PROPERTIES OF RIBOSOMES FROM GROUP A STREPTOCOCCI[1]

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The presence in E. Coli of ribosomes with discrete sedimentation properties (Tissieres and Watson, 1958) and the isolation of four particles characterized by sedimentation coefficients 30S, 50S, 70S, and 100S has been described (Tissieres et al. 1959). In preparations of ribosomes from calf liver (Sherman and Petermann, 1961), rat liver (Petermann and Hamilton, 1957), rabbit appendix (Takata and Ozawa, 1957) and calf thymus (Hess, Utsunomiya and Lagg, unpublished) turbidity associated with a second component in electrophoretic patterns has been observed. This report concerns the isolation and properties of ribosomes from Streptococcus pyogenes[2] essentially free of material causing turbidity.

Thawed cells, washed three times with potassium phosphate buffer (pH 7.6, containing 0.005 M $MgSO_4$, 0.05 M NaCl, total ionic strength 0.10), were used for extraction. About 12 gms of wet cells suspended in 20 ml. buffer were subjected to 120 min. sonic radiation (Hess and Slade, 1955) and centrifuged twice at 23,000 x g for 30 min.[3] The supernatant was centrifuged for 90 min. at 105,000 x g to obtain a pellet, called 105P, amounting to about 205 mg. dry weight. Electrophoretic and sedimentation characteristics of the material in

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[2]S. pyogenes (type 3), harvested during exponential growth phase and obtained as frozen cells from Difco Laboratories, Detroit.

[3]The sediment resuspended in buffer and exposed to additional sonic energy provided further extractable material having the properties described in this report.

this pellet are seen in Fig. 1 and Fig. 2, respectively[4]. The sedimentation coefficient, S^{o}_{20W} at infinite dilution, for the major component was found to be 50S.

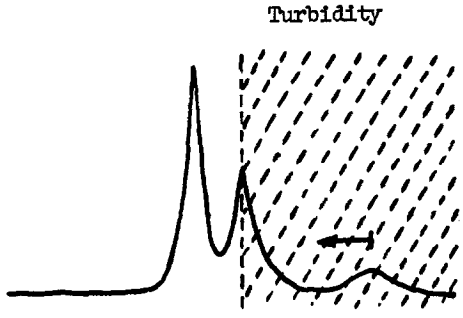

Fig. 1

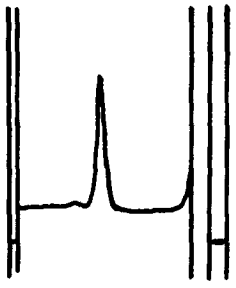

Fig. 2

Fig. 3

Fig. 1. Electrophoretic pattern from the ascending limb for S. pyogenes ribosomes in potassium phosphate buffer pH 7.6. 0.1 μ containing 0.005M Mg^{++}.
Fig. 2. Ultracentrifugal pattern for the ribosomes in the same buffer.
Fig. 3. Electrophoretic pattern for the material remaining in supernatant after pH of the solution used to obtain Fig. 1 is lowered from 7.6 to 5.6.

Samples pipetted from the faster moving boundary region in the ascending limb of the electrophoresis cell were examined in the Spinco Model E ultracentrifuge. The patterns obtained were the same as seen in Fig. 2, except that less material accumulated at the bottom of the cell. The ratio of the absorbance $\frac{280\ m\mu}{260\ m\mu}$ of pipetted samples was 0.50, indicating a high % of RNA. Samples removed from the slower moving boundary in the descending limb, however, contain randomly aggregated material which sediments in the analytical centrifuge before the speed of the rotor reaches 33,000 RPM. The absorbancy ratio $\frac{280\ m\mu}{260\ m\mu}$ of such samples, was 0.74 indicating the presence of about 9.0% RNA.

[4] The electrophoretic patterns were enantiographic and therefore patterns from the descending limb are not shown

When fraction 105P was suspended in the buffer described above and the pH lowered to 5.6, a precipitate formed. This precipitate has the electrophoretic and absorbancy properties of the material comprising the slower moving boundary in the electrophoretic pattern obtained with 105P (Fig. 1), while the supernatant corresponds to the materials giving the faster moving boundary (Fig. 3). In the ultracentrifuge, also, only one major component, the 50S particles was observed. The supernatnat solution obtained at pH 5.6 containing the 50S particles was dialyzed against water and lyophilized. The dry material was soluble in water as well as in the buffer used in the study and contained 58% RNA. The sedimentation characteristics of the 50S particles were unaltered by dialysis and lyophilization.

When the Mg^{++} concentration in the phosphate buffer used in the extraction was varied from 0.001 to 0.01 M, 30S, 50S, and 70S particles were found. In all cases 50S particles predominated, although the amount of 70S component increased with increased Mg^{++} concentration. When tris buffer at pH 7.6 was used for extraction, Mg^{++} concentration was varied from 0.01 to 0.05 M. In this instance 50S, 70S, and 100S particles were observed, 70S particles predominated, and only a trivial amount of 100S particles was detected.

Significant aspects are summarized below:

(a) The material causing turbidity in microsomal fraction 105P obtained from various sources can be distinguished from ribosomes in terms of RNA content and electrophoretic mobility. This material is not readily removed from ribosomes by differential centrifugation. In the case of S. pyogenes, however, the material causing turbidity in fraction 105P can be separated from 50S ribosomes by precipitation at pH 5.6.

(b) Ribosomes prepared from S. pyogenes remain soluble after dialysis against water and lyophilization. Ultracentrifugal and electrophoretic analyses suggest furthermore that the structural integrity of the 50S particle has not been altered by such treatment.

(c) No appreciable amount of particles with sedimentation coefficients higher than 70S is present in fractions prepared from S. pyogenes even in

Mg^{++} concentrations as high as 0.05M. This finding is in contrast to ribosome fractions prepared from E. coli (Tissieres et al. 1959).

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