SHORT COMMUNICATIONS

BBA 73034

Partial solubilization of protein and 5'-nucleotidase from microsomal membranes of the rat liver by ultrasonic irradiation

5'-Nucleotidase (5'-ribonucleotide phosphohydrolase, EC 3.1.3.5) of the rat liver has been shown to be associated with the microsomal membranes[1,2]. This report presents evidence for the partial dissociation and solubilization of 5'-nucleotidase and other proteins from microsomal membranes by means of ultrasonic irradiation.

Liver microsomes were prepared according to the method of PALADE AND SIEKEVITZ[3] from female Sprague–Dawley rats weighing approx. 250 g. The microsomal pellets were washed twice by suspension and recentrifugation in 0.25 M sucrose. The resulting pellets were resuspended in 0.076 M Tris–citrate (pH 8.7) (Buffer T) or in 0.05 M Tris–HCl (pH 7.8) containing 0.05 M KCl and 0.1 mM $MgCl_2$ (Buffer TKM). One ml of this suspension (designated M-1) contained microsomes obtained from 1 or 2 g of the original liver. Aliquots of M-1 were subjected to ultrasonic irradiation for 20 min at 20000 cycles/sec at the peak power output of MSE ultrasonic power unit (60 W). The loss of 5'-nucleotidase activity by this procedure was negligible. Ultrasonically treated M-1 (designated M-2) was used without further fractionation in some experiments. In others, M-2 was centrifuged for 1 h at 105000 $\times$ g and the supernatant (designated M-2/S) was used.

Starch-gel electrophoresis[4] of these microsomal preparations gave results shown in Fig. 1. Rat serum was used as a marker and the gels were stained for protein with naphthol blue black. M-1 separated into 2 bands (A in Fig. 1), a cathodic band (f) that migrated with a mobility similar to serum γ-globulin and a diffuse anodal band (e) in the general region of β-globulins. M-2 or M-2/S yielded, in addition to these 2 bands, 4 new bands on the anodic side of the gel (B in Fig. 1). Two (a, b) of these new bands had a mobility greater than serum albumin, and the remaining two (c, d) migrated in the region between albumin and α_2-globulin.

Microsomal membranes were also solubilized[3] by suspending M-1 in a solution of sodium deoxycholate at a final sodium deoxycholate concentration of 1 % (w/v). The ribosomes were then removed by centrifugation for 1 h at 105000 $\times$ g. The electrophoretic pattern of sodium deoxycholate-solubilized microsomal membranes (C in Fig. 1) was identical to that of M-2 and M-2/S, indicating that the 4 protein bands separated from these latter preparations originated from microsomal membranes and not from ultrasonically disrupted ribosomes.

The various microsomal preparations suspended in Buffer TKM were subjected to zone sedimentation through a 5–20 % sucrose gradient layered over a cushion of 50 % sucrose. Effluent fractions collected from the bottom of each tube were analyzed for protein and 5'-nucleotidase[1]. On centrifuging M-1 (Fig. 2A), the membrane-containing fraction sedimented rapidly and accumulated as a sharp, turbid zone near

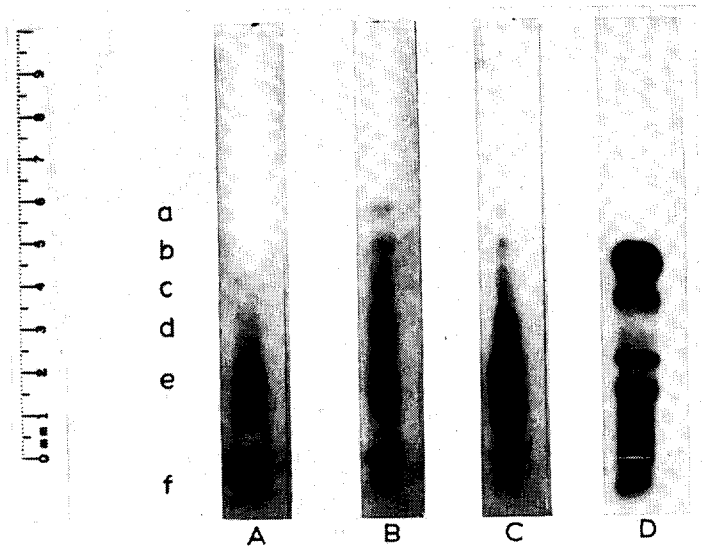

Fig. 1. Starch-gel electrophoresis of microsomal preparations and rat serum. The wells contained 0.04 ml (1.4 mg protein) of: M-1 (A); M-2/S (B); sodium deoxycholate-solubilized microsomal membranes (C); and 1:3 dilution of rat serum (D). 8 V/cm were applied for 3 h. The gel and all the materials were prepared in Buffer T. The origin is at 0 cm. The anode is at the top.

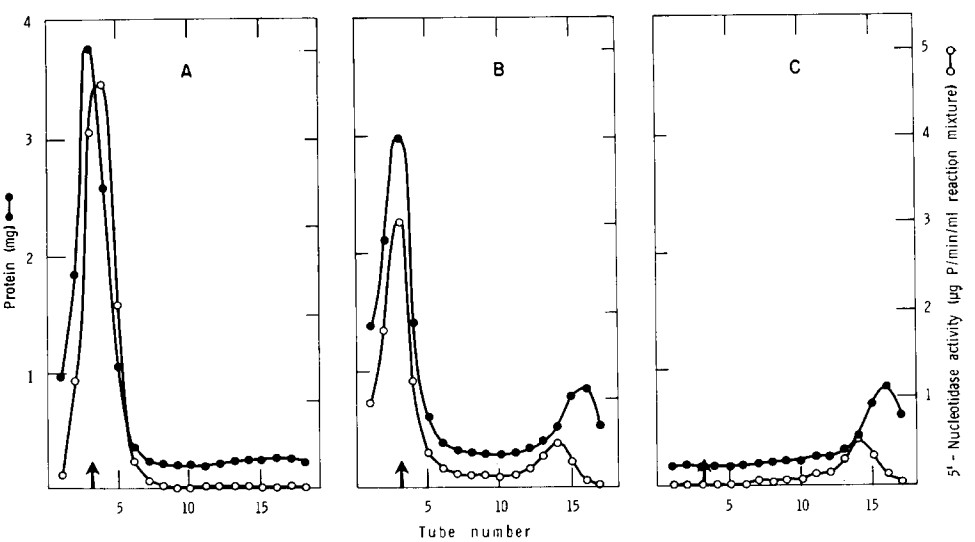

Fig. 2. Centrifugation of microsomal preparations through density-gradient solutions of sucrose. 1 ml of M-1 (A), M-2 (B), and 165000 × *g* supernatant (C) of M-2 was layered over 27 ml of gradient solution of sucrose prepared as described in the text. The 3 tubes were placed in a Spinco SW 25.1 rotor and centrifuged at 23000 rev./min for 18 h. The effluent fractions (1.6 ml) are numbered from the bottom of the tube, and arrow indicates the position of the 20–50% sucrose interface. The overall recovery of protein (●—●) in effluent fractions averaged 88%. The recovery of 5′-nucleotidase (○—○) averaged 95%.

the 20–50 % sucrose interface. Free ribosomes were pelleted at the bottom of the tube by this procedure. As demonstrated previously[1], the zone of 5′-nucleotidase activity corresponded to that of the membrane-containing fraction. Upon examination in the electron microscope[5], M-1 consisted of a collection of membrane vesicles (85–170 mμ in diameter) and free ribosomes typical of liver microsomal fraction.

Simultaneous sedimentation analysis of ultrasonically treated M-1 (M-2, Fig. 2B) containing the same amount of protein as M-1 showed a slight decrease in the size of the peaks near the 20–50 % sucrose interface and the presence of slow zones of solubilized protein and 5′-nucleotidase in the upper portion of the tube. Preliminary centrifugation of M-2 for 1 h at 165000 × *g* completely eliminated the interface material without affecting the position and the magnitude of the slow zones of protein and 5′-nucleotidase (Fig. 2C). The pellet that had been removed by the preliminary centrifugation, when examined in the electron microscope, consisted of tightly packed fragments of microsomal membranes and free ribosomes. A large proportion of these membrane fragments were rounded into small vesicles with an average diameter of about 30 mμ.

The $s_{20,w}$ values of the solubilized protein and 5′-nucleotidase, determined as a separate experiment according to the method of MARTIN AND AMES[6], were 3.9 and 10.9 S, respectively, when calculated with yeast alcohol dehydrogenase as a marker. The 10.9-S 5′-nucleotidase represented about 14 % of the total 5′-nucleotidase activity present in M-2.

Antibodies to M-2/S were produced[7] in 2 rabbits by intravenous injection, in graded doses, of a total of 45 mg protein of M-2/S. Fig. 3 shows a representative precipitin pattern observed in double diffusion studies performed in Ouchterlony plates. Pooled antiserum to M-2/S (AS) in the center well was allowed to react with M-1 (I), M-2/S (II), sodium deoxycholate-solubilized microsomal membranes (III), and 105000 × *g* supernatant of rat-liver homogenate (IV) in the peripheral wells. The outermost precipitin band (A) appeared as a split band in each of the four reactions but fused at the tips, indicating the presence of a serologically identical antigen in the preparations in the peripheral wells. Of the other 3 bands formed between AS and M-1 (I), Band B was not seen in any of the other reactions and may represent an antigen that is labile to solubilization procedures. Band C split at the tip into

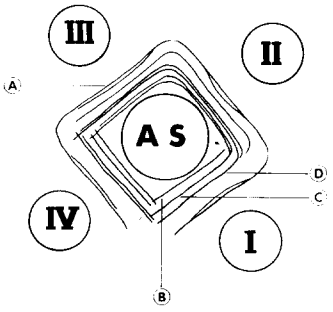

Fig. 3. Precipitin reaction of M-1 (I), M-2/S (II), sodium deoxycholate-solubilized microsomal membranes (III), and 105000 × *g* supernatant of rat-liver homogenate (IV) with rabbit antisera (AS) prepared against M-2/S. 0.2 ml of undiluted antisera was placed in the center well. Peripheral wells contained 0.1 ml of microsomal preparations (I–III, about 26 mg protein/ml). The Ouchterlony plate was incubated for 48 h at 37°. Contact print was made from the plate using an ultraviolet lamp, and the precipitin lines were traced with the aid of a photographic enlarger.

2 components and fused with 2 bands formed between AS and M-2/S (II). These 2 bands, in turn, fused with 2 similar bands formed between AS and sodium deoxycholate-solubilized microsomal membranes (III). Likewise, Band D split into at least 3 components and fused with 3 similar bands between AS and sodium deoxycholate-solubilized membranes (III).

Dissociation of a single antigenic material into smaller units that contain different antigenic determinants has been shown[8] to result in the splitting of precipitin bands in double diffusion studies in an Ouchterlony plate. The band-splitting noted in our studies indicate that ultrasonic irradiation of M-1 resulted in the dissociation of membrane antigens into several antigenic subunits. That these precipitin bands are indeed due to membrane antigens is shown by their reaction of identity with bands formed between AS and sodium deoxycholate-solubilized microsomal membranes.

AS reacted with 105000 × *g* supernatant of rat-liver homogenate (IV) to yield 4 precipitin bands in addition to the Band A. These 4 bands, however, showed no interaction with any of the bands formed between AS and various microsomal preparations. The M-2/S preparation used for immunization may have contained a trace of cell sap, enough to produce an antibody response in the rabbits but not enough to form precipitin bands on the Ouchterlony plate.

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The isolation of rat liver plasma membrane fragments

Preparations of liver plasma membranes may be obtained from crude nuclear fractions by modifications of the method of NEVILLE[1] (*e.g.* ref 2). Such preparations have been extensively studied both in the electron microscope and by analysis of gross composition and enzymic content[2–5]. KAMAT AND WALLACH[6] have shown that