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Beef Pancreas Ribosomes: Stability*

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Received September 4, 1962

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

* This work was supported in part by grants from the National Institutes of Health, United States Public Health Service (A-803) and the United States Atomic Energy Commission AT(11-1)-305. A preliminary report of this work was presented at the meeting of the American Society of Biological Chemists, Atlantic City, N. J., April 10 to 14, 1961 (Madison and Dickman, 1961). A portion of the data of this paper is taken from a thesis (Madison, 1962) submitted to the Department of Biological Chemistry, University of Utah College of Medicine, by James T. Madison in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

can be followed readily by this means. An increase in absorbancy at 260 mμ (A_{260}) is closely associated with aggregation in this system. This change is termed the hyperchromic effect and is generally considered to be due to breakage of H-bonds in the RNA (Hall and Doty, 1959). A decrease in the A_{260}/A_{235} ratio also furnishes a very sensitive index of aggregation, since an increase in turbidity would result in more light of shorter wave lengths being scattered (Bayzer and Schauenstein, 1955). Finally, chemical and enzymatic analyses of dialyzable and/or sedimentable components have been useful in evaluating the sequence and quantitative significance of a number of reactions.

Materials and Methods

The ribosomes were prepared from microsomes by treatment with deoxycholate as described in the preceding paper (Madison and Dickman, 1963). Pancreas ribosomes were washed once with 1 mM phosphate, while those from rat liver were washed three times and dialyzed overnight against 1 mM phosphate–0.5 mM Mg^{++}. Analyses and ultracentrifugation were also carried out as in the previous paper.

The absorbancy of dilute (initial A_{260}, 0.3 to 0.5) ribosomal suspensions was determined at intervals with a Beckman DU spectrophotometer. The results are expressed as per cent increase in A_{260}, *i.e.*, $[(A_t - A_0)/A_0] \times 100$, as a function of time. In this equation A_t signifies the A_{260} at time t, and A_0 signifies that at the beginning of the experiment.

Normal rabbit serum or rabbit serum containing antibodies to pancreatic RNase or chymotrypsinogen A was dialyzed at 4° against distilled H_2O. Soybean trypsin inhibitor and crystalline ribonuclease were purchased from the Worthington Biochemical Corporation. The sulfated polysaccharides were dissolved in 1 mM phosphate, *p*H 7.4. Their preparation is described by Bernfeld *et al.* (1960).

Results

(a) *Ultracentrifugation of Incubated Beef Pancreas Ribosomes*

Ninety per cent of a freshly prepared suspension of beef pancreas ribosomes sedimented as 80 S particles (Fig. 1A). Aggregates formed, however, on incubation at 26° in 1 mM phosphate. After 15 minutes, 80 S particles had decreased to 80% of the total, with the remainder having aggregated to 117 S (Fig. 1B). No 80 S particles remained after 30 minutes of incubation, and 88% of the suspension sedimented as 252 S material (Fig. 1C). At the end of 45 minutes, aggregation was practically complete. Eighty-four per cent of the RNA sedimented so rapidly in the ultracentrifuge that no peaks were resolved.

(b) *Spectral-Absorbance Studies*

(1) *Ultraviolet Spectra.*—The ultraviolet spectrum of beef pancreas ribosomes resembles that of a typical nucleoprotein, with the absorbancy peak close to 260 mμ (Fig. 2). The ratio of absorbancy at 260 mμ to that at 235 mμ (A_{260}/A_{235}) was 1.5 to 1.6. The A_{260}/A_{280} ratio was 1.7. An absorbancy index, $A^{1\%}_{1\,cm}$ = 89.2 ± 2.9, was calculated from the A_{260} and the amount of nucleoprotein as determined by RNA and protein analyses. This value is lower than that of 120 reported for rat liver ribosomes (Hamilton and Petermann, 1959) and probably reflects the lower nucleic acid content of the pancreas particles.

(2) *Hyperchromicity.*—The increase in absorbancy of nucleic acid solutions when heated or treated with hydrogen bond (H-bond) breaking substances, *e.g.* urea, has been interpreted as being due to decreases in the number of nucleotide base pairs present in the structure (Shugar, 1960). Hall and Doty (1959) have studied certain of these transformations in intact beef liver ribosomes.

An increase in absorbancy, however, may also result from an increase in light scattering due to aggregation of particles. An experimental distinction between a hyperchromic effect due to aggregation on the one hand and H-bond breakage on the other can generally be readily accomplished by absorbance measurements at two wave lengths. An increase in light scattering will decrease the A_{260}/A_{235} ratio (Doty and Edsall, 1951), whereas the ratio will increase if H-bonds are broken.

A spectral-absorbancy curve of ribosomes which had been incubated 2 hours at 26° is shown in Figure 2. This treatment resulted in an increase of A_{260}/A_{235} from 1.54 to 1.65. The A_{260} value increased 17% over this period, while A_{300} actually decreased. Absorbancy measurements were continued into the visible range to determine if increased light scattering might be responsible for the increased absorbancy in the ultraviolet. Since scattered light should also be detectable at higher wave lengths (Bayzer and Schauen-

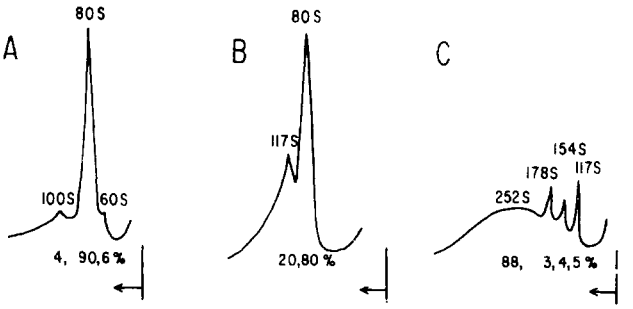

FIG. 1.—Sedimentation patterns of washed beef pancreas ribosomes, incubated at 26° in 1 mM phosphate, *p*H 7.2. (*A*) zero time, (*B*) 15 minutes, (*C*) 30 minutes. The sedimentation coefficient in Svedberg units is listed above each peak and the percentage of the total material just below.

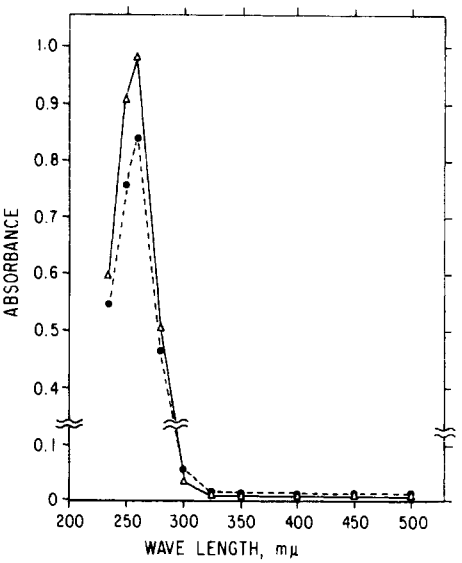

FIG. 2.—Spectral absorbancy curves of beef pancreas ribosomes in 1 mM phosphate, *p*H 7.8. ●– – –●, zero time; △———△, incubated 2 hours at 26°.

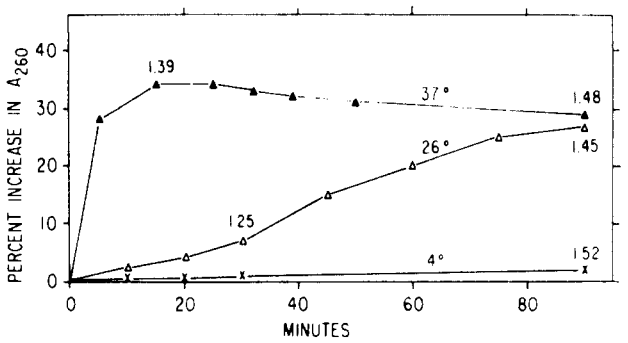

FIG. 3.—Influence of temperature on the A_{260} of beef pancreas ribosomes suspended in 1 mM phosphate, *p*H 7.8. × — ×, 4°; △— —△, 26°; ▲ —— —▲, 37°. The numbers refer to the A_{260}/A_{235} ratio at the indicated time. At zero time it was 1.55.

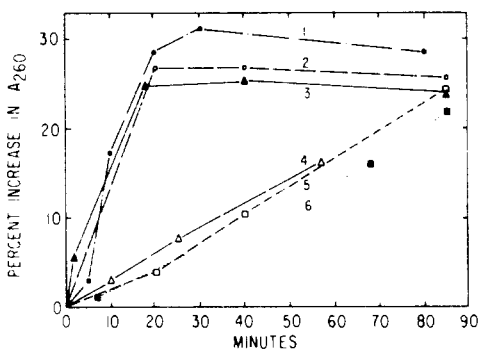

FIG. 4.—Hyperchromicity of beef pancreas ribosomes as influenced by ionic strength. Washed ribosomes were resuspended in the following solutions, *p*H 7.8, 26°. *1*, 0.005 mM phosphate (1.5×10^{-5}); *2*, 1 mM phosphate–50 mM KCl (5×10^{-2}); *3*, 10 mM phosphate (3×10^{-2}); *4*, 1 mM phosphate–5 mM KCl (1.8×10^{-2}); *5*, 1 mM phosphate (3×10^{-3}); *6*, 0.1 mM phosphate (3×10^{-4}). The approximate ionic strength of each solution is given in parentheses.

stein, 1955; Leach and Scheraga, 1960), the results eliminate this factor as a major contributor to the increased A_{260} under these conditions.

Determination of A_{260}/A_{235} at shorter time intervals, however, revealed that the ratio decreased for the first few minutes at 26°, then increased. These relationships are presented in Figure 3. From Figure 1 it is known that aggregation occurred readily at 26°, and this might be responsible for a certain portion of the A_{260} increase. Further aggregation took place on continued incubation, and A_{260} continued to rise. Although the data at hand do not permit quantitative calculation of the contribution of aggregation compared to that of H-bond breakage in absorbancy increases, semiquantitative estimates can be made. At 26°, A_{260}/A_{235} fell to 1.25 in 30 minutes, with an A_{260} increase of only 7% (Fig. 3). As shown in Figure 1, no 80 *S* particles remained at this time, most of them having aggregated to 252 *S* material. Thus aggregation to this extent has only a minor effect on A_{260} but a major one on absorbancy ratio. Further aggregation occurred during the ensuing 60 minutes at 26°, yet this cause of decreased ratio was overcome by H-bond breakage, and the absorbancy ratio actually increased to 1.45. It would appear from this analysis that, in most of the experiments to be described, the increase in A_{260} was due to helix coil transformation rather than to aggregation.

(3) *Ionic Strength.*—The results of a study of

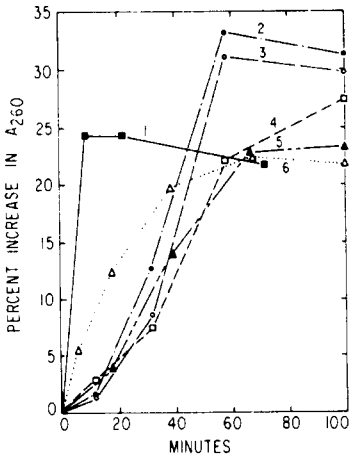

Fig. 5.—Effect of *p*H on A_{260} of beef pancreas ribosomes. Washed ribosomes were resuspended in 1 mM phosphate and adjusted to the indicated *p*H values. A_{260}/A_{235} ratios were determined at zero time (1.55), and the ratios after 100 minutes of incubation at 26° are in parentheses. *1*, *p*H 5.1 (1.24); *2*, *p*H 6.5 (1.36); *3*, *p*H 6.9 (1.37); *4*, *p*H 7.4 (1.40); *5*, *p*H 7.8 (1.45); *6*, *p*H 8.6 (1.49).

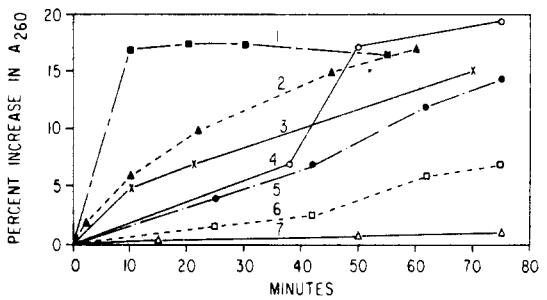

FIG. 6.—Effect of Mg^{++} and Ca^{++} on A_{260} of beef pancreas ribosomes. Solvent 1 mM phosphate, *p*H 7.8: *1*, no addition, 37°; *2*, 1 mM Mg^{++}, 37°; *3*, 0.5 mM Ca^{++}, 26°; *4*, no addition, 26°; *5*, 0.01 mM Mg^{++}, 26°; *6*, 0.1 mM Mg^{++}, 26°; *7*, 0.5 mM Mg^{++}, 26°.

stability of beef pancreas ribosomes as affected by the ionic strength of the medium are presented in Figure 4. As judged by the rate of increase in A_{260}, the particles were most stable in the range 0.0003 to 0.018 ionic strength. The stability decreased markedly in more concentrated (10 mM phosphate), or less concentrated (0.005 mM phosphate) solutions. For routine handling, therefore, beef ribosomes were washed and resuspended in 1 mM phosphate.

(4) *pH.*—The influence of *p*H on the rate of increase in A_{260} is shown in Figure 5. Beef pancreas ribosomes were most stable at a *p*H of 7.4 to 7.8. An increase to *p*H 8.6, or a decrease to *p*H 5.1, increased the rate of development of the hyperchromic effect. The decrease in A_{260}/A_{235} ratio and the larger increase in A_{260} at 100 minutes at lower *p*H indicates that as the *p*H was decreased aggregation and the amount of light scattered increased.

(5) *Magnesium and Calcium Ions.*—The influence of various concentrations of magnesium and calcium ions on the chromicity of beef pancreas ribosomes is shown in Figure 6. A magnesium concentration of 0.5 mM almost completely prevented the hyperchromic effect at 26°, but at 37° considerably greater magnesium concentrations were required. Magnesium was roughly ten times more effective at 26° than it was at 37°. Although Ca^{++} also inhibited the development of hyperchromicity, it was only about one tenth as effec-

tive as Mg^{++} at 26°. It can be calculated that the solubility product constants of $CaHPO_4$ and $MgHPO_4$ have not been exceeded with the concentrations utilized in this experiment (Van Wazer, 1958). It is also known that the soluble complex of calcium and phosphate dissociates to a greater extent than that of magnesium (Greenwald *et al.*, 1940). As shown in the accompanying paper (Madison and Dickman 1963), the particles contain much more Mg^{++} than Ca^{++}.

The effect of EDTA on the absorbance of beef pancreas ribosomes is shown in Figure 7. At 26°, 0.1 to 1.0 mM EDTA significantly increased the rate of hyperchromic development. Millimolar EDTA had a much smaller effect at 4° than at 26°. As judged from the original A_{260} value, however, aggregation occurred rapidly even at lower temperature. The addition of an equal molar concentration of Mg^{++} greatly reduced this disruptive influence of EDTA. It is probable that Mg^{++} stabilizes this RNA as it does that of tobacco mosaic virus (Boedtker, 1960), by neutralization of charges of the phosphate group. Its withdrawal by EDTA results in H-bond breakage and concomitant increase in chromicity.

(6) *Urea.*—Urea strongly accelerated hyperchromicity (Fig. 8). Even at 4° the presence of 4 M urea increased the A_{260} reading at zero time by almost 10%. Absorbancy of this solution increased 25% in 30 minutes, after which it leveled off. In accordance with the greater instability of the control at 26°, the addition of urea exerted a much larger effect at this temperature than at 4°. Two molar urea, for example, elicited a 42% hyperchromic effect in 12 minutes at 26°. Millimolar magnesium ion exerted a considerable stabilizing influence on the particles. Its presence in 2 M urea prevented the original A_{260} increase, and the A_{260}/A_{235} ratio did not drop to so low a value. These results also suggest that Mg^{++} binding helps maintain helical structure of RNA in ribosomes.

(7) *RNase and Anti-RNase Serum.*—The addition of RNase to ribosomes at 4° (Fig. 9*A*) did not produce hyperchromicity, but at 26° the A_{260} increased at approximately double the rate of the control (Fig. 9*B*). Normal serum exerted a slight stabilizing effect on the ribosomes at 26°, as did antichymotrypsinogen serum (not shown in figure). In this connection it might be mentioned that the addition of 10 μg per ml of soybean trypsin inhibitor did not lessen the development of hyperchromicity. The influence of anti-RNase serum is especially noteworthy. It stabilized the ribosomes completely at 26° and was almost as effective at 37°. This antibody has been shown to be effective as an inhibitor of RNase by Brown *et al.* (1959). Whether it precipitated the enzyme on the surface of the particles or in solution was not determined. These data indicate that most if not all of the instability of beef pancreas ribosomes results from their content of RNase.

The A_{260} of completely hydrolyzed beef pancreas ribosomes is also included in Figure 9. A total increase in A_{260} of about 45% was found after alkaline hydrolysis of ribosomes that had been incubated with or without the anti-RNase serum. This hydrolysis produced a further 15% increase in the A_{260} of ribosomes that had been previously incubated at 26° at 90 minutes. This extra hyperchromic effect is undoubtedly due to hydrolysis of the RNA to mononucleotides and compares closely to that found for purified calf liver RNA by Hall and Doty (1959). Since beef pancreas ribosomes have been shown to contain large amounts of RNase by direct analysis (Madison and Dickman, 1963), it would appear that the effect of the *added* RNase is due less to its enzymatic activity than to its properties as a

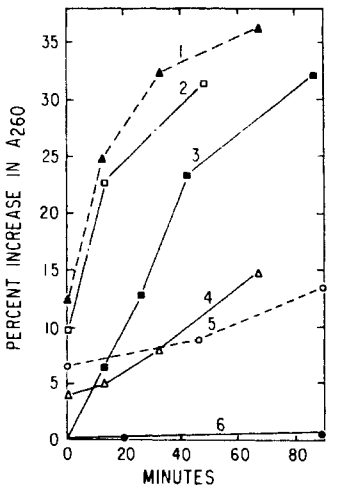

FIG. 7.—Influence of EDTA on A_{260} of beef pancreas ribosomal suspensions. Solvent, 1 mM phosphate, *p*H 7.8. *1*, 0.5 mM EDTA, 26°; *2*, 0.1 mM EDTA, 26°; *3*, no addition, 26°; *4*, 1 mM EDTA, 1 mM Mg^{++}, 26°; *5*, 1 mM EDTA, 4°; *6*, no addition, 4°.

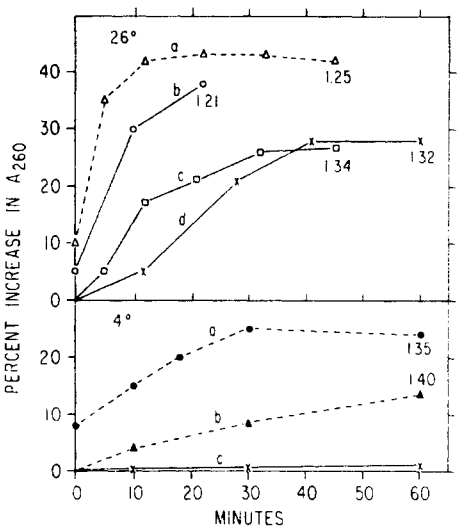

FIG. 8.—Hyperchromic effect of urea on beef pancreas ribosomes. Ribosomes were suspended in 1 mM phosphate, *p*H 7.8. At 4°: *a*, 4 M urea; *b*, 2 M urea; *c*, no addition. At 26°: *a*, 2 M urea; *b*, 1 M urea; *c*, 2 mM urea, 1 mM Mg^{++}; *d*, no addition. Numbers refer to A_{260}/A_{235} at the time indicated. The ratio at zero time was 1.45.

basic protein. The correspondence between the magnitude of the hyperchromic effects after the alkaline hydrolysis of beef liver RNA and of these particles also suggests that RNA hydrolysis is incomplete in 1 mM phosphate at 26°.

(8) *Ribonuclease Inhibitors.*—Sulfated polysaccharides and polyvinylalcohol sulfate are potent inhibitors of ribonuclease (Fellig and Wiley, 1959). These compounds also prevented the hyperchromic effect of beef pancreas ribosomes at low ionic strength, as can be seen from Figure 10. Increased ionic strength, which decreases the ability of the polyanions to inhibit ribonuclease, also destroyed their ability to prevent the increase in A_{260}. At a polysulfate concentration of 5 μg per ml, the RNA-inhibitor ratio was about 6, which is a relatively large amount of inhibitor. Fellig and Wiley (1959) found that at an RNA-inhibitor ratio of 100 all the substances tested here, except the sulfated dextran, completely inhibited RNase. At 5 μg per

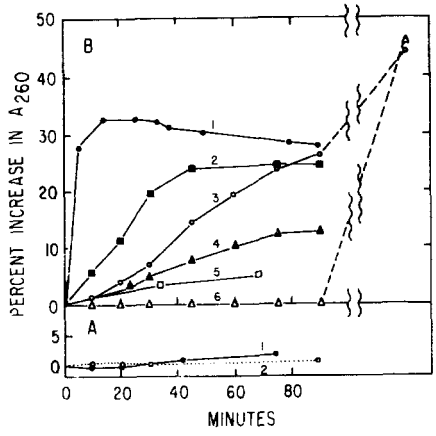

FIG. 9.—Influence of RNase and anti-RNase serum on A_{260} of beef pancreas ribosomes. All ribosomes were suspended in 1 mM phosphate, *p*H 7.8, prior to the incubations. (*A*) at 4°: *1*, RNase 1 μg/ml; *2*, no addition. (*B*) *1*, no addition, 37°; *2*, RNase 1 μg/ml, 26°, *3*, no addition, 26°; *4*, 0.1 ml normal serum per 10 ml ribosomal suspension, 26°; *5*, 0.1 ml anti-RNase serum per 10 ml ribosomal suspension, 37°; *6*, 0.1 ml anti-RNase serum per 10 ml ribosomal suspension, 26°. The two interrupted dashed lines indicate A_{260} after hydrolysis in 0.01 M KOH at 95° for 2.5 hours. The solutions were adjusted to *pH* 7.8 before absorbancy determinations.

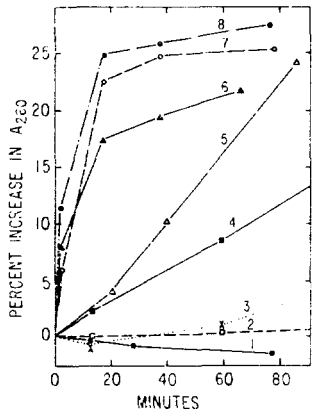

FIG. 10—Effect of sulfated polymers on hyperchromicity of beef pancreas ribosomes. Washed ribosomes were resuspended at 26° in 1 mM phosphate, *p*H 7.8, which contained the following substances at a concentration of 5 μg/ml: *1*, polyvinyl-alcohol sulfate (SEL II); *2*, cellulose sulfate (SCEL III); *3*, sulfated corn amylose (SAM IV); *4*, dextran sulfate (SDEX VIII); *5*, no addition. Three tubes contained 0.05 M KCl in addition to 5 μg/ml of the following: *6*, SEL II; *7*, SAM IV; *8*, SCEL III.

ml all the substances, except the sulfated dextran, effectively prevented the hyperchromic effect. At 10 μg per ml, however, dextran sulfate was also an effective inhibitor. Sulfated cellulose at 0.1 μg per ml (RNA-inhibitor ratio of about 250) had no effect on the A_{260} increase.

(9) *Dialysis.*—The stability of beef pancreas ribosomes at 4° was also studied by dialyzing the particles overnight. About 10 ml of a ribosomal suspension, plus yeast RNA and crystalline ribonuclease where indicated, were placed inside a Visking dialysis casing. The samples were dialyzed with gentle rocking against 200 ml of the solution indicated. The results in Table I indicate that very little ultraviolet-absorbing material was lost upon overnight dialysis against 1 mM phosphate. Addition of 10 μg of RNase per ml had very little effect. The addition of purified yeast RNA, however, resulted in the appearance of 15.7 units of dialyzable 260 mμ absorbing material. About this same amount of dialyzable material was produced from the RNA by 0.1 mg of crystalline RNase, which is approximately the amount of enzyme that was present in the pancreas ribosomes. The appearance of dialyzable material was associated with a significant increase in the total A_{260} absorbing material recovered. The addition of RNA (134 units) to ribosomes (70 units) gave a total of 223 units, a 9% increase. The addition of ribonuclease increased the A_{260} of the RNA from 134 to 153 units, a 14% increase. Apparently the ribosomal RNase does not attack the RNA of the particles under these conditions but is able to hydrolyze the added yeast RNA. Further support for this hypothesis is afforded by the equality of dialyzable material from ribosomes alone or ribosomes plus RNase.

TABLE I
DIALYSIS OF BEEF PANCREAS RIBOSOMES

Ten ml of washed beef pancreas ribosomes (1 mg/ml) were dialyzed at 4° with gentle rocking against 200 ml 1 mM phosphate, *p*H 7.4, for 12 hours. Other additions were made as indicated. Absorbancy units were calculated by multiplying the A_{260} of the respective solutions by their volumes.

Material Dialyzed	Absorbancy Units		
	Dialyzable	Nondialyzable	% Dialyzable
Ribosomes	1.2	67	1.8
Ribosomes + 0.1 mg RNase	1.7	70	2.4
Ribosomes + 6 mg RNA	15.7	207	7.0
6 mg RNA	2.1	132	1.6
0.1 mg RNase + 6 mg RNA	16.3	137	10.6

(c) *Sedimentation of Components*

The sedimentability of several ribosomal components after beef pancreas ribosomes had been incubated for various lengths of time at 26° is shown in Figure 11. Freshly prepared beef pancreas ribosomes were incubated in 1 mM potassium phosphate, *p*H 7.3. At the end of the incubation the tubes were cooled in an ice bath and centrifuged at 4° for 90 minutes at 85,000 × *g*. There was no significant change in the distribution of the various components after 90 minutes at 4°. At 26°, however, 89% of the ribosomal RNase was solubilized after 60 minutes; the orcinol-positive material gradually became nonsedimentable, while the majority of the protein recovered was sedimentable even after 90 minutes. Data from a similar experiment at 37° are also shown. At this temperature 90% and 69% of the RNase and RNA, respectively, were soluble after incubation for 15 minutes. Most of the recovered protein, however, remained sedimentable until the particles had been incubated for more than 60 minutes.

The results in Figure 11 are based on the amount of each component that was recovered. The recovery of RNase activity and orcinol-reacting material was quite high—between 80 and 100%. The proteins, however, were rapidly destroyed, as can be seen from Figure 12. At 37° about 50% of the protein was lost in 15 minutes, while at 26° the loss was only a little slower. At 4° less than 15% of the protein was lost in 90 minutes.

Discussion

Incubation of beef pancreas ribosomes at 26° or 37° resulted in separation of the RNA from the protein, aggregation of the protein, limited hydrolysis of both RNA and protein, and a 20 to 25% increase in chromicity. At 26° aggregation occurred before appreciable amounts of RNA had dissociated from the protein, since after 45 minutes of incubation aggregation was complete but 84% of the RNA was still bound to the aggregated material.

The value of 89 for $A^{1\%}_{cm}$ of beef pancreas ribosomes is significantly lower than that of 120 found for rat liver ribosomes (Hamilton and Petermann, 1959) or 112 for reticulocyte ribosomes (Ts'o and Vinograd, 1961). The absorbancy index of beef pancreas ribosomal RNA, after extraction with phenol, was 204, which checks closely with that calculated for beef liver by Hall and Doty (1959). On the basis of the RNA and protein contents given in the preceding paper (Madison and Dickman, 1963) and $A^{1\%}_{cm}$ of 19 at 260 mμ for the ribosomal protein, together with the experimentally determined absorbancy index for the RNA, the observed A_{260} of the pancreas ribosomes can be accounted for.

A 10% increase in A_{260} occurred immediately upon the addition of 4 M urea at 4°, 2 M urea at 26°, or 1 mM EDTA at 26°. This increase would correspond to about one fourth of the nucleotides of the ribosomal RNA being in a helical configuration (Doty *et al.*, 1959). This percentage agrees well with the estimate of Hall and Doty (1959) for the fraction of the RNA of calf liver ribosomal RNA that is present in a helical form.

Since the hyperchromicity of beef pancreas ribosomes can be prevented by agents that inhibit RNase (sulfated polymers and anti-RNase serum) but not by trypsin inhibitor or antichymotrypsinogen serum, it is likely that the RNase, not proteolytic enzymes, is mainly responsible for the instability of these particles. $CaCl_2$ and $MgCl_2$ probably prevent the hyperchromic effect by helping to maintain the helical structure of the ribosomal RNA. The studies on hyperchromicity as well as the dialysis experiments indicate that the beef pancreas ribosomes were virtually completely resistant to hydrolysis by RNase at 4°.

Do the data obtained in this study relate directly to the inactivity of these ribosomes in incorporating amino acids into protein? Beef pancreas ribosomes are quickly and irreversibly aggregated under conditions for optimal incorporation by liver ribosomes. In the presence of the quantity of RNase absorbed on the nucleoprotein the RNA is rapidly hydrolyzed and separated from the protein. These results thus point clearly to what must be achieved to obtain beef pancreas ribosomes that are active in amino acid incorporation: (1) removal of the absorbed basic proteins, especially RNase, and (2) stabilization of ribosomes, rather than their precipitation, by Mg^{++} and an ionic strength of 0.1. Experimental approaches to these objectives are suggested by these studies and are presently being attempted.

Acknowledgments

We wish to thank the Joe Doctorman and Son Packing Company for gifts of beef pancreas, Dr. Emil L. Smith for the use of the model E ultracentrifuge, Mr. Douglas Brown for his aid in obtaining and measuring sedimentation patterns, and Dr. William Cobain for directions in the preparation of chymotrypsinogen A antiserum. We want to thank Dr. Ray Brown for a gift of ribonuclease antiserum and directions for its preparation, and Dr. Peter Bernfeld for gifts of sulfated polysaccharides.

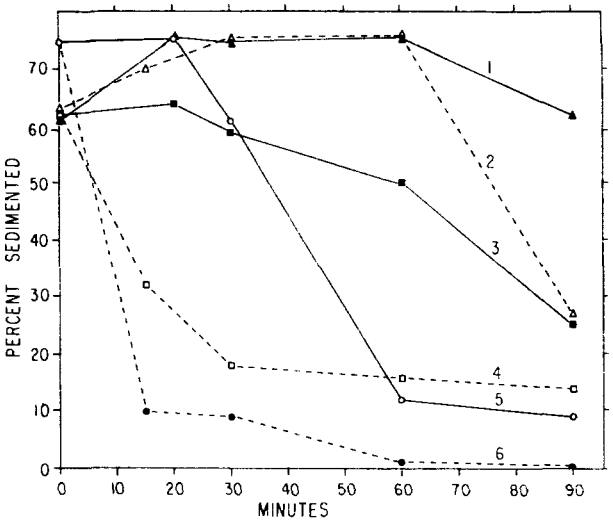

Fig. 11.—Sedimentation of beef pancreas ribosomal components on incubation. Washed ribosomes were resuspended in 1 mM phosphate, *pH* 7.8, and incubated at 26° or 37°. The suspensions were then sedimented at 85,000 × *g* for 90 minutes and both supernatant solutions and sediments were analyzed for protein, RNA, and RNase. The percentage sedimented is calculated from the total amount of each component which was found at the end of each incubation period. *1*, protein, 26°; *2*, protein, 37°; *3*, RNA, 26°; *4*, RNA, 37°; *5*, RNase, 26°; *6*, RNase, 37°

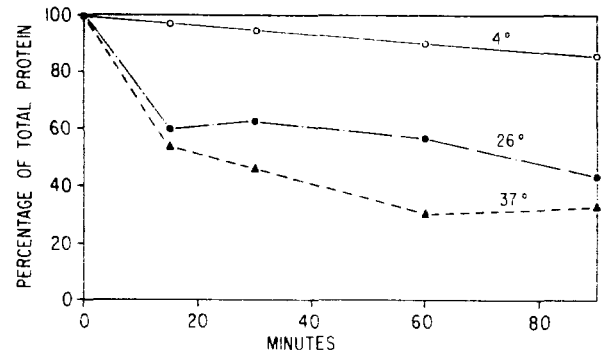

Fig. 12.—Loss of protein on incubation of beef pancreas ribosomes. Ribosomes were incubated as in Figure 11. Per cent of protein refers to the total protein in the supernatant and in the pellet relative to that at zero time. Incubation temperatures: O——O, 4°; ●— · —●, 26°; ▲------▲, 37°.

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The End-Groups of Tobacco Mosaic Virus RNA. II. Nature of the 3'-Linked Chain End in TMV and of Both Ends in Four Strains*

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Received October 4, 1962

Exhaustive digestion of C^{14}-labeled tobacco mosaic virus (TMV) RNA by snake venom phosphodiesterase usually yields about 0.5 moles of guanosine, cytidine, and uridine and 1.5 mole of adenosine. This strongly suggests that adenosine represents the 3'-linked ("left") end-group of the intact RNA chain, as it was previously shown to represent the 5'-linked ("right") end of the chain. Analysis of four quite different strains of TMV by both phosphodiesterase and alkaline degradation indicates that adenosine probably represents the terminal residue of each of these, notwithstanding the fact that some of the strains show differences from common TMV in regard to the end-group of their protein.

The ribonucleic acid of the tobacco mosaic virus (TMV-RNA) represents a chain molecule composed of about 6400 nucleotides. Studies of the nature of its end-groups have become practical only since virus preparations of high specific radioactivity became available. It was thus demonstrated with P^{32}-labeled RNA that the 2'- and 3'-positions of the 5'-linked end, as well as the 5'-position of the 3'-linked end of the chain, were not phosphorylated (Fraenkel-Conrat and Singer, 1962). It was further shown by alkaline degradation of C^{14}-labeled RNA that the 5'-linked end (*i.e.*, the right end by the customary methods of symbolizing polynucleotide structures) was an adenosine residue (Sugiyama and Fraenkel-Conrat, 1961). Various other methods have confirmed this and supplied information concerning the nature of the neighboring residues, which appear to be pyrimidines (Whitfeld, 1962, and unpublished data).

Snake venom phosphodiesterase supplies a tool to identify the nature of the 3'-linked end of the chain, since this terminal residue, if unphosphorylated in the 5'-position, should be present as the only nucleoside upon complete digestion of the RNA by phosphodiesterase. The problems inherent in this approach, and the tentative identification of the "left" terminal nucleotide of TMV-RNA, represents the main subject of this paper. Common TMV, as well as four typical strains, were investigated in this regard, and the strains were also subjected to alkaline degradation to ascertain their 5'-linked ("right") terminal residue.

Methods and Materials

The conditions of biosynthesis of C^{14}-TMV of 10^6 to 10^7 cpm/mg have been reported (Sugiyama and Fraenkel-Conrat, 1961). The virus was isolated by conventional methods of differential centrifugation. The preparation of TMV-RNA of highest possible purity in terms of nuclease contaminants has been previously described (Fraenkel-Conrat *et al.*, 1961). The other prerequisite for this work is the availability of snake venom phosphodiesterase of highest purity in terms of the absence of 5'-nucleotidase and of any nucleases or phosphatases splitting ribose 5'-phosphate bonds. A series of highly purified samples of this diesterase was kindly placed at our disposal by Dr. M. Laskowski (Felix *et al.*, 1960; Williams *et al.*, 1961). The conditions of enzyme treatment are indicated in the tables and text.

The isolation and identification of terminal nucleosides and nucleoside diphosphates was achieved by the same methods as previously described (Sugiyama and Fraenkel-Conrat, 1961). Known amounts of the unlabeled carrier compounds were added to the enzymatic digest and then reisolated by a series of purification steps. These usually consisted of (1) paper electrophoresis at *p*H 7.4, (2) two-dimensional chromatography, (3) desalting by charcoal adsorption and elution, and (4) paper electrophoresis at *p*H 3.5. The recovery of the absorbance of the nucleoside markers supplied the necessary correction factors for losses incurred during the complicated and time-consuming procedure of reisolation of the pure compound. The radioactivity found associated with each of these markers indicated the extent to which the given compound had been derived from the labeled TMV-RNA. The specific radioactivities of each nucleoside, which in some virus preparations differed appreciably one from another, were determined in separate experiments and used in these calculations.

Alkaline degradation was performed in the manner previously described (Sugiyama and Fraenkel-Conrat, 1961).

Results and Discussion

A. The 3'-Linked End-Group of TMV-RNA.—Degradation of a monodisperse terminally unphos-

* Aided by grant G-9745 from the National Science Foundation and by grant E-1267 from the National Institute of Allergy and Infectious Diseases, United States Public Health Service. A preliminary report of this work was presented at the 140th meeting of the American Chemical Society (Abstracts of Papers, p. 55C).

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