REMOVAL OF STRUCTURAL PROTEINS FROM RIBOSOMES BY TREATMENT WITH SODIUM DEOXYCHOLATE IN THE PRESENCE OF EDTA

Sjur OLSNES*

Norsk Hydro's Institute for Cancer Research, The Norwegian Radium Hospital, Montebello, Oslo 3, Norway

Received 4 March 1970

1. Introduction

Sodium deoxycholate is widely used to liberate ribosomes bound to the endoplasmic reticulum. Under the conditions mostly used the detergent does not produce major change in the structure of ribosomes [1]. However, in the course of a study of the contamination of polysomes by ribonucleoproteins of nuclear origin [2], evidence was obtained that when polysomes were treated with deoxycholate in the presence of EDTA, the sedimentation properties of the subunits obtained were lower than expected. A closer examination of this phenomenon revealed that treatment of polysomes by deoxycholate in the presence of EDTA results in the release of part of the structural proteins from the ribosomal subunits. Since EDTA and deoxycholate are commonly used in the study of ribosomes a report of these findings seems warranted.

2. Materials and methods

Polysomes were isolated from rat liver by the use of sodium deoxycholate as previously described [2]. The polysome pellet was resuspended in a 0.15 M KCl solution, containing 0.001 M $MgCl_2$ and 0.01 M triethanolamine (pH 7.5).

Density gradient centrifugation in sucrose and in $CsCl_2$ was carried out as previously described [2].

Protein was determined by the method of Lowry et al. [10].

Total ribosomal proteins were prepared according to Leboy et al. [3]. Prior to disc electrophoresis, the protein containing solution was dialyzed for 24 hr against 6 M urea, containing 1 M sucrose and 0.14 M mercaptoethanol (pH 7.5, adjusted with conc. CH_3COOH).Polyacrylamide gel electrophoresis was carried out as described earlier [3, 4] in 5 × 100 mm gels containing 13% acrylamide and 6 M urea at constant current (3 mA per tube) for 2 hr. The large pore gel was omitted [5].

3. Results

Fig. 1. shows the sedimentation rate of ribosomal subunits after treatment of polysomes by EDTA in the presence and absence of sodium deoxycholate. Whereas the ribosomal subunits obtained by treatment of polysomes with EDTA alone sediment at a rate corresponding to about 50 S and 30 S, as usually found, the subunits obtained when sodium deoxycholate as well was present sediment considerably slower (at about 35 S and 25 S). If the polysomes were treated with sodium deoxycholate and the deoxycholate was removed by sedimenting the polysomes through 0.5 M sucrose, subsequent treatment with EDTA gave subunits sedimenting at normal rates (50 S and 30 S). It is thus clear that sodium deoxycholate and EDTA must be present together to alter the sedimentation rate of the subunits.

The effect of EDTA and deoxycholate on the buoyant density of the subunits was studied by $CaCl_2$ density gradient centrifugation. When the polysomes were treated with EDTA alone, two peaks at $\rho = 1.61$ and $\rho = 1.53$ were found (fig. 2). In contrast, when sodium

* Fellow of the Norwegian Cancer Society.

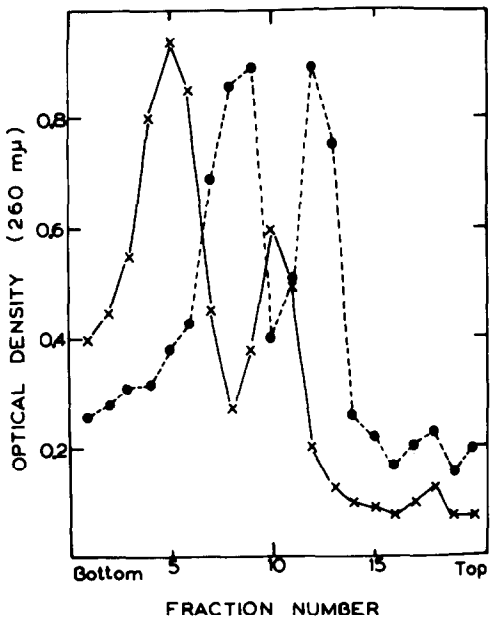

Fig. 1. Effect of deoxycholate and EDTA on the sedimentation rate of ribosomal subunits. Polysomes were treated with deoxycholate and EDTA (• - - - •) or with EDTA alone (x——x). The EDTA concentration was 0.02 M and the deoxycholate concentration 1%. The preparation was layered on top of a 0.5-0.1 M sucrose-gradient containing 0.15 M KCl, 0.02 M EDTA and 0.01 M triethanolamine (pH 7.5). The gradients were centrifuged in the SW-65 rotor at 300,000 *g* (R_{av}) for 2 hr.

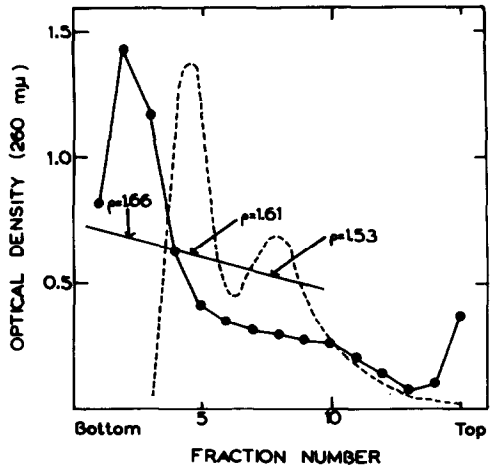

Fig. 2. Effect of EDTA and sodium deoxycholate on the buoyant density of ribosomal subunits. Polysomes were treated with deoxycholate and EDTA (•——•) or with EDTA alone (- - - - -). The EDTA concentration was 0.02 M and the concentration of sodium deoxycholate 1%. The preparations were fixed with formaldehyde and subjected to centrifugation in CsCl gradients.

deoxycholate was added, a peak with buoyant density about $\rho = 1.66$ and a broad shoulder in the less dense region was observed. As RNA has a higher buoyant density ($\rho = 1.90$) than protein ($\rho = 1.25$) [6] the increase in the density observed on treatment with sodium deoxycholate in the presence of EDTA indicates that proteins had been removed from the ribosomal subunits. Direct evidence for the release of protein was obtained by measuring the protein content of the supernatant after sedimentation of the ribosomal subunits. The value found (20% of the total ribosomal protein) was in good agreement with that calculated on the basis of the increase in buoyant density of the ribosomal subunits (18% of the structural protein).

The non-ionic detergents, Triton X-100 and Brij-58 were found not to remove protein from ribosomes in the presence of EDTA, and also to protect against

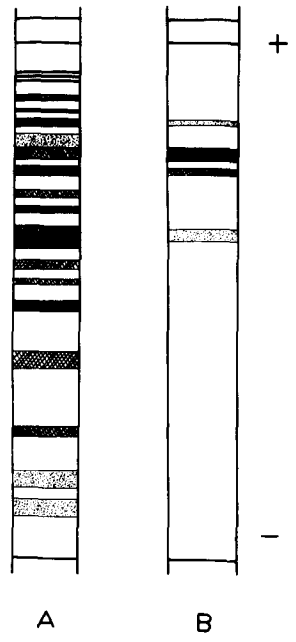

Fig. 3. Polyacrylamide gel electrophoresis of ribosomal proteins. (A) total ribosomal proteins, (B) proteins from the supernatant after treatment of polysomes with 0.02 M EDTA and 1% sodium deoxycholate and sedimentation of the ribosomal subunits.

the degradative action of sodium deoxycholate.

The protein released by treatment with sodium deoxycholate in the presence of EDTA was studied by polyacrylamide gel electrophoresis (fig. 3). A suspension of polysomes was treated with 1% sodium deoxycholate in the presence of 0.02 M EDTA. The ribosomal subunits were removed by centrifugation through 0.5 ml of 1 M sucrose at 88,000 *g* (R_{av}) for 15 hr in a 2 ml tube with adapter to Rotor 50 (Spinco). The supernatant containing the liberated proteins was dialyzed against 0.002 M TEA (pH 8.2) for 48 hr in order to remove sodium deoxycholate, and against 6 M urea containing 1 M sucrose and 0.14 M mercaptoethanol (pH 7.5) prior to electrophoresis. It is evident (fig. 3) that only a small number of the different ribosomal proteins were released by treatment with sodium deoxycholate in the presence of EDTA.

4. Discussion

Treatment with sodium deoxycholate alone, in contrast to other anionic detergents, such as sodium dodecyl sulfate, does not produce major changes in the ribosomes. However, it is clear from the present data that deoxycholate in the presence of EDTA will remove a considerable amount of the structural protein. It has been suggested [7] that the sodium deoxycholate molecule, being built from four fused rings, may be sterically hindered from penetrating into the ribosomes. In the presence of EDTA the structure of the ribosomal subunits becomes looser [8]. Conceivably, this change will permit the sodium deoxycholate molecules to penetrate deeper into the ribosomal subunits and gain better access to the proteins. In this connection it is of interest that small changes in the ribosomal structure induced by treatment with oxidizing agents like NaOCl or H_2O_2 will make the ribosomes vulnerable to treatment with sodium deoxycholate, an effect which is prevented by elevated magnesium ion concentrations [9].

It has been shown previously [2, 3] that sodium deoxycholate effectively removes protein from RNA-protein complexes other than ribosomes. Thus, the resistance of ribosomes to sodium deoxycholate in the presence of magnesium ions seems to represent an exception among ribonucleoprotein complexes.

Acknowledgement

The author is indebted to Prof. A.Pihl for his valuable criticism and advice.

References

[1] M.L.Petermann, The Physical and Chemical Properties of Ribosomes (Elsevier, Amsterdam, 1964).
[2] S.Olsnes, Biochim. Biophys. Acta, in press.
[3] P.S.Leboy, E.C.Cox and G.Flaks, Proc. Natl. Acad. Sci. U.S. 52 (1964) 1367.
[4] R.A.Reisfeld, U.J.Lewis and D.E.Williams, Nature 195 (1962) 281.
[5] S.Hjerten, S.Jerstedt and A.Tiselius, Anal. Biochem. 11 (1965) 219.
[6] R.P.Perry and D.E.Kelley, J. Mol. Biol. 16 (1966) 255.
[7] P.O.P.Ts'o, Ann. Rev. Plant Physiol. 13 (1962) 45.
[8] M.G.Hamilton and M.E.Ruth, Biochemistry 8 (1969) 851.
[9] A.L.Golub and J.S.Clegg, Biochim. Biophys. Acta 182 (1969) 121.
[10] O.H.Lowry, N.J.Rosebrough, A.L.Farr and R.J.Randall, J. Biol. Chem. 193 (1951) 265.