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Beef Pancreas Ribosomes: Isolation and Properties*

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Ribonucleoprotein particles (ribosomes) have been isolated after treatment of beef pancreatic microsomal preparations with deoxycholate. Suspensions of these ribosomes in 1 mM phosphate, *p*H 7.4, consist almost exclusively of 80 *S* particles. In contrast to rat liver ribosomes, however, the beef pancreas particles were aggregated by low concentrations of Mg^{++}, by ionic strength of 0.1, or by EDTA. Spermine also precipitated these ribosomes, with the release of large amounts of protein into solution. Free electrophoresis demonstrated that the pancreatic ribosomes were less acidic than their liver homologs. This could be accounted for by the presence of considerable quantities of the basic proteins ribonuclease and chymotrypsinogen A in association with beef particles. The addition of ribonuclease or lysozyme to rat liver ribosomes resulted in a suspension which closely resembled beef pancreas ribosomes in a number of characteristics. The data obtained in this comparison of beef pancreas and rat liver ribosomes suggest that the extreme instability of beef pancreas ribosomes is most likely due to the presence of ribonuclease, which was adsorbed in the course of isolation.

Ribonucleoprotein particles (ribosomes) have been isolated from a variety of sources and intensively examined since they were first shown to be the site of amino acid incorporation into protein (Littlefield *et al.*, 1955; Roberts, 1958). Siekevitz and Palade (1958) reported that ribosomes of pancreas became labeled more rapidly than other intracellular fractions when guinea pigs were injected with leucine-C^{14}. Dickman *et al.* (1962a) measured the rate of incorporation of valine-C^{14} and tryptophan-C^{14} into the proteins of various intracellular fractions by beef pancreas slices and reached similar conclusions. Despite these evidences of considerable protein-synthesizing ability, isolated microsomal fractions or ribonucleoprotein particles from the pancreas of most species are inactive in the incorporation of amino acids into protein. There must be some factor or condition which is responsible for this inactivity. In this and the succeeding paper we describe the isolation of beef pancreas ribosomes and compare a number of their properties and characteristics with those from rat liver.

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Materials and Methods

(a) *Preparation of Ribosomes*

Pancreas was removed from cattle as soon as possible after slaughter and placed in ice-cold 0.25 M sucrose for transport to the laboratory. The tissue was trimmed of fat and connective tissue and forced through a prechilled French tissue press at a pressure between 2,000 and 10,000 p.s.i. All subsequent operations were performed at a temperature of 0–4°. The pulp was dispersed in sufficient ice-cold 0.25 M sucrose to

produce a 10% (v/v) homogenate. Rat liver homogenates were prepared similarly with a Potter-Elvejhem homogenizer equipped with a Teflon pestle. Fractionation was carried out by centrifugation for 13 minutes at 14,000 × *g* in a Spinco model L centrifuge. The microsomal fraction was then sedimented by centrifugation of the supernatant for 30 minutes at 100,000 × *g*. It was resuspended in one-tenth the original volume of 1 mM potassium phosphate, *p*H 7.8 (with liver microsomes the phosphate contained 0.5 mM Mg^{++}) and was brought to 0.5% deoxycholate concentration. The suspension cleared markedly on this addition. The *p*H of the solution was between 7.2 and 7.4. The ribosomes were collected by centrifugation for 60 minutes at 100,000 × *g* and were washed in 10 mM phosphate, *p*H 7.4.

(*b*) *Analytical Methods*

Protein was determined by a modification of the method of Nayyar and Glick (1954) with bovine plasma albumin as a standard, and total N was estimated by the micro-Kjeldahl method (Hiller *et al.*, 1948). RNA was determined with orcinol reagent (Schneider, 1957) with yeast RNA as standard. Phospholipid was extracted by the procedure of Schneider (1957) and total P determined by the method of Gomori (1942) after digestion with H_2SO_4 and H_2O_2. A factor of 25 was used to convert phospholipid P to phospholipid.

Ca and Mg were titrated with EDTA, with Eriochrome black T used as indicator, as described by Robinson and Rathbun (1959).

RNase was assayed at *p*H 5 by the method of Dickman *et al.* (1956, 1962b) and chymotrypsinogen by the method of Schwert and Takenaka (1955) after activation with trypsin (Hirs, 1955).

Sedimentation patterns were produced with a Spinco Model E ultracentrifuge at 20° and 29,500 rpm, photographs being taken every 2 minutes. The movement of the peaks was observed with schlieren optics and their magnitude determined with a microcomparator. The patterns shown were obtained from photographs taken from 6 to 8 minutes after maximum speed was reached. Sedimentation coefficients have been extrapolated to that at infinite dilution, since the sedimentation rates were significantly affected by concentration.

Free electrophoresis (Spinco Model H) was carried out at 1.5° in 50 mM KCl–1 mM phosphate, *p*H 7.2. The conductivity of the ribosomal suspension was used in the calculation of mobilities.

Results

(*a*) *Composition of Ribosomes*

The compositions of beef pancreas and rat liver ribosomes are summarized in Table I. The pancreas particles contained more protein and nitrogen than those from liver. The relatively large amounts of RNase and chymotrypsinogen A associated with the pancreas ribosomes were not removed by five washings with 1 mM phosphate, *p*H 7.4. The adsorption of basic proteins in the course of isolation is probably responsible for the higher protein content of the beef particles.

Approximately 4% of the dry weight of the pancreas ribosomes was soluble in hexane. This suggests that most or all of the lipid present was phospholipid. The phosphorus content of the particles could be completely accounted for by RNA and phospholipid. The magnesium-calcium ratio on a molar basis was about 25. This ratio is considerably higher than that of 7 for rat liver (Thiers and Vallee, 1957) or 5.5 for pea seedling ribosomes (Ts'o *et al.*, 1958). However, two out of three pancreas preparations contained no detectable calcium. The molar ratio of magnesium to phosphorus was 0.15, which is quite similar to that found in guinea pig pancreas ribosomes by Siekevitz and Palade (1960).

Table I

Composition of Beef Pancreas and Rat Liver Ribosomes

Beef pancreas ribosomes were washed once with 1 mM phosphate, *p*H 7.3. Liver ribosomes were washed three times and dialyzed overnight against 1 mM phosphate–0.5 mM Mg^{++}, *p*H 7.3. Analytical procedures are given in the text. Percentages are calculated on a dry weight basis.

Component	Beef Pancreas		Rat Liver	
	No. of Analyses	%	No. of Analyses	%
RNA	18	38.5 ± 5.1[a]	2	45.0
Protein	18	56.4 ± 5.1	2	47.5
Phospholipid	5	5.5 ± 1.1		5 to 10[b]
RNase	21	1.16 ± 0.46	2	0.001
Chymotrypsinogen	2	1.4 to 2.9		—
N	3	19.1 ± 1.9	2	15.2
P	4	4.15 ± 0.64		—
Mg	3	0.48 ± 0.11		—
Ca	3	0.019 ± 0.032		—

[a] Mean ± standard deviation. [b] Hamilton and Petermann (1959).

About one third of the protein of pancreas ribosomes was soluble in 95% ethanol if the particles had been previously precipitated with trichloracetic acid (Table II). Extraction with 100% ethanol or ethanol-ether (3:1) at 37° rather than 4° did not affect the amount of protein soluble in the organic solvent. A second and a third extraction with ethanol solubilized very little additional bromsulphalein-reacting material. No protein was extracted with ethanol, however, if the trichloroacetic acid precipitation was omitted. These results are similar to those of Michael (1962) on serum albumins.

(*b*) *Sedimentation Patterns*

(1) *Dialysis of Ribosomes.*—The sedimentation pattern of a freshly prepared suspension of ribosomes is

Table II

Solubility of Beef Pancreas Ribosomal Protein in Organic Solvents

Washed beef ribosomes in 1 mM phosphate, *p*H 7.2, were precipitated with trichloroacetic acid at 4°. Both treated and control samples were extracted once with the indicated solution, then centrifuged. The supernatant solutions were evaporated and the protein contents of the residues from precipitates and supernatants were determined with bromsulphalein (Nayyar and Glick, 1954).

Treatment of Ribosomes	Extraction Conditions		Protein Solubilized (% of total)
	Solvent	Temp.	
Control	50% EtOH	4°	0
	75% EtOH	4°	0
	95% EtOH	4°	0
Precipitated with 5% trichloroacetic acid	95% EtOH	4°	31
	95% EtOH	37°	34
	EtOH-Et_2O (3:1)	4°	29
	EtOH-Et_2O (3:1)	37°	31

shown in Figure 1A. The bulk of the particles (88%) have a sedimentation coefficient of 80 S with small amounts sedimenting faster or slower. Other preparations were even more homogeneous. In Figure 4A, for example, 80 S particles constituted 96% of the sedimenting material. Overnight dialysis of three-times-washed particles against 1 mM phosphate, pH 7.4, resulted in considerable aggregation (Fig. 1B). The 80 S material now constituted only 20% of the total, the remainder being divided between 117 S and 145 S particles. These results contrast strikingly with those obtained with ribosomes from other sources. Both Hamilton and Petermann (1959) and Tissieres and Watson (1958) observed considerable dissociation of rat liver and *E. coli* ribosomes upon dialysis against Mg^{++}-free solutions.

In Figure 2 the effects of dialysis against a Mg^{++}-containing solution are compared with dialysis against a Mg^{++}-free solution. Beef pancreas ribosomes were markedly aggregated by 0.1 mM Mg^{++} (Fig. 2B), whereas the sedimentation coefficients of rat liver particles were unaffected by 0.5 mM Mg^{++} (Fig. 2D).

(2) *EDTA.*—The influence of EDTA on the ultracentrifugal patterns of beef pancreas and rat liver ribosomes is shown in Figure 3. Components of beef pancreas particles aggregated on treatment with 2 mM EDTA (Fig. 3A), in contrast to the dissociation found with those from liver (Fig. 3B). Analysis showed that 70% of the pancreas RNA had become nonsedimentable upon centrifugation for 60 minutes at 105,000 $\times g$. These data indicate that the aggregated material consisted primarily of protein. At 26° no concentration of EDTA was found that would produce nucleoprotein subunits from 80 S particles of beef pancreas. The removal of Mg^{++} from the nucleoprotein at 26° possibly permits some hydrolysis of RNA by the adsorbed RNase. The ribosomal structural proteins, their positive charges no longer neutralized by the negatively charged RNA, rapidly associate and aggregate. The partially degraded RNA remains in the supernatant solution. Some 50 S particles were formed at 4° in 2 mM EDTA (Fig. 3C). Ts'o (1958) has reported a similar effect of temperature on pea seedling ribosomes exposed to EDTA.

(3) *Deoxycholate.*—Subunits of 50 S and 30 S were produced by the addition of deoxycholate to isolated beef pancreas ribosomes (Fig. 4). An increased deoxycholate concentration did not increase the degree of dissociation. It is of interest that a deoxycholate-EDTA mixture (Fig. 4D), while not changing the extent to which the 80 S particles were dissociated, increased the relative amount of the 30 S component. This result suggests that Mg^{++} and phospholipid are both involved in the maintenance of the structure of 50 S ribosomes, since 30 S particles were not formed by 2 mM EDTA in the absence of deoxycholate.

(4) *Ionic Strength.*—The presence of 0.1 M KCl resulted in the production of a 120 S component from pancreatic 80 S particles as shown in Figure 5. The effect of the added salt was very rapid, since an identical pattern was obtained when the salt had been added 2 hours prior to centrifugation. Treatment of beef pancreas ribosomes with 0.1 M KCl at 4° for 3 hours released more than half of the RNase. This result suggests that basic proteins are bound by electrostatic forces to the negatively charged ribosomes. An ionic strength of 0.1 did not cause aggregation of rat liver ribosomes.

(c) *Precipitation of Ribosomes*

(1) *Alkaline Earths.*—The precipitation of ribo-

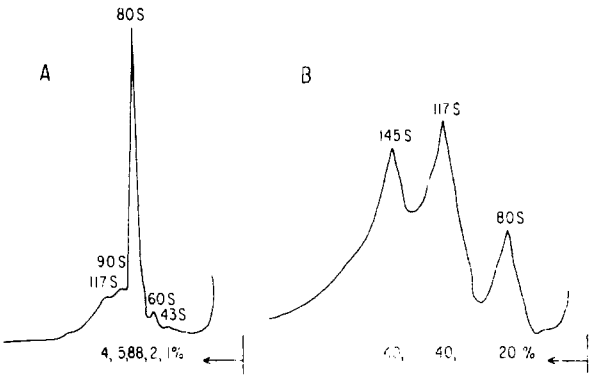

FIG. 1.—Effect of dialysis on sedimentation pattern of beef pancreas ribosomes. Ribosomes, washed three times in 1 mM phosphate, pH 7.4, were (A) resuspended in 1 mM phosphate or (B) dialyzed overnight at 4° against 1 mM phosphate, then centrifuged and resuspended in this solution. The numbers above the peaks are the calculated sedimentation coefficients in Svedberg units (S). Numbers below the diagrams indicate the approximate percentage of the total area under each peak.

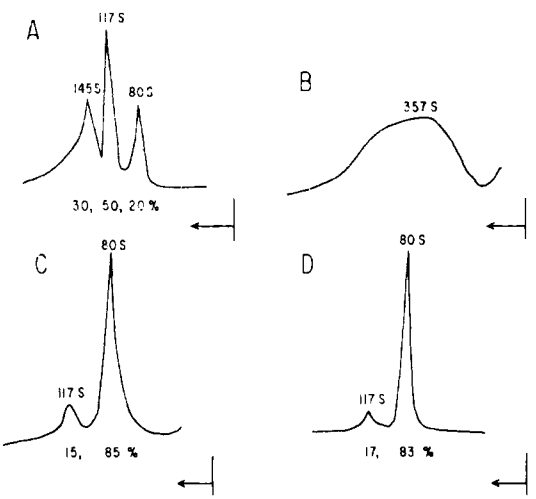

FIG. 2.—Influence of Mg^{++} on sedimentation patterns of beef pancreas and rat liver ribosomes. Beef pancreas ribosomes were resuspended in (A) 1 mM phosphate or (B) 1 mM phosphate–0.1 mM Mg^{++}. Rat liver ribosomes were resuspended in (C) 1 mM phosphate or (D) 1 mM phosphate–0.5 mM Mg^{++}. Beef pancreas ribosomes had been washed three times and then dialyzed overnight at 4° against the indicated solution. All suspensions were between pH 7.2 and 7.4.

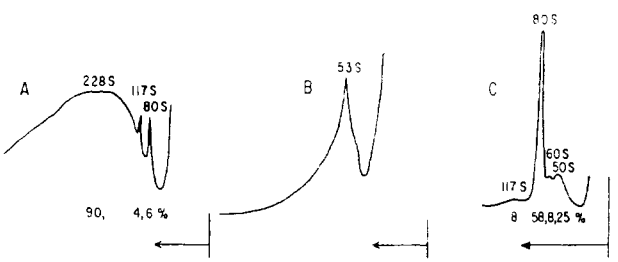

FIG. 3.—Influence of EDTA on sedimentation patterns of beef pancreas and rat liver ribosomes. (A) Pancreatic ribosomes suspended in 1 mM phosphate, 2 mM EDTA, 20°. (B) Hepatic ribosomes suspended in 1 mM phosphate, 5 mM EDTA, 20°. (C) Pancreatic ribosomes in 1 mM phosphate, 2 mM EDTA, 4°. All suspensions were at pH 7.2. EDTA was added immediately before centrifugation. Sedimentation coefficients in (C) are corrected for increased viscosity of water at 4°.

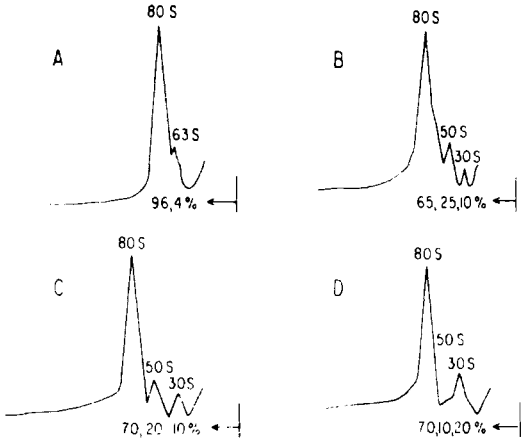

FIG. 4.—Sedimentation patterns of beef pancreas ribosomes in deoxycholate. All suspensions were in 1 mM phosphate, *p*H 7.2 – 7.4, which contained the following concentrations of deoxycholate: (*A*) none; (*B*) 13 mM; (*C*) 36 mM; (*D*) 26 mM deoxycholate-0.5 mM EDTA.

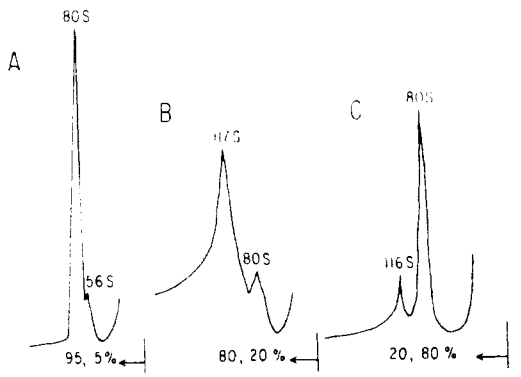

FIG. 5.—Effect of ionic strength on sedimentation patterns of ribosomes. Beef pancreas particles resuspended in (*A*) 1 mM phosphate, (*B*) 1 mM phosphate–0.1 M KCl. Rat liver particles were resuspended in (*C*) phosphate–0.1 M KCl. The KCl was added to the suspensions immediately before centrifugation.

somes by $MgCl_2$ is shown in Figure 6. Millimolar Mg^{++} or Ca^{++} almost completely precipitated the beef pancreas particles, while 0.1 mM concentrations did not. The results of a similar experiment with rat liver ribosomes are also included in Figure 6. The liver particles required about a 10-fold larger concentration of Mg^{++} to precipitate them than did those from pancreas. Takanami (1960) has reported that rat liver ribosomes purified by prior precipitation with $MgCl_2$ required about 30 mM Mg^{++} or 10 mM Ca^{++} for complete precipitation.

Addition of crystalline pancreatic ribonuclease, egg white lysozyme, or a mixture of ribonuclease, chymotrypsinogen A, and trypsinogen (not included in Figure 6) to rat liver ribosomes at 4° reduced the magnesium concentration required to precipitate the ribosomes to about one fourth that required to precipitate untreated particles. RNase assays of the precipitated ribosomes showed that 87% of the added enzyme had become adsorbed. Since there was no decrease in the amount of the A_{260} absorbing material which was precipitated, RNase did not cause significant hydrolysis of ribosomal RNA at this temperature. It would seem that RNase was effective under these conditions as a basic protein rather than because of its hydrolytic activity.

The differences in capability among these basic proteins is especially intriguing. Egg white lysozyme

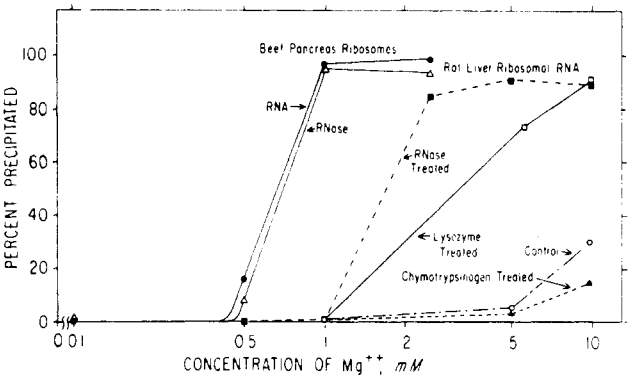

FIG. 6.—Precipitation of ribosomes by Mg^{++}. Washed beef pancreas ribosomes were resuspended in 1 mM phosphate, *p*H 7.2, and brought to the indicated Mg^{++} concentration. The solutions were allowed to stand for 1 hour at 4° and were then centrifuged for 30 minutes at 600 × *g*. ●———●, RNA; △– – –△, RNase. Washed rat liver ribosomes were resuspended in 1 mM phosphate, *p*H 7.2, and 10 μg/ml of the following proteins was added: ■– – –■, RNase; □———□, lysozyme; ▲– – –▲, chymotrypsinogen A; ○———○, none. After 1 hour at 4° the solutions were centrifuged for 1 hour at 85,000 × *g* and the ribosomes were resuspended in 1 mM phosphate. Mg^{++} was added as indicated and the suspensions treated as described above. RNA was determined in the pellets and supernatant solutions.

appeared as active as RNase, while chymotrypsinogen slightly decreased precipitability of the ribosomes. In this connection the results of Brachet (1956) on the effects of addition of basic proteins to onion root tips are of interest. He found that RNase, histone, and salmine all strongly inhibited phenylalanine incorporation into proteins. Cytochrome *c*, however, although it penetrated into the cells and deeply stained the roots, hardly inhibited incorporation. Becker and Green (1960) likewise inhibited protein synthesis in ascites tumor cells with protamine and histone.

In work with cell-free systems De Kloet *et al.* (1962) reported that RNase, inactivated RNase, and protamine all inhibited amino acid incorporation into protein by yeast protoplasts. The fact that active RNase inhibited at much lower concentrations than did the inactivated enzyme suggested that enzyme action promotes inhibition. On the other hand, this inhibition by RNase had previously been found by the same authors (De Kloet *et al.*, 1961) to be reversed by polymethacrylic acid.

(2) *Spermine.*—Beef pancreas ribosomes also were precipitated with spermine, as shown in Figure 7. In contrast to results obtained with $MgCl_2$, precipitation with the organic base resulted in the solubilization of more than 80% of the RNase. Spermine also has been reported to release enzymes from guinea pig pancreas ribosomes (Siekevitz, 1961; Siekevitz and Palade, 1962).

(*d*) *Electrophoresis*

The aggregation of beef pancreas ribosomes by 0.1 M KCl and their precipitation by lower concentrations of Mg^{++} than was required by the liver particles suggested that the pancreas ribosomes had a lower net negative charge than those from liver. The descending electrophoretic patterns of the two suspensions are shown in Figure 8. Beef pancreas ribosomes demonstrated a major electrophoretic component with a mobility of -7×10^{-5} cm²/volt/second, while the major component of rat liver ribosomes had an elec-

trophoretic mobility of about -11×10^{-5} cm²/volt/second. This latter value is very similar to that reported by Tashiro and Inouye (1959) and Takanami (1960). The large peak at the origin of the rat liver pattern was probably glycogen, since these animals had been starved for only 8 hours before being sacrificed.

Since there was no magnesium in the medium it is possible that some dissociation of the liver ribosomes occurred during electrophoresis. It is unlikely, however, that the greater electrophoretic mobility of the rat liver ribosomes was due to a magnesium content lower than that of the beef pancreas particles. The latter were prepared in, and washed with, a magnesium-free medium, while the rat liver particles were prepared in, and dialyzed against, a medium containing 0.5 mM $MgCl_2$. They were placed in a magnesium-free medium just before electrophoresis.

Discussion

Although there is a superficial similarity in the properties of the 80 *S* ribosomes prepared from beef pancreas homogenates to those from other tissues and species, the major conclusion to be drawn from the data is that they differ significantly from other ribosomes in a number of characteristics. (1) It has been observed repeatedly that dialysis of ribosomes against Mg-free solutions results in dissociation to smaller particles (Chao and Schachman, 1956; Gillchriest and Bock, 1958; Petermann *et al.*, 1958; Tissieres and Watson, 1958; Hamilton and Petermann, 1959). Beef pancreas ribosomes aggregated under these conditions. (2) The role of Mg^{++} in stabilization of 70 *S* to 80 *S* particles is also well documented (Roberts, 1958; Takanami, 1960; Ts'o and Vinograd, 1961), yet beef pancreas ribosomes were aggregated by as little as 10^{-4} M Mg^{++}.

These effects, in conjunction with the decreased electrophoretic mobility and higher protein content of pancreas ribosomes, suggest that basic proteins have become adsorbed on the particles in the course of isolation. Beef pancreas is known to contain relatively large amounts of the basic proteins RNase, chymotrypsinogen A, and trypsinogen, and these proteins have been shown to be associated with washed ribosomes. Furthermore, the addition of these proteins to liver ribosomes resulted in a shift of their properties to those more closely resembling beef pancreas ribosomes. The adsorption of proteins on particles of liver and other tissues has been demonstrated repeatedly (Beinert, 1951; Schneider and Hogeboom, 1952; Paigen, 1956; Hamilton and Petermann, 1959; Petermann and Pavlovec, 1961). In an important paper Keller and Cohen (1961) showed that C^{14}-labeled chymotrypsinogen A was readily bound to beef pancreas ribosomes in the presence of deoxycholate. The present work confirms and extends these findings. The magnitude of this adsorption is so great that it leads to the hypothesis that adsorption of basic proteins, especially RNase, is the major factor responsible for the instability of beef pancreas ribosomes as ordinarily isolated.

The ribosomes as prepared in this work contained approximately 1% of RNase and larger amounts of trypsinogen and chymotrypsinogen A. Can the anomalous reactions of the particles be explained by the presence of these enzymes, especially RNase? As demonstrated in the following paper (Madison and Dickman, 1963), the RNase associated with the particles remains essentially inactive at ice-bath temperatures. Thus stability differences at 4° probably are due to basic proteins competing with Mg^{++} for intraparticle ligand functions. At 26° or 37°, however, the

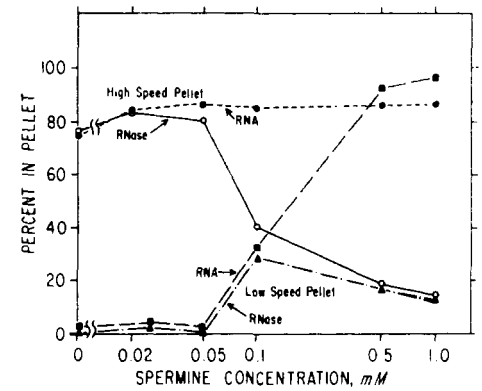

FIG. 7.—Effect of spermine on sedimentation of beef pancreas ribosomal components. Ribosomes, suspended in 1 mM phosphate, *p*H 7.3, were treated with various concentrations of spermine for 1 hour at 4°, then centrifuged. ▲———▲, RNase activity; ■———■, RNA measured by A_{260} after centrifugation for 30 minutes at 600 × *g*; O———O, RNase activity; ●———●, RNA measured by A_{260} after centrifugation for 90 minutes at 85,000 × *g*.

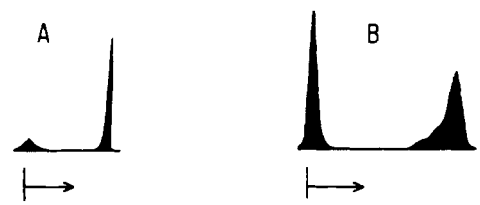

FIG. 8.—Electrophoresis of ribosomes. (*A*) Descending pattern from beef pancreas in 0.05 M KCl–1 mM phosphate, *p*H 7.1, photographed after 110 minutes at 16 milliamp. (*B*) Rat liver ribosomes had been washed twice and dialyzed overnight against 1 mM phosphate–0.5 mM Mg^{++}. They were centrifuged and resuspended in 0.05 M KCl–1 mM phosphate, *p*H 7.1. The descending pattern was photographed after 120 minutes at 16 milliamp. The large peak at the origin is probably glycogen.

change in composition of the aggregates and the appearance of acid-soluble nucleotides in the supernatant strongly suggest that RNase has hydrolyzed sufficient RNA to allow the protein moieties to separate from it and to coalesce.

Acknowledgments

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Beef Pancreas Ribosomes: Stability*

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The stability of beef pancreas ribosomes was studied under various conditions. Incubation of the particles at 26° in 1 mM phosphate, *p*H 7.4, resulted in complete loss of 80 *S* material in 30 minutes. This was accompanied by a 7% hyperchromic effect and a decrease in A_{260}/A_{235} ratio from 1.55 to 1.25. Longer incubation periods at 26° or at 37° resulted in hyperchromic effects in the range 25–40% and an increased A_{260}/A_{235} ratio. Analysis of aggregated material demonstrated that it was mostly protein. Ribosomal RNA had become partially hydrolyzed during incubation and was solubilized. Stability was greatest at an ionic strength of 0.0003 to 0.05 and between *p*H 6.5 and *p*H 7.8. The addition of 2 M urea at 4° or 1 M urea at 26° increased the rate of development of hyperchromicity. The presence of 0.1 mM ethylenediaminetetraacetate markedly decreased stability. Both these effects could be at least partially counteracted by Mg^{++}. Divalent ions by themselves decreased the hyperchromic effect but caused precipitation at low concentrations. The most effective stabilizing agent studied was antiribonuclease serum. Ribonuclease inhibitors of the type of sulfated polysaccharides were also effective in solutions of low ionic strength. The results demonstrate that beef pancreas particles are so unstable primarily because of their content of ribonuclease.

Many of the properties of ribosomes from a wide variety of sources are now well established, and the factors which determine *in vitro* stability are presently under investigation (Roberts, 1958). The stability and functional activity of such multicomponent particles can be affected by reversible association-dissociation type reactions or by more extensive, essentially irreversible degradative processes. In a previous investigation beef pancreas microsomal fraction was treated with detergents and/or chelating agents and their effects on the sedimentation coefficients, hyperchromicity, and composition of the insoluble material were studied (Dickman *et al.*, 1962). In this publication the effects of a variety of treatments on the stability and properties of the isolated ribosomal particles are reported.

The stability of ribosomes can be measured in a number of ways. Sedimentation patterns furnish visual evidence of association-dissociation phenomena, and aggregation to form larger and larger conglomerates

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