrane showed negligible fixation of complement when tested in the presence of nuclear membrane.

Specificity of anti-rat liver nuclear membrane sera

The anti-rat liver nuclear membrane sera collected in the second and the fifth

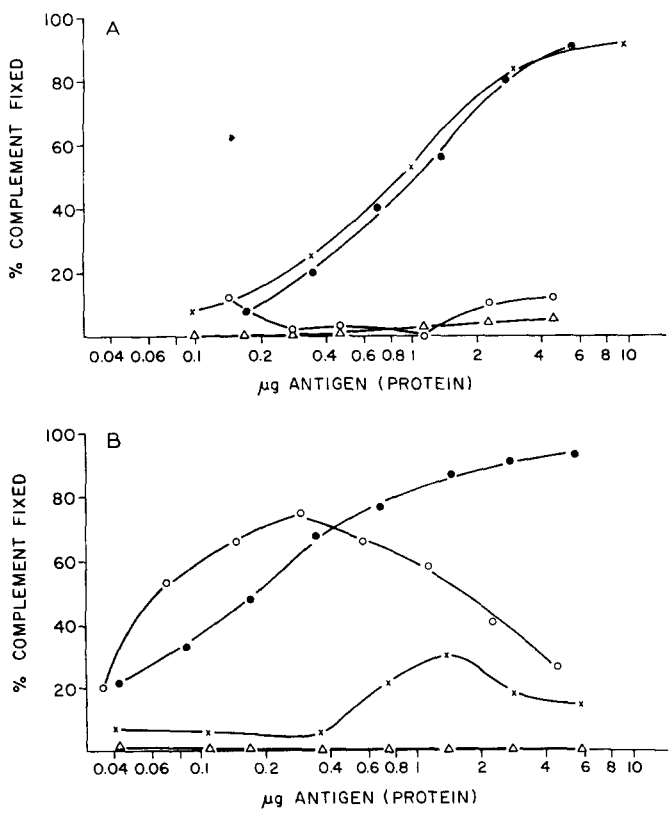

Fig. 3. Comparison of anti-rat liver nuclear membrane sera obtained after two weeks of injection in two rabbits. Sera were tested diluted 1/400 in the complement fixation assay against rat liver (●) nuclear membrane, (○) rough endoplasmic reticulum, and (×) plasma membrane. All membranes were also assayed against sera obtained prior to immunization (△). Figs A and B represent data from different rabbits.

week of immunization were found to have different immunological specificity. One rabbit had produced by the second week of immunization a serum which fixed complement in the presence of rat liver nuclear and plasma membrane to approximately the same degree, while little complement fixation was observed for rat liver rough endoplasmic reticulum (Fig. 3A). In contrast, serum taken the second week of immunization from another rabbit, immunized identically, fixed complement in the presence of nuclear membrane or rough endoplasmic reticulum. Only slight complement fixation was observed when plasma membrane was used (Fig. 3B). 50 % complement fixation occurred with the nuclear membrane at approximately 1.0 and 0.2 μg membrane protein per assay in each rabbit, respectively.

On the other hand, analysis of sera obtained in the fifth week of immunization showed little distinction between the two rabbits. These antibodies reacted similarly when tested against various membranes. Rat liver nuclear membrane reacted in the complement fixation assay (Fig. 4) with 50 % complement fixation occurring at 0.07 μg membrane protein per 1.4 ml assay volume. A similar reactivity was observed when a liver nuclear membrane preparation isolated from another rat strain (Charles River CD strain) was tested (not shown). Rat liver rough endoplasmic reticulum and mitochondria also reacted to a high extent with the anti-nuclear membrane sera, with 50 % complement fixation at 0.08 and 0.1 μg protein, respectively. Rat liver plasma membrane, smooth endoplasmic reticulum and rat muscle sarcoplasmic reticulum reacted to a lesser extent with antibodies, showing 50 % fixation of complement at 0.25, 0.6 and 0.46 μg protein, respectively. These anti-nuclear membrane sera did not fix complement in the presence of rabbit muscle sarcoplasmic reticulum, calf thymus nuclear membrane or sheep red blood cell membrane (Fig. 4). The nonreactivity of rabbit muscle sarcoplasmic reticulum membrane contrasts with the amount of complement fixation observed for this membrane from rat muscle, suggesting an immunochemical species specificity of membrane.

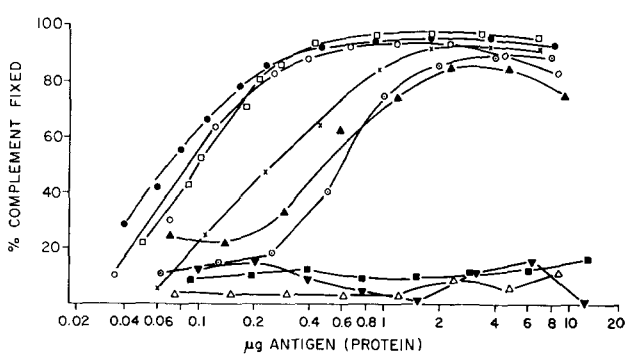

Fig. 4. Complement fixation by various membrane preparations in the presence of anti-rat liver nuclear membrane sera after a booster injection in the fifth week. Immune sera were tested (dilution 1/400) against (●) rat liver nuclear membrane, (○) rat liver rough endoplasmic reticulum, (⊙) rat liver smooth endoplasmic reticulum, (□) rat liver mitochondria, (×) rat liver plasma membrane, (▲) rat muscle sarcoplasmic reticulum, (△) rabbit muscle sarcoplasmic reticulum, (■) calf thymus nuclear membrane and (▼) sheep red blood cell membrane. The values represent the average of four experiments, and were essentially identical in the two immunized rabbits represented in Fig. 4. Reactivity of all membranes was negligible with sera obtained prior to immunization.

Absorption of anti-nuclear membrane sera

In an attempt to characterize further the anti-nuclear membrane antibodies, immune sera of two rabbits after five weeks of immunization were absorbed with various membrane preparations. The remaining antibodies were then tested. The data are expressed in Table II as the membrane protein concentration necessary to give 50 % fixation of complement.

In one rabbit (A), absorption with rat liver nuclear membrane essentially eliminated all antibodies which reacted with the membranes when added at a concentration of 40 μg membrane protein. Similar treatment of immune sera with various

TABLE II

COMPLEMENT FIXATION BY ANTI-NUCLEAR MEMBRANE SERA ABSORBED WITH VARIOUS MEMBRANE FRACTIONS

Results are expressed as the concentration of membrane protein required for 50 % complement fixation (μg protein/1.4 ml assay volume). Anti-rat liver nuclear membrane sera diluted 400-fold with the dilution buffer were treated with the indicated amounts of protein. The mixtures were incubated overnight at 4 °C and then centrifuged for 30 min at 100000 × g. Under these conditions, all free and antibody-bound membranes sediment. The control sera to which no membrane was added were unaffected by centrifugation. 0.2 ml aliquots of the supernatants were used in the complement fixation assay described in Materials and Methods.

Organelle (μg/ml)	Antigen					
	Nuclear membrane	Rough endoplasmic reticulum	Mitochondria	Plasma membrane	Sheep red blood cell	Rabbit sarcoplasmic reticulum
Absorbed by Serum A						
None						
0	0.08	0.10	0.10	0.25	> 20	> 20
Nuclear membrane						
10	2.8	–	–	–	–	–
20	6.5	–	–	–	–	–
30	7.4	–	–	–	–	–
40	7.2	> 20	> 20	–	–	–
Rough endoplasmic reticulum						
12	0.15	3	> 20	–	–	–
24	0.29	8	> 20	–	–	–
58	0.58	> 20	> 20	–	–	–
Mitochondria						
30	0.23	6	3	–	–	–
60	0.40	15	15	–	–	–
Sheep red blood cell						
28	0.10	0.11	–	–	> 20	–
56	0.11	0.11	–	–	> 20	–
Rabbit sarcoplasmic reticulum						
58	0.05	0.04	–	–	–	> 20

Table II continued

Organelle (μg/ml)	Antigen					
	Nuclear membrane	Rough endoplasmic reticulum	Mitochondria	Plasma membrane	Sheep red blood cell	Rabbit sarcoplasmic reticulum
Absorbed by Serum B						
None						
0	0.09	0.11	0.12	0.27	> 20	> 20
Nuclear membrane						
10	> 10	–	–	> 20	–	–
20	> 10	–	–	> 20	–	–
30	> 15	–	–	> 20	–	–
40	> 10	> 20	> 20	> 20	–	–
Rough endoplasmic reticulum						
12	0.20	6	5	0.6	–	–
24	0.24	> 20	> 20	> 20	–	–
58	0.38	> 20	> 20	> 20	–	–
Mitochondria						
30	0.08	15	1.5	> 20	–	–
60	0.09	20	2.5	> 20	–	–
Sheep red blood cell						
28	0.10	0.11	–	–	> 20	–
56	0.10	0.11	–	–	> 20	–
Rabbit sarcoplasmic reticulum						
58	0.06	0.08	–	–	–	> 20

amounts of rough endoplasmic reticulum or mitochondria from rat liver resulted in the loss of antibodies fixing complement in the presence of rough endoplasmic reticulum, plasma membrane and mitochondria. However, antibodies specific for the nuclear membrane remained with 50 % complement fixation occurring at about 0.3 μg membrane protein.

The serum of a second rabbit (B) reacted similarly to that from the first when absorbed with and tested against nuclear membrane, endoplasmic reticulum and mitochondria.

Absorption of the two anti-nuclear membrane sera with rabbit muscle sarcoplasmic reticulum or sheep red blood cell membranes had no effect, which supports the fact that nonabsorbed immune sera did not fix complement in the presence of these membranes. It appears therefore that treatment of anti-nuclear membrane sera with rough endoplasmic reticulum or mitochondria can yield antisera which are specific for the liver nuclear membrane.

Comparison of antigenic determinants of chromatin and nuclear membrane

The complement fixation by liver nuclear membrane antisera in the presence

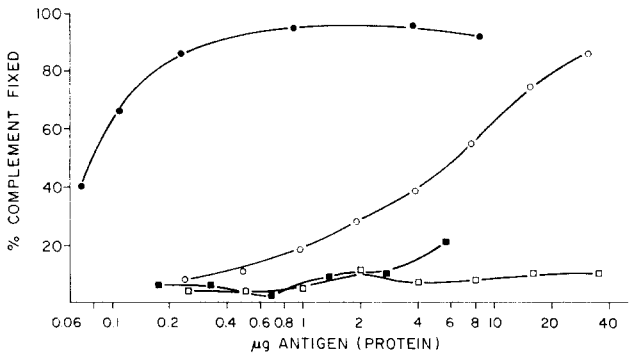

Fig. 5. Complement fixation by anti-rat liver nuclear membrane sera in the presence of nuclei and nuclear components of rat liver. (For details see Materials and Methods.) Complement fixation observed for antisera to rat liver nuclear membrane (dilution 1/400) is illustrated in the presence of rat liver (●) nuclear membrane, (○) whole nuclei, (□), chromatin and (■) a nonhistone protein · DNA complex.

of several nuclear components was tested as illustrated in Fig. 5. Whole nuclei fixed complement in the presence of the antisera, so that about 7 μg total nuclear protein was required for 50 % fixation as compared with 0.08 μg protein of purified nuclear membrane. Liver chromatin and a nonhistone protein · DNA complex did not show appreciable complement fixation. This complex could not be tested at protein concentrations above 5 μg because of anticomplementarity. These results suggest that the antigenic determinants of liver nuclear membrane are practically absent in our preparations of liver chromatin or nonhistone protein · DNA complexes.

DISCUSSION

In order to obtain a highly purified nuclear membrane preparation for use in the production of antibodies, the method of Bornens [12] was extended by subjecting the membrane preparation to sucrose gradient centrifugation. The purified rat liver nuclear membrane was then found to contain very low amounts of plasma and inner mitochondrial membranes and chromatin components as judged by electron microscopy, sodium dodecyl sulfate-polyacrylamide gel electrophoresis and chemical and enzyme analysis.

The purified liver nuclear membrane proved to be an effective immunogen which readily induced complement-fixing antibodies in rabbits. The antibodies reacted not only with the nuclear membrane but also with other subcellular membranes derived from rat tissue. The cross-reaction could be due either to contamination of the nuclear membrane preparations with other membrane fragments, or to the presence of common antigenic components in the membrane fractions. Considering the effectiveness of nuclear membrane in removing the cross-reacting antibodies as compared to the larger quantity of mitochondria required to remove these antibodies (cf. Table II), it appears that contamination is probably not the explanation for the observed cross-reactions. Enzyme analysis indicated the presence of less than 1 % of mitochondria and 4 % of plasma membrane in purified nuclear membrane. It seems therefore more likely that the cross-reactions are due to the presence of similar

antigenic structures in the membranes derived from rat.

Similarities in membrane composition and function between nuclear membrane and endoplasmic reticulum [1, 30, 34, 35] as well as outer mitochondrial membrane [36] have been reported. Also, similar biogenesis of these membranes has been proposed [3, 37]. The common antigenic components in these membranes do not appear to be phospholipid, since the anti-liver nuclear membrane sera did not fix complement in the presence of rabbit sarcoplasmic reticulum or sheep red blood cell membrane. On the other hand, complement fixation by rat liver plasma membrane or rat muscle sarcoplasmic reticulum could possibly be explained by contaminating endoplasmic reticulum in these membrane preparations.

There are several groups of antigens localized on membranes. The Forssman antigen is a glycolipid associated with proteins of the sheep red blood cell and other cell membranes [38]. However, no complement fixation was observed by sheep red blood cell membrane when tested against anti-nuclear membrane sera, suggesting that the Forssman antigen is not involved. Histocompatibility antigens have been identified in the rat as being primarily located on plasma membrane [39, 40]. It is unlikely that the immunochemical reactivity observed in this study was due to these antigens. Only low concentrations of these antigens are reported to be present in endoplasmic reticulum [40], lysosomes [41, 42] or nuclear membrane [43] of several rat tissues, including liver. Unusually low concentrations of histocompatibility antigens were reported to be present in muscle [41]. Moreover, we have found that antibody produced against liver nuclear membrane prepared from Sprague-Dawley rats fixed complement to the same extent in the presence of a similar liver nuclear membrane preparation from a different rat strain (Charles River CD strain). It therefore appears that we are dealing with an as yet undefined group of antigens common to membranes from the same species.

Although marker enzymes have been established for many subcellular fractions, no clear marker has been identified for the nuclear membrane. Studies reported here indicate that a population of antibodies specific for nuclear membrane can be produced in the rabbit. These antibodies were identified following absorption of antisera with endoplasmic reticulum or mitochondria and indicate the presence of antigenic sites specific for nuclear membrane. We therefore propose that these antisera may serve as a tool for detection of nuclear membrane in various subcellular preparations. Using this probe it was found, for example, that chromatin preparations do not contain appreciable amounts of nuclear membrane. This immunochemical method is of considerable advantage for its high sensitivity.

ACKNOWLEDGEMENT

This work was supported by USPHS Grants HD-05384, HL-15341 and HL-14214.

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