Canine Pancreatic Ribosomes

I. PREPARATION AND SOME PROPERTIES*

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SUMMARY

Purified microsomal and postmicrosomal ribosomes have been prepared from dog pancreas by a method involving magnesium precipitation and treatment with sodium deoxycholate. The purified ribosomes were shown to be substantially free from contamination with supernatant protein in an experiment employing a radioactive supernatant fraction. The purified particles appear to contain 60% RNA when pancreatic ribosomal protein is used as the protein standard in the Lowry procedure. Different values are obtained, however, when either bovine serum albumin or bovine pancreatic chymotrypsinogen is used as standard.

The monoribosome behaves ultracentrifugally as an 80 S species in the presence of 0.1 M KCl and as a 74 S species in its absence. Lyophilization causes partial dissociation of the monoribosome to ribosomal subunits, complete dissociation being effected by ethylenediaminetetraacetate at a concentration of 2 μmoles per mg of ribosome. The large and small subunits sediment, respectively, as 50 S and 33 S species in the presence of 0.1 M KCl and as 34 S and 29 S species in the absence of KCl. The individual subunits have been isolated by density gradient centrifugation, and their chemical composition has been studied.

The pancreas offers certain special advantages for studies on protein synthesis. The acinar cells of this gland are richly endowed with protein-synthesizing organelles, and in many species the protein products of synthesis and secretion have been analyzed in detail (1). The activities of the acinar cells are under nervous as well as hormonal control and can be affected by a variety of drugs.

A major disadvantage of this tissue is the nearly ubiquitous presence of RNase among the secretion products of the gland, hindering both structural and functional studies of pancreatic ribosomes. However, young pigeons (2), dogs (3–5), and rabbits (5) contain little or no pancreatic ribonuclease, and several investigators have succeeded in isolating pancreatic polysomal systems capable of incorporating amino acids into protein. They have also studied some of the functional properties of these systems (2, 4, 5). To date, little work has been done on the structural aspects of purified pancreatic ribosomes.

In the present work we will describe the purification of canine pancreatic ribosomes and some of the chemical and physicochemical properties of the purified particles. In an accompanying paper (6), we will describe the protein moieties of these ribosomes in greater detail.

EXPERIMENTAL PROCEDURE

Materials

Frozen pancreatic glands from male dogs were purchased from Pel Freez Biologicals, Inc., Rogers, Arkansas. Bovine chymotrypsinogen A (EC 3.4.4.5) and STP were purchased from Worthington. Crystalline bovine serum albumin was obtained from Sigma, and RNA was a gift from Dr. M. P. Gordon. Magnesium was used in the form of magnesium acetate. Penicillin dihydrochloride, purchased from Nutritional Biochemicals, was used as a 0.075 M solution adjusted to pH 7.5 with N KOH. EDTA was used as a 0.4 M solution, pH 7.5. Uniformly labeled L-phenylalanine-14C, 366 mC per mmole, was purchased from New England Nuclear. All solutions were prepared with deionized water. All other chemicals were of reagent grade.

Preparation of Ribosomes

The method of preparation of dog and rabbit pancreatic ribosomes is based on previous experiments with bovine ribosomes (7, 8) and is a modification of procedures applied earlier by Takanami (9) and Tashiro and Siekevitz (10). To simplify

* The abbreviations used are: STP, soybean trypsin inhibitor; DOC, sodium deoxycholate; ATE, N-acetyl-L-tyrosine ethyl ester.
Preparation of Crude Cell Fractions—Frozen dog pancreas (51 g), trimmed of gross fat and connective tissue, was divided into 3-g portions. Each was finely chopped and immediately placed in 30 ml of ice-cold 0.88 m sucrose containing 0.02% (w/v) STI. All subsequent procedures were carried out in the cold (at about 2°C).

The tissue was homogenized in a homogenizer of the Potter-Elvehjem type with a Pyrex glass tube and Teflon pestle. The homogenates were filtered through gauze and centrifuged at 105,000 × g for 45 min to remove the heavier particles. Crude microsomes were then collected by centrifugation at 78,000 × g for 105 min, and crude postmicrosomal ribosomes were sedimented from the microsomal supernatant by centrifugation at 78,000 × g for 18 hrs. The supernatant remaining was designated as the postmicrosomal supernatant.

Microsomal Ribosomes—The crude microsomal pellets, resuspended by gentle homogenization in 0.88 m sucrose containing 0.02% STI (225 ml), were centrifuged at 105,000 × g for 60 min and were designated as washed microsomes. The washed microsomes were resuspended in 4 ml of 1.0 mM potassium phosphate buffer at pH 7.5, and the ribonucleoprotein particles were precipitated by the addition of 2 mM magnesium acetate. After standing for 10 min on ice, the white flocculent precipitate was collected by centrifugation at 7,000 × g for 10 min, washed twice by resuspension in 40 ml of 1 mM phosphate buffer, pH 7.5, containing 10 mM magnesium, and resedimented. The washed pellet was resuspended in 12 ml of 1 mM phosphate buffer, pH 7.5, containing 0.05 mM magnesium, dialyzed for 24 hrs against three changes of 2 liters of the same buffer, and clarified by centrifugation at 151,000 × g for 50 min. The DOC-treated microsomal pellets were resuspended in 36 ml of 1 mM potassium phosphate buffer at pH 7.5, and the ribosome pellets were precipitated by the addition of 2 mM magnesium acetate. After standing for 10 min on ice, the white flocculent precipitate was collected by centrifugation at 7,000 × g for 10 min, washed twice by resuspension in 40 ml of 1 mM phosphate buffer, pH 7.5, containing 10 mM magnesium, and resedimented. The washed microsomes were resuspended in 36 ml of 1 mM phosphate buffer, pH 7.5, containing 2 mM magnesium added immediately before use, and sedimented by centrifugation at 151,000 × g for 30 min. The microsomal pellets were resuspended in 4 ml of 1 mM phosphate buffer, pH 7.5, containing 2 mM magnesium, dialyzed for 60 min against three changes of 2 liters of the same buffer, and clarified by centrifugation at 151,000 × g for 50 min. The final preparation was a suspension of purified microsomal ribosomes. Approximately 0.8 mg of microsomal ribosomes was obtained from 1 g of wet tissue.

Postmicrosomal Ribosomes—The crude postmicrosomal ribosomes were resuspended by gentle homogenization in 36 ml of 1 mM phosphate buffer, pH 7.5, and precipitated by the addition of 4 ml of 1.0 mM magnesium acetate. The magnesium precipitate was sedimented and washed as described in the microsome purification procedure. The washed pellets were resuspended in 60 ml of 0.01 M DOC in 1 mM phosphate buffer, pH 7.5, containing 2 mM magnesium added immediately before use, and sedimented by centrifugation at 151,000 × g for 50 min. The ribonucleoprotein pellets were then resuspended in 12 ml of 1 mM phosphate buffer, pH 7.5, containing 0.5 mM magnesium, dialyzed against three changes of 2 liters of the same buffer for 24 hours, and clarified by centrifugation at 32,000 × g for 10 min. The final preparation was a suspension of purified postmicrosomal ribosomes. A yield of about 2 mg of postmicrosomal ribosomes per g of wet tissue was usually obtained.

Purified ribosomal suspensions were either stored for not more than 2 or 3 days on ice or lyophilized and stored at -20°C.

The effectiveness of the purification procedure in the removal of adsorbed supernatant protein for the ribosomes was shown by two experiments employing radioisotopes which are presented under “Results.”

Preparation of Subunits—Lyophilized ribosomes, resuspended in a volume of water equal to the original suspension, were dissociated by subunits by the addition of EDTA to a final concentration of 2.0 mmoles of EDTA per mg of ribosomes. The subunits were immediately either studied by analytical ultracentrifugation or separated by density gradient centrifugation as described below. The subunits could not be pelleted from the sucrose solution by centrifugation at 105,000 × g for 20 hrs, a similar problem having been encountered by Pestka and Nirenberg (11) with ribosomes from Escherichia coli. Accordingly, the fractions corresponding to the subunit peaks were pooled and dialyzed for 48 hrs against four changes of 2 liters of 1 mM phosphate buffer, pH 7.5, containing 0.1 mM magnesium for the small subunit and 1 mM magnesium for the large subunit (12). The dialyzed suspensions, shown to be sucrose-free, were lyophilized and stored at -20°C.

Centrifugal Methods

Analytical Ultracentrifugation—Sedimentation analyses were performed in the cold (5-7°C) with a Spinco model E analytical ultracentrifuge with the use of the schlieren optical system. Photographic plates were measured on a Nikon Profile Projector. The observed sedimentation coefficients were corrected to Σφ,w on the assumption that the partial specific volume was 0.66
A plot of $s_{1/2}$ against concentration by the method of least squares was used to calculate $s_{1/2}$ and the concentration dependence. The concentration of each component in the samples analyzed was determined by enlarging the photographic plates about 10-fold, tracing the peaks, and weighing the areas of paper corresponding to each peak. The method assumes that the refractive index of each subunit is the same. No correction for the Johnston-Ogston (14) effect was made.

Preparative Ultracentrifugation—This was performed in a Spinco model L-2 ultracentrifuge at 2°C, and the centrifugal forces were measured by a modification of the orcinol procedure (17) with occasional 60 fractions by the method of Oumi and Osawa (16). Gradients which were centrifuged at 25,000 rpm (SW 25.2 rotor) for 11 hours. Gradients were fractionated into about 40 or 50 fractions by the method of Oumi and Osawa (16), and the optical density at 200 nm was measured on either diluted fractions or diluted aliquots of fractions.

Analytical Methods

Chemical Determinations—Chemical determinations were performed on aliquots of whole ribosomes or subunits. RNA was measured by a modification of the orcinol procedure (17) with purified RNA as standard.

Protein was determined by the procedure of Lowry et al. (18). In order to assess the influence of the protein standard employed on the apparent protein content of ribosomes, standard curves were constructed with (a) bovine serum albumin in 0.9% sodium chloride solution, standardized by Kjeldahl nitrogen determination; (b) the basic pancreatic protein, bovine chymotrypsinogen A, standardized by means of its extinction coefficient (19); and (c) ribosomal protein extracted from purified canine pancreatic ribosomes with 12.8 N acetic acid (6) and quantified by amino acid analysis. Spectral constants of the purified ribosomes were determined in 1 mm potassium phosphate buffer, pH 7.5, containing 0.5 mm Mg++. Isotope Analysis—Samples of 1 ml each of the ribosome suspensions or appropriate supernatant fractions were placed in vials and dissolved in 1 ml of Hyamine 10X (Packard) by either standing at room temperature overnight or if necessary by heating at 60°C for up to 90 min. Then 10 ml of Bray’s solution (20) were added, and the solutions were incubated at 37°C for 2 hours and cooled. The samples were counted in a Packard Tri-Carb liquid scintillation counter. The channels ratio method (21) was used to determine counting efficiency in all samples.

The authors wish to express their thanks to Dr. Darrell H. Spackman of the University of Washington School of Medicine for the amino acid composition of the protein from canine pancreatic ribosomes.
Experiment A describes the purification of ribosomes from the pancreas of a dog which had received an intravenous injection with \( ^{14}C \)-labeled phenylalanine. Experiment B describes the purification of ribosomes from an unlabeled dog pancreas which was homogenized in the postmicrosomal supernatant from Experiment A. The designation of the fractions is shown in Fig. 1 and the purification is described under "Experimental Procedure."

### Purification of canine pancreatic ribosomes

#### Table I

| Fraction                  | Experiment A | | | Experiment B | | |
|---------------------------|--------------|----------|----------|--------------|----------|
|                           | Total radioactivity | Total protein | Specific radioactivity | SPC | Total radioactivity | Total protein | Specific radioactivity | SPC |
| Postmicrosomal supernatant| 37,033        | 280.8    | 1219    | 132          | 338         |
| Postmicrosomal             |              |          |         |              |            |
| Crude ribosomes            | 443,232      | 532.8    | 832     | 68           |            |
| Mg\(^{++}\) supernatant    | 373,402      | 388.0    | 1,297   |              |            |
| First wash                 | 20,328       | 15.6     | 1,303   |              |            |
| Second wash                | 4,496        | 4.0      | 1,124   |              |            |
| DOC supernatant            | 37,932       | 68.4     | 555     |              |            |
| Purified ribosomes         | 10,387       | 53.4     | 195     |              |            |
| Microsomal                 |              |          |         |              |            |
| Crude ribosomes            | 13,631       | 40.3     | 338     | 28           |            |
| DOC supernatant            | 89,332       | 98.0     | 912     |              |            |
| Mg\(^{++}\) supernatant    | 5,992        | 14.0     | 428     |              |            |
| First wash                 | 248          | 1.2      | 207     |              |            |
| Second wash                | 216          | 2.0      | 168     |              |            |
| Purified ribosomes         | 1,097        | 14.3     | 139     | 11           |            |

\[
\text{SPC} \times 100 = \frac{\text{specific activity of ribosomal protein}}{\text{specific activity of postmicrosomal supernatant protein}}
\]

### Residual Enzymatic Activity

A suspension containing 1 mg of purified postmicrosomal ribosomes showed no detectable amylase activity in an assay system which would have revealed less than 0.4 mg of this enzyme. Since 1 mg of ribonuclease particles contains 0.4 mg of protein (see below), less than 0.1% of the protein could be accounted for as active amylase. A suspension containing 2 mg of purified ribosomes showed no detectable activity when assayed for chymotrypsin against ATE. Based on the assumption that the specific activity of dog chymotrypsin is similar to that of the bovine enzyme, less than 0.1% of the ribosomal protein was active chymotrypsin.

### Chemical Composition of Ribosomes

Table II presents the gross chemical composition and the extinction coefficient of purified postmicrosomal ribosomes from dog pancreas. A mean value of 60.4 ± 1.7% RNA was found; the mean absorbance of a 1% solution of ribosomes at 260 mg was 123 ± 8.6.

Comparison of the chemical composition of ribosomes from different sources or preparative procedures or from both has been difficult because investigators have employed different methodology, and even when the same analytical procedures have been used, different materials have often been employed as standards. The influence of the protein standard employed is shown in Fig. 2 which presents standard curves for several proteins commonly employed in the Lowry procedure. Ribosomal protein, extracted from purified pancreatic ribosomes with 12.8 N acetic acid (6), has a more limited range of linearity than either bovine serum albumin or the basic pancreatic protein, chymotrypsinogen A. Over the linear range, ribosomal protein yields more color per unit weight than bovine serum albumin but less than bovine chymotrypsinogen A. The presence of puromycin during the purification of the ribosomes in Experiment B caused no significant change in the structural ribosomal protein as judged by zone electrophoretograms.
TABLE II
Chemical and spectral properties of canine postmicrosomal ribosomes

All analyses were performed on fresh preparations of ribosomes unless otherwise indicated.

<table>
<thead>
<tr>
<th>Preparation No.</th>
<th>(RNA × 100)/ (RNA + protein)</th>
<th>Ribonucleoprotein, A660 at 260 nm</th>
</tr>
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<tbody>
<tr>
<td>43</td>
<td>63.8</td>
<td>136.3</td>
</tr>
<tr>
<td>43*</td>
<td>59.6</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>59.7</td>
<td>122.0</td>
</tr>
<tr>
<td>46</td>
<td>59.7</td>
<td>138.1</td>
</tr>
<tr>
<td>46*</td>
<td>59.7</td>
<td>142.3</td>
</tr>
<tr>
<td>47</td>
<td>59.5</td>
<td></td>
</tr>
<tr>
<td>47*</td>
<td>63.5</td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>59.3</td>
<td>124.8</td>
</tr>
<tr>
<td>50</td>
<td>59.4</td>
<td>119.1</td>
</tr>
<tr>
<td>51</td>
<td>61.4</td>
<td>141.0</td>
</tr>
<tr>
<td>52</td>
<td>59.6</td>
<td>132.0</td>
</tr>
</tbody>
</table>

Mean value . . . . . . 60.4 132.0
Standard deviation . . 1.7 8.6
Coefficient of variance . . 2.9% 6.8%

* Preparations of lyophilized ribosomes.

and 64% if chymotrypsinogen A was employed as a standard protein.

The differences in the color yields of chymotrypsinogen and ribosomal protein are not due to the acidic conditions employed for the extraction of ribosomal protein. Chymotrypsinogen, dissolved in 12.8 N acetic acid, gave color values in the Lowry protein test which were identical with those obtained with chymotrypsinogen dissolved in neutral buffer. However, since color development in the Lowry procedure is highly pH dependent, it is necessary to neutralize the acid introduced with sodium hydroxide solution.

**Sedimentation Characteristics of Canine Ribosomes and Their Subunits**

**Fresh Ribosomes**—Fresh preparations of ribosomes in 1 mM potassium phosphate buffer, pH 7.5, containing 0.5 mM magnesium were studied by analytical ultracentrifugation and were shown to consist mainly of undissociated monoribosomes with a small amount of component sedimenting more slowly as well as with traces of a component sedimenting more rapidly (Fig. 3a).

The same result was obtained in the presence or absence of 0.1 M potassium chloride. Addition of EDTA to a concentration of 2.5 μmoles per mg of ribosomes caused complete dissociation to subunits in the presence of 0.1 M potassium chloride. Addition of EDTA to a concentration of 2.5 μmoles per mg of ribosomes caused complete dissociation to subunits in the presence of 0.1 M potassium chloride.

In the absence of potassium chloride, however, dissociation did not go to completion (Fig. 3b). The proportion of the larger to the smaller subunit was 2.0 ± 0.11:1.0 as shown by measurements of the areas. Although no correction was made for the Johnston-Ogston effect (14), other workers have reported similar values with or without this correction factor (10).

**Lyophilized Ribosomes**—Lyophilized ribosomes, previously dialyzed against 1 mM phosphate buffer, pH 7.5, containing 0.5 mM magnesium, and resuspended in a volume of water equal to that of the original suspension, were found to be partially dissociated into subunits (Fig. 3d). EDTA at a concentration as low as 1 μmole per mg of ribosomes caused complete dissociation to subunits in some preparations. Complete dissociation was always achieved at a concentration of 2 μmoles per mg of ribosomes, even in the absence of potassium chloride.

Dissociation of ribosomes caused by lyophilization can be partially reversed by increasing the magnesium concentration of

![Fig. 2. Standard protein curves obtained by means of the Lowry protein procedure with: ●, bovine chymotrypsinogen A; ▲, canine pancreatic ribosomal protein; and ■, bovine serum albumin. The values used to construct the curves were the average of six determinations in the case of chymotrypsinogen, four determinations in the case of ribosomal protein, and duplicate determinations in the case of bovine serum albumin.](http://www.jbc.org/)

![Fig. 3. Sedimentation patterns of postmicrosomal ribosomes.](http://www.jbc.org/)
the solution. Fig. 4 shows the effect of increasing magnesium concentration on the proportions of the whole ribosome and its subunits. At a concentration of 1.5 mM magnesium, the monoribosome reaches a maximum concentration and the small subunit is no longer detectable. Dimers, trimers, and higher aggregates begin to form at this concentration, and in 5 mM magnesium, the preparation is almost completely aggregated. Hence, partial reversal of dissociation by lyophilization can be effected by an increase in magnesium concentration. The results suggest that this partial reversal of dissociation is similar to that effected by magnesium on dissociated mammalian ribosomes formed by chelating agents described by Tashiro and Siekevitz (10) and Petermann and Hamilton (26).

**Sedimentation Coefficients**—The sedimentation behavior of undissociated ribosomes was studied by analytical ultracentrifugation. The value of $s_{20,w}$ for undissociated ribosomes in 1 mM potassium phosphate buffer, pH 7.5, containing 0.5 mM magnesium was $73.8 \pm 0.4$ S (eight runs) in the absence of potassium chloride and $79.7 \pm 0.7$ S (six runs) in the presence of 0.1 M potassium chloride. The ribosomes also exhibited different concentration dependencies in the two solutions, the equation for the regression line being $s_{20,w} = (73.8 \pm 0.4) - (1.83 \pm 0.05) c$ in the absence of KCl and $s_{20,w} = (79.5 \pm 0.7) - (1.17 \pm 0.13) c$ in the presence of 0.1 M KCl, where $c$ is the concentration of ribonucleoprotein in milligrams per ml (see Fig. 5).

![Fig. 4](image-url)

**Fig. 4.** Effect of increasing magnesium ion concentration on reversal of dissociation of postmicrosomal ribosomes by lyophilization. Experiments were performed in 1 mM potassium phosphate buffer, pH 7.5.

![Fig. 5](image-url)

**Fig. 5.** Concentration dependence of the sedimentation coefficient of canine pancreatic ribosomes. The ribosomes were suspended in 1 mM potassium phosphate buffer, pH 7.5, containing 5 mM magnesium in the presence (●—●) and absence (O—O) of 0.1 M KCl. The ribonucleoprotein concentration is the concentration of total ribonucleoprotein which in these experiments is the same as the concentration of species present in the sedimenting region of that species, i.e. all species of $s_{20,w}$ equal to or smaller than the species listed.

### Table III

<table>
<thead>
<tr>
<th>Sedimentation characteristics of subunits of canine pancreatic ribosomes</th>
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<tbody>
<tr>
<td>Method of formation and subunit</td>
</tr>
<tr>
<td>--------------------------------</td>
</tr>
<tr>
<td>Lyophilization</td>
</tr>
<tr>
<td>Small...</td>
</tr>
<tr>
<td>Fresh + EDTA</td>
</tr>
<tr>
<td>Small...</td>
</tr>
<tr>
<td>Fresh in 0.1 M KCl + EDTA</td>
</tr>
<tr>
<td>Small...</td>
</tr>
</tbody>
</table>

The more slowly sedimenting species present in preparations of undissociated ribosomes has a sedimentation coefficient of 58.7 ± 0.7 S and probably represents the unresolved mixture of large and small subunits. The absorption at 260 mp shown in Fig. 6 indicates a higher proportion of protein per unit of RNA in the small subunit than in the large subunit. This observation is the same as that reported by Tashiro and Siekevitz (27) for guinea pig liver ribosomes.

**Nature of Subunits**

**Sucrose Density Gradient Centrifugation**—The large and small subunits, formed by complete dissociation of lyophilized ribosomes with EDTA as described under "Experimental Procedure," were separated on sucrose gradients. The profile of color formation in the Lowry protein determination and in materials absorbing at 260 mp shown in Fig. 6 indicates a higher proportion of protein per unit of RNA in the small subunit than in the large subunit. This observation is the same as that reported by Tashiro and Siekevitz (27) for guinea pig liver ribosomes.

In order to evaluate the purity of the subunit preparation, fractions corresponding to the small subunit, the large subunit, and also the more rapidly sedimenting part of the large subunit were pooled (see Fig. 6). Then 0.5 ml of each preparation was diluted to 2.0 ml with 1 mM phosphate buffer, pH 7.5, and each was resedimented on a preparative sucrose gradient as described under "Experimental Procedure." The ultraviolet profiles of the gradients formed are shown in Fig. 7. The small subunit preparation is contaminated with less than 5% of the large subunit, but the peak fractions of the large subunit preparation contain about 7% contamination by the small subunit. The more rapidly sedimenting portion of the large peak resedimented at the same rate as the peak fractions of this component upon
Fig. 6. Sucrose density gradient centrifugation of lyophilized ribosomes dissociated by the addition of EDTA (2.0 mM per mg of ribosomes). Preparations were centrifuged at 25,000 rpm (SW 25.2) for 12 hours in sucrose gradients as described under "Experimental Procedure." A, fractions pooled for subsequent analyses; B, peak fractions of the larger subunit; C, smaller subunit; —, optical density per ml at 260 nm; - - - , protein concentration (micrograms per ml).

Chemical Composition of Isolated Subunits Lyophilized sucrose-free preparations of the small subunit are readily soluble in water, while similar preparations of the large subunit are insoluble and form a fine suspension. The composition of these subunit preparations was determined by chemical analyses, and the results are presented in Table IV. In calculating the data, it was assumed that the color yield per unit weight of protein of each subunit is the same as that of total ribosomal protein. Within the limits of experimental error of the assay procedures used, the individual subunits appear to have the same chemical composition, but may have a slightly higher proportion of RNA (about 64%) than the undisassociated ribosomes. Thus, the analytical determinations on isolated subunits did not support the impression gained from analyses of successive fractions along the sucrose gradient that the smaller subunit contained a higher proportion of protein. It was reported recently (28) that color development in the Lowry reaction was progressively reduced by increasing the concentration of sucrose. Accordingly, tests were conducted to see if this could account for the present findings. However, the color yield of canine pancreatic ribosomal protein was not affected by the concentrations of sucrose used in the density gradient centrifugations.

DISCUSSION

Structural studies on ribosomes are of limited significance unless the particles can be shown to be free from substantial contamination by nonribosomal proteins. Although the definition of ribosomal protein remains somewhat arbitrary, the protein presently known to be associated with ribosomes includes the nascent polypeptide chains, adsorbed supernatant protein, and various protein factors functional in protein synthesis, as well as the structural protein which is an integral part of the ribosome. Experiments reported here indicate that ribosomes prepared by the present purification procedures are substantially free from relocated supernatant protein and from those enzymes such as amylase and chymotrypsin which are known to be major products of synthesis by the ribosomes.

Experiments employing labeled supernatant fraction demonstrated that the specific activity of the protein removed by magnesium precipitation and two subsequent washes was equal to that of free supernatant protein which, in itself, is a strong indication of the efficiency of the method. Subsequent washing with DOC removed additional radioactive protein with lesser specific radioactivity. The nature of the latter material is not known, but it may correspond to residual supernatant protein diluted with either membranous protein or some of the more loosely bound ribosomal components.

In radioactive Experiment B, where the ribosomes became labeled in vitro by a recombination experiment, a maximum 3% of the ribosomal protein from purified ribosomes could be accounted for as adsorbed supernatant protein. In Experiment A, however, where labeling was accomplished in vivo, the calculated contamination was as high as 16%. The difference may be due to the incorporation of the isotope into structural protein in Experiment A and hence might be used as a measure of turnover of the ribosomal protein in dog pancreas.

The presence of puromycin in Experiment B could also have contributed to the lowering of the specific activity of the protein of purified postmicrosomal ribosomes by liberation of nascent polypeptide chains (29). However, postmicrosomal ribosomes are known to be less active in vivo in protein synthesis than...
Were not already saturated with unlabeled supernatant protein. Lyophilization of the ribosomes caused partial dissociation of postmicrosomal supernatant, thus ensuring that the ribosomes which the values are lowered to 34 S and 29 S. Work, unlabeled tissue was homogenized directly in labeled respectively, in the presence of 0.1 M magnesium, and ribosomes were isolated from the mixture. In the present work, unlabeled tissue was homogenized in labeled postmicrosomal supernatant, thus ensuring that the ribosomes were not already saturated with unlabeled supernatant protein.

The present method produces ribosomes containing 60% RNA and 40% protein, with canine ribosomal protein as the standard in the Lowry procedure. Brilliant and Dickman (33) describe canine pancreatic polysomes containing 45% RNA with bovine serum albumin as a standard. Tashiro and Siekevitz (10) found guinea pig liver ribosomes to contain 56% RNA with hepatic ribosomal protein as a standard, and Petermann and Pavlovec (34) report 50% RNA in Jensen's sarcoma ribosomes with bovine serum albumin as protein standard.

However, small increases in the percentages of ribosomal RNA reported by different workers cannot alone be regarded as an indication of the preparation of purer ribosomes. The present paper had demonstrated the importance of the selection of the standard protein. Although this was shown only for the Lowry method where color yield is markedly enhanced by sequences of amino acids containing functional side chains (35), variations depending on the standard protein could be expected to occur as well when total nitrogen determinations or other assay methods are used. Apparent differences in RNA content might also be observed depending on whether the orcinol reaction, total phosphate, or the extinction coefficient at 260 nm is used to estimate the RNA (36). Moreover, although most investigators report data from the assay of whole ribosomes, some workers extract the RNA from the ribonucleoprotein particles before assay (37), a technique which may not always be quantitative.

The extinction coefficient of a 1% solution of the purified dog pancreatic monoribosomes, 132 ± 8.6, is similar to that recorded for purified ribosomes from guinea pig liver, 135 ± 3, in a similar solvent (10), but is somewhat higher than that of polysomes from dog pancreas, 112, in a solvent of higher ionic strength (33). However, the value of the extinction coefficient is subject to the same variation with methodology as the gross chemical composition discussed above, and furthermore, configurational changes dependent upon the ionic strength of the solvent might be reflected in the absorbance at 260 μm.

The undissociated dog pancreatic monoribosome is typical of mammalian ribosomes in its hydrodynamic behavior, and it sediments as an 80 S species in the presence of 0.1 M KCl. However, in the absence of KCl it sediments as a 74 S species. Differences in sedimentation characteristics in lower and higher ionic strength buffers could result either from electrostatic retardation or from a change in configuration at low ionic strength, the ribosome becoming less compact in the absence of KCl. The present indications are that pancreatic ribosomes prepared by the present method are exhibiting conformational sensitivity to ionic strength rather than electrostatic retardation. Although Petermann and Pavlovec (34) report sedimentation coefficients of 79 to 80 S for Jensen's sarcoma ribosomes at both the higher and lower ionic strengths, hepatic ribosomes described by Tashiro and Siekevitz (10) seem to exhibit a trend similar to the effect described here.

The dissociation of the ribosomes by EDTA is comparable with that described by Tashiro and Siekevitz (10) and by Petermann and Pavlovec (34) all of whom worked with mammalian ribosomes. Fast and slow sedimenting subunits are formed which have sedimentation coefficients of 50 S and 33 S, respectively, in the presence of 0.1 M KCl, and in the absence of which the values are lowered to 34 S and 29 S.

Lyophilization of the ribosomes caused partial dissociation to fast and slow sedimenting subunits with sedimentation coefficients of 51 S and 34 S, the values being similar to those for subunits formed by EDTA treatment in the presence of 0.1 M KCl. The data suggest that the sedimentation behavior of subunits depends more on the method of preparation than on the ionic strength of the solvent in which they are contained. Hence, differences in the sedimentation coefficient are more readily explained by conformational changes or flexibility (38) than by electrostatic retardation. A reduction in the sedimentation coefficient of the large subunit upon the removal of magnesium ions by EDTA and a lowering of ionic strength has already been well characterized (10, 34) as has the occurrence of the small subunit in two discrete forms, 33 S and 29 S (34).

The dissociation of ribosomes can be effected by a variety of treatments. Dissociation by removal of divalent cations by chelating agents such as EDTA is well known. More recently, dissociation by polyvinyl sulfate by a mechanism not involving removal of magnesium ions (39) and dissociation by increased temperatures by a process not involving weakening of the magnesium binding alone have been reported (40, 41). Low temperature freezing has been reported to facilitate dissociation of guinea pig hepatic ribosomes (10), the process frequently being accompanied by turbidity upon thawing. Canine pancreatic ribosomes can be freeze-dried and resuspended after lyophilization without any increase in turbidity but with dissociation, the extent of which varied from preparation to preparation. It thus appears that the dissociation of ribosomes does not involve simply the removal of magnesium ions, but that other physical forces are also involved. This is consistent with the suggestion of Petermann and Pavlovec (41) that dissociation of ribosomes might occur secondarily to conformational changes.

The present communication describes the preparation and chemical composition of the individual ribosomal subunits free from significant contamination by each other. The apparent discrepancy in chemical composition of the smaller species, as indicated by analyses of the isolated subunits compared with analyses of fractions along a sucrose gradient, is difficult to explain. The small subunit may indeed contain a higher proportion of protein than the large subunit, but some of the protein might be lost during isolation. Alternatively, the absorbance of 260 μm might not yield a valid measurement of the RNA concentration.

In the present work the distinction between microsomal and postmicrosomal ribosomes is purely operational, based on differential centrifugation. The relatively larger yield of postmicrosomal relative to microsomal ribosomes may have resulted from the mechanical detachment of these ribosomes from the
endoplasmic reticulum during preparation. Although postmicrosomal ribosomes were employed in most of the experiments reported, parallel observations were made with microsomal ribosomes.

The purification procedures reported here have been successfully applied to the preparation of postmicrosomal ribosomes from rabbit pancreas. Rabbit ribosomes had a comparable chemical composition and behaved similarly to canine particles in their sedimentation characteristics, in dissociation by EDTA, and in lyophilization. The protein moieties of purified dog and rabbit pancreatic ribosomes are described in an accompanying paper (6).

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