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. They contain less than 0.1% of digestive enzymes known to be major products of synthesis by the gland. 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 KC1 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 pmoles 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. Puromyein dihydrochloride, purchased from Nutritional Biochemicals, was used as a 0.075 M solution adjusted to pH 7.5 with NaOH. 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

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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 27,000 x g for 45 min to remove the heavier particles. Crude microsomes were then collected by centrifugation at 78,000 x g for 105 min, and crude postmicrosomal ribosomes were sedimented by centrifugation at 105,000 x g for 60 min. 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 27,000 x g for 30 min to remove heavier particles. The microsomes were sedimented by centrifugation at 105,000 x g for 60 min and were designated as washed microsomes. The washed microsomes were resuspended in 40 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 x 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 ribonucleoprotein particles were precipitated by the addition of 4 ml of 1 mM magnesium acetate. After standing for 10 min on ice, the white flocculent precipitate was collected by centrifugation at 7,000 x 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 hours against three changes of 2 liters of the same buffer, and clarified by centrifugation at 32,000 x g for 10 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.

Postmicrososomal 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. After standing for 10 min on ice, the white flocculent precipitate was collected by centrifugation at 7,000 x g for 10 min, washed twice by resuspension in 40 ml of 1 mM phosphate buffer, pH 7.5, containing 10 mM magnesium for 15 minutes, and resedimented. The washed precipitate was resuspended in 12 ml of 1 mM phosphate buffer, pH 7.5, containing 0.05 mM magnesium, dialyzed for 24 hours against three changes of 2 liters of the same buffer, and clarified by centrifugation at 32,000 x g for 10 min. The final preparation was a suspension of purified microsomal 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 to subunits by the addition of EDTA to a final concentration of 2.0 mM 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 x g for 20 hours, 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 hours 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 S20,w on the assumption that the partial specific volume was 0.66
A plot of $s_{20,w}$ against concentration by the method of least squares was used to calculate $s_{20,w}$ 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 area 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°, and the centrifugal forces and the optical density at 260 nm was measured on either diluted gradients which were centrifuged at 25,000 rpm (SW 25.2 rotor) for 11 hours. Gradients were fractionated into about 40 or suspensions (20 mg in 1 ml of buffer) were layered onto the placed in the mixing chamber, and 26 ml of 5% (w/v) sucrose in the same buffer were placed in the adjacent chamber. Ribosome layered onto the gradients which were centrifuged at 39,000 rpm for 11 hours and cooled. The samples were counted in a Packard Tri- analysis of the University of Washington School of Medicine for Spackman and Martin and Ames (15). For analytical gradients, 2.2 ml of 20% (w/v) sucrose in 1 mm potassium phosphate buffer at pH 7.5 were placed in the mixing chamber, and 2.3 ml of 5% (w/v) sucrose in the same buffer were placed in the adjacent chamber. Ribosome suspensions (1 to 2 mg in 0.1 ml of buffer) were layered onto the gradients which were centrifuged at 39,000 rpm (SW 30 rotor) for 3 hours. For preparative gradients, 24 ml of 20% (w/v) sucrose in 1 mm phosphate buffer at pH 7.5 were placed in the mixing chamber, and 26 ml of 9% (w/v) sucrose in the same buffer were placed in the adjacent chamber. Ribosome suspensions (20 mg in 1 ml of buffer) were layered onto the gradients which were centrifuged at 25,000 rpm (SW 25.2 rotor) for 11 hours. Gradients were fractionated into about 40 or occasionally 60 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° for up to 90 min. Then 10 ml of Bray’s solution (20) were added, and the solutions were incubated at 37° 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 analysis of the amino acid composition of the protein from canine pancreatic ribosomes. Samples were counted for a sufficient length of time to give less than 5% counting error.

Enzyme Assays—Whole ribosomes were tested for residual amylase activity by the method described by Fisher and Stein (22) and for chymotryptic activity by the null point titrimetric method with ATE as substrate (23).

RESULTS

Purity of Ribosomes

Elimination of Relocated Protein—The efficiency of the purification procedures in removing adsorbed supernatant protein from the ribosomes was evaluated by two experiments with the use of radioisotopes. The data are summarized in Table I. A male dog (34 kg), fasted for 24 hours and then fed, received an injection intravenously 20 min later with 100 μC of L-phenylalanine-14C in 5 ml of Hendry’s (24) isosmotic sodium phosphate buffer, pH 7.4. The dog was killed 90 min after injection of the isotope, and the pancreas (57 g) was quickly removed and immediately frozen on Dry Ice. In Experiment A, the labeled pancreas was homogenized and microsomal and postmicrosomal ribosomes were prepared as described under “Experimental Procedure.” The specific radioactivities of protein in the crude and purified ribosomes were determined together with the specific radioactivity of the protein removed at all stages of purification.

Of the isotope contained in the postmicrosomal supernatant of Experiment A, 98% was present in the form of protein precipitable by 5% trichloracetic acid. The specific radioactivity of this supernatant protein (1219 dpm per mg of protein) was used to estimate the contamination of crude and purified ribosomes by supernatant protein on the assumption that all of the radioactivity of the ribosomes was due to adsorbed supernatant protein and that the specific radioactivity of adsorbed supernatant protein was the same as that of free supernatant protein.

Crude postmicrosomal ribosomes, which are harvested directly from the cell supernatant, are more heavily contaminated with supernatant protein than crude microsomal ribosomes, which have been exposed to DOC in the initial step of their isolation (7, 8). In Experiment A, the specific radioactivity of crude postmicrosomal ribosomes was 832 dpm per mg of protein. Thus, relocated supernatant protein could account for as much as 68% of the protein of crude postmicrosomal ribosomes. The specific radioactivity of the protein removed by magnesium precipitation and by the two subsequent washes was equal to that of free supernatant protein, this being a strong indication of the efficiency of the purification procedures. An additional complement of radioactive protein with lower specific radioactivity was removed by the DOC wash. The final preparation of purified postmicrosomal ribosomes had a specific activity of 196 dpm per mg. If this were due exclusively to protein adsorbed from the supernatant, it could represent a 16% contamination.

Crude microsomal ribosomes contain less adsorbed supernatant protein (28%), much of the radioactivity having been removed by the initial exposure to DOC (cf. DOC supernatant in Table I). Magnesium precipitation and the ensuing washes resulted in further purification, but, as in the case of the postmicrosomal ribosomes, the final product was radioactively labeled and could contain up to 11% supernatant protein.

However, Hirsch and Hiatt (25) recently showed that 14C-arginine was incorporated into the structural protein of rat liver.

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Experiment A describes the purification of ribosomes from the pancreas of a dog which had received an intravenous injection with L-phenylalanine-¹⁴C 90 min before being killed. 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."

### Table I

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Experiment A</th>
<th>Experiment B</th>
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<tbody>
<tr>
<td></td>
<td>Total radioactivity</td>
<td>Total protein</td>
</tr>
<tr>
<td></td>
<td>dpm</td>
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</tr>
<tr>
<td>Postmicrosomal supernatant</td>
<td>443,232</td>
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<tr>
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<td>Mg²⁺ supernatant</td>
<td>373,402</td>
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<td>First wash</td>
<td>20,328</td>
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<td>Second wash</td>
<td>4,496</td>
<td>4.0</td>
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<td>DOC supernatant</td>
<td>37,932</td>
<td>68.4</td>
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<td>Purified ribosomes</td>
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<tr>
<td>Microsomal</td>
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<tr>
<td>Crude ribosomes</td>
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<td>DOC supernatant</td>
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<td>Purified ribosomes</td>
<td>1,987</td>
<td>14.3</td>
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* Supernatant protein contamination (SPC) = \( \frac{\text{specific activity of ribosomal protein}}{\text{specific activity of postmicrosomal supernatant protein}} \) × 100

Ribosomes to a significant level during 3 hours in vivo. Hence the possibility existed that the radioactivity of the purified ribosomes in Experiment A was due partially to synthesis of ribosomal protein de novo rather than to adsorbed supernatant protein. Accordingly, a second experiment was carried out. In Experiment B, the postmicrosomal supernatant of the homogenate from Experiment A was centrifuged at 105,000 \( \times g \) for 3 hours in vivo to purify the ribosomes. Thus, the specific activity of dog chymotrypsin was 132 ± 8.6. A 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 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.
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>(\text{RNA} \times 100)</th>
<th>((\text{RNA} + \text{protein}))</th>
<th>(\text{Ribonucleoprotein, } %)</th>
<th>(\text{at } 260\text{ m})</th>
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<tr>
<td>43</td>
<td>63.8</td>
<td>59.6</td>
<td>136.3</td>
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<td>43*</td>
<td>66.4</td>
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<tr>
<td>52</td>
<td>59.4</td>
<td>59.4</td>
<td>132.0</td>
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</tr>
</tbody>
</table>

Mean value: 60.4
Standard deviation: 1.7
Coefficient of variance: 2.9%

* 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 \(\text{N}\) 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 \(\text{pH}\) 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, \(\text{pH} 7.5\), 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 mM potassium chloride. Addition of EDTA to a concentration of 2.5 \(\mu\)moles per mg of ribosomes caused complete dissociation to subunits in the presence of 0.1 mM potassium chloride. Addition of EDTA at a concentration as low as 0.1 \(\mu\)mole per mg of ribosomes caused complete dissociation in the presence or absence of 0.1 mM 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 ± 0.1:1.0 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, \(\text{pH} 7.5\), 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. 3c). EDTA at a concentration as low as 1 \(\mu\)mole per mg of ribosomes caused complete dissociation to subunits in some preparations. Complete dissociation was always achieved at a concentration of 2 \(\mu\)moles 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 the incubation medium.
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 ± 0.4 S (eight runs) in the absence of potassium chloride and 79.7 ± 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 $s_{20,w} = (73.8 ± 0.4) - (1.83 ± 0.05) c$ in the absence of KCl and $s_{20,w} = (79.5 ± 0.7) - (1.17 ± 0.13) c$ in the presence of 0.1 M potassium chloride. The ribosomes were separated on sucrose gradients. The profile of color formed is shown in Fig. 7. The small subunit consists of the more slowly sedimenting portion of the large peak resedimented and also the more rapidly sedimenting part of the large subunit. This observation is the same as that reported by Tashiro and Siekevitz (27) for guinea pig liver ribosomes.

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 subunits and dimers of small subunits reported in mammalian ribosomes by Tashiro and Siekevitz (10).

The values of $s_{20,w}$ and the concentration dependence of subunits prepared by lyophilization or by EDTA in the presence and absence of 0.1 M KCl are presented in Table III. The values of $s_{20,w}$ for the large subunit (50 to 51 S), the small subunit (33 to 34 S), and the concentration dependencies are similar whether the ribosomes result from lyophilization or EDTA treatment in 0.1 M KCl. Subunits formed by EDTA in the absence of KCl not only show a difference in concentration dependence but also have distinctly lower sedimentation coefficients, the value of $s_{20,w}$ for the large subunit being lowered to 34 S and the small subunit to 29 S.

These observations are relevant to the purity of the subunit preparation. 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

### Table III

<table>
<thead>
<tr>
<th>Nature of subunits</th>
<th>Sedimentation characteristics of subunits of canine pancreatic ribosomes</th>
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<tbody>
<tr>
<td>Method of formation</td>
<td>Equation of concentration dependence</td>
</tr>
<tr>
<td>and subunit</td>
<td>$s_{20,w}$</td>
</tr>
<tr>
<td>Lyophilization</td>
<td>$s_{20,w} = 50.6 - (0.90 ± 0.13) c$</td>
</tr>
<tr>
<td>Large</td>
<td>$s_{20,w} = 34.2 - (0.53 ± 0.20) c$</td>
</tr>
<tr>
<td>Small</td>
<td>$s_{20,w} = 29.2 - (0.55 ± 0.18) c$</td>
</tr>
<tr>
<td>Fresh + EDTA</td>
<td>$s_{20,w} = 50.4 - (1.03 ± 0.17) c$</td>
</tr>
<tr>
<td>Large</td>
<td>$s_{20,w} = 32.8 - (0.55 ± 0.10) c$</td>
</tr>
<tr>
<td>Small</td>
<td>$s_{20,w} = 29.0 - (0.55 ± 0.10) c$</td>
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</table>

### 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.
of the larger subunit; C, the smaller subunit; p, optical density
A, the faster sedimenting portion of the large subunit; B, the peak
fractions of the large subunit were rejected in subunit prepara-
tions. Centrifugation was at 25,000 rpm (SW 25.2) for 11
hours on a preparative gradient as described under “Experi-
mental Procedure.” I, fractions pooled for subsequent analyses;
L and S indicate positions of the large and small subunits, re-
spectively. Centrifugation was at 25,000 rpm (SW 25.2) for 11
hours in sucrose gradients as described under “Experi-
mental Procedure.” ---, fractions pooled for subsequent analyses;
A, the faster sedimenting portion of the large subunit; B, the peak
of the larger subunit; C, the smaller subunit; ——, optical density
per ml at 260 nm; — — —, protein concentration (micrograms per ml).

![Fig. 6](image)

**Fig. 6.** Sucrose density gradient centrifugation of lyophilized ribosomes dissociated by the addition of EDTA (2.0 \( \mu \)M per mg of ribosomes). Preparations were centrifuged at 28,000 rpm (SW 25.2) for 12 hours in sucrose gradients as described under “Experimental Procedure.” ---, fractions pooled for subsequent analyses; A, the faster sedimenting portion of the large subunit; B, the peak fractions of the larger subunit; C, the smaller subunit; ——, optical density per ml at 260 nm; — — —, protein concentration (micrograms per ml).

![Fig. 7](image)

**Fig. 7.** Analysis of ribosomal subunits by sucrose gradient centrifugation. A, faster sedimenting portion of the larger subunit; B, peak fractions of the larger subunit; C, smaller subunit. L and S indicate positions of the large and small subunits, respectively. Centrifugation was at 25,000 rpm (SW 25.2) for 11 hours on a preparative gradient as described under “Experimental Procedure.”

**TABLE IV**

<table>
<thead>
<tr>
<th>Preparation No.</th>
<th>(RNA X 100)/(RNA + Protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Large subunit</td>
</tr>
<tr>
<td>46</td>
<td>64.2</td>
</tr>
<tr>
<td>47</td>
<td>62.1</td>
</tr>
<tr>
<td>51</td>
<td>64.3</td>
</tr>
</tbody>
</table>

recentrifugation and was considerably less contaminated by small subunits. Accordingly, the more slowly sedimenting fractions of the large subunit were rejected in subunit prepara-
tions.

**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 undissociated 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 demon-
strated 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 ac-
counted for as adsorbed supernatant protein. In Experiment
A, however, where labeling was accomplished in vivo, the cal-
culated 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
micosomal ribosomes (30, 31), and little effect would be expected on the final results in this case. It will be shown in an accompanying paper (6) that the protein composition of pancreatic ribosomal particles is not detectably altered by the puromycin treatment.

Removal of radioactive supernatant protein from unlabeled ribosomes was used earlier by Kurland (32) as a criterion of purification of E. coli ribosomes. In Kurland's experiment a homogenate prepared from unlabeled cells was mixed in the proportion of 2:1 with supernatant prepared from labeled cells, and ribosomes were isolated from the mixture. In the present work, unlabeled tissue was homogenized directly 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. Breillatt and Dickman (33) describe a method for increasing the yield of a ribosome preparation from bovine globulin to 85%. The method of Kurland (34) report 50% RNA in Jensen's sarcoma ribosomes with bovine serum albumin as protein 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 method produces ribosomes containing 60% RNA and 40% protein, with canine ribosomal protein as the standard in the Lowry procedure. Breillatt and Dickman (33) describe a method for increasing the yield of a ribosome preparation from bovine globulin to 85%. The method of Kurland (34) report 50% RNA in Jensen's sarcoma ribosomes with bovine serum albumin as protein 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.

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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).

REFERENCES
