ISOLATION OF RNA-CONTAINING PROTEINS FROM PIG EYE LENSES USING A ZINC-HEAT PROCEDURE

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1. Introduction

The zinc-heat procedure is a simple and convenient method to precipitate proteins from homogenates of various organs [9,13]. Some of the proteins remaining in the supernatant of pig eye lens homogenates after the zinc-heat precipitation are simultaneously purified to an extent which makes it possible to crystallize them. So far one crystallized protein containing 1–3% RNA was obtained [1]. We now report further investigations on this protein, the isolation of RNA-containing protein particles and the crystallization of leucine aminopeptidase of pig eye lenses.

2. Materials and methods

RNA determination: The orcinol method of Mejbaum [2] was used in a modification described by Militzer [3].

DNA determination: DNA was measured with a modified version [4] of the method of Dische [5].

Reagents: All chemicals were reagent grade and acrylamide [6] and urea were further purified by recrystallization. *N,N'*-Tetramethylene-ethylenediamine and Coomassie brillant blue G 250 were obtained from Serva, Heidelberg. Acrylamide, SDS and 2-mercaptoethanol were products of Ferak, Berlin. Urea was purchased from VEB Laborchemie, Apolda. Indol and orcinol came from Merck, Darmstadt. Disc-electrophoresis at pH 9.2 was carried out according to Matson [7] and at pH 6.8 according to Swank and Munkres [8].

3. Results

3.1. *Purification procedure*

The procedure already described [1,9] was slightly modified. Pig eye lenses (12 g per 100 ml) are homogenized in a 0.9% NaCl solution. While the pH of the homogenate is kept constant between 7.2 and 7.4 by the addition of 0.1 N NaOH, 1.0 M $ZnSO_4$ is added dropwise under continuous stirring until the concentration of Zn^{++} is between 0.006 and 0.007 M. The supernatant of the zinc precipitation is then brought to 52–54°C for 15 to 20 min followed by centrifugation. This supernatant is concentrated 10 times at 30°C in a vacuum evaporator and dialyzed against 0.01 M Tris–HCl buffer of pH 8.0.

Within three weeks the nucleoprotein of fig.1 crystallizes and in some cases nucleoprotein particles appear as a faint precipitate prior to crystallization.

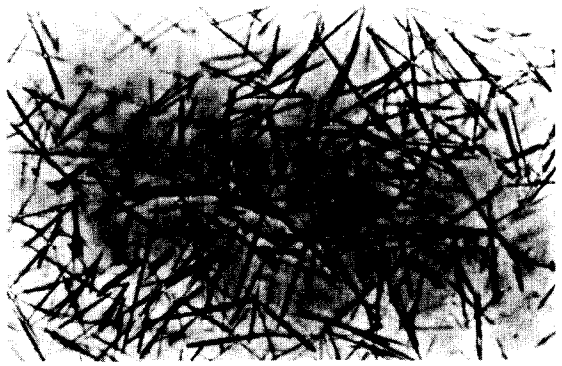

Fig.1. Crystallized nucleoprotein from pig eye lenses. Phase contrast photograph. Magnification × 128.

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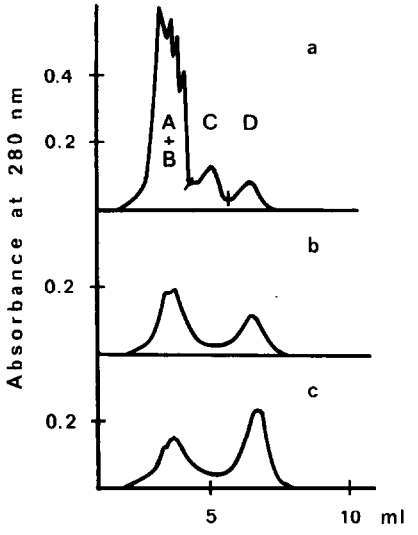

Fig.2. Elution profiles of pig eye lens homogenates on Sephadex G-75 in the course of the purification procedure. Column: 0.9 × 30 cm; Eluant: 0.05 M Tris–HCl buffer pH 7.2 (a) 0.2 ml of a homogenate of 12 g lenses and 100 m' 0.9% NaCl (b) Supernatant after the zinc precipitation, lyophilized, dialyzed twice against 10 times the volume of eluant and diluted to the same optical density at 280 nm as the raw homogenate (c) Supernatant after zinc-heat precipitation treated like b.

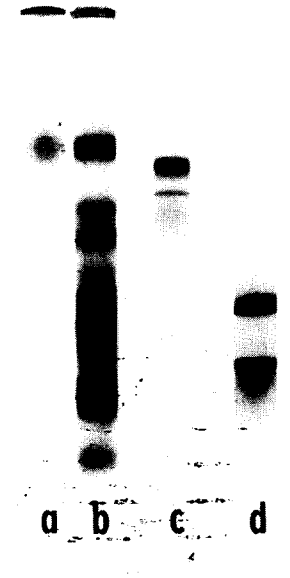

Fig.3. Polyacrylamide gel electrophoresis of nucleoprotein I and II. The electrophoresis was carried out at 0.16 M Tris–Glycine buffer pH 9.0 in 7% gels. (a) Nucleoprotein II (b) Nucleoprotein I (c and d) Nucleoprotein II incubated with 1% mercaptoethanol and 1% SDS prior to electrophoresis. The anode is at the bottom. Only a and b were run at the same time.

After removing both nucleoproteins we were able to crystallize LAP* by concentrating the supernatant in a vacuum evaporator and dialyzing it against a 40% ammonium sulfate solution at 4°C. The shape of the LAP crystals and the electrophoretic mobility on polyacrylamide gels of the LAP thus obtained is equal to those of LAP from bovine eye lenses [10,11]. They have an activity against L-leucineamide of C_1 = 40 to 60.

To follow the purification procedure we used the method of Björk [12] for separating calf lens proteins on Sephadex G-75 (fig.2). Homogenates of calf and pig lenses showed elution profiles similar to those reported by Björk who found that peak A - B contained α- and β-crystallins, peak C δ-crystallins and peak D dialyzable material.

All protein fractions were found to be diminished after the zinc-heat precipitation while fraction D increased relatively and changed its composition during the procedure, so that it could no longer be removed by dialysis. Its u.v.-absorption maxima and minima shifted from 279/254 nm via 259/245 nm after zinc precipitation to 259/235 nm after zinc-heat precipitation.

3.2. *Nucleoprotein I*

The material precipitating first at pH 8.0 appears as small particles of homogeneous shape under the light microscope. Like the crystallized nucleoprotein its solubility is low between pH 6 and 8, and high in 0.1 N HCl. It is also soluble in 0.25 M Tris–HCl or Davis buffer of pH 9.0, but tends to precipitate again after a few days. This nucleoprotein contains about 2–3% RNA. All preparations showed a common pattern of 6–7 bands in polyacrylamide gel electrophoresis. One of the bands had the same mobility as the crystallizing nucleoprotein (fig.3). This was also found when the electrophoresis was carried out with SDS.

* LAP: leucine aminopeptidase (EC 3.4.1.1)

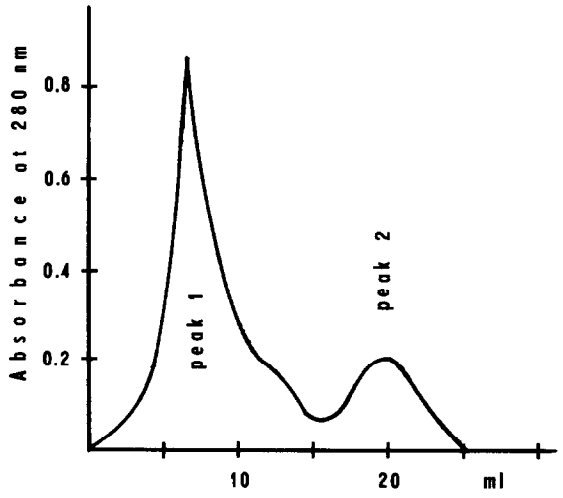

Fig.4. Chromatography of nucleoprotein II on a column (0.9 × 60 cm) of Sephadex G-100. Eluant: 0.01 N HCl. Flow rate: 5 ml/hr.

3.3. *Crystallized nucleoprotein (Nucleoprotein II)*

The crystallized nucleoprotein separated into two fractions on Sephadex G-100 (fig.4), the first peak showing a protein absorption spectrum, the second a nucleotide absorption spectrum (fig.5).

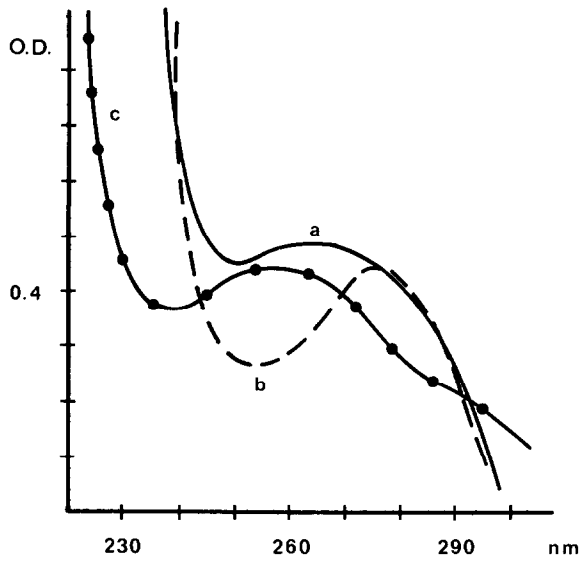

Fig.5. Ultraviolet absorption spectra. (a) Whole nucleoprotein II (b) Peak 1 shown in fig.4. (c) Peak 2 shown in fig.4. All material was dissolved in 0.01 N HCl.

The protein moved as a broad but single band in polyacrylamide gels at pH 9.2 (fig.3a). In the presence of mercaptoethanol and SDS the protein was separated into a main fraction, a leading smaller fraction, and several slowly moving weak bands differing in intensity from preparation to preparation (fig.3c). In a few preparations the main fraction seemed to be composed of two bands (fig.3d).

At pH 6.8 the electrophoretic pattern included the main fraction and its di-, tetra-, and octamers, when the 7.5% gels contained 0.1% SDS and 8 M urea. In addition very faint bands were found moving down the gels ahead of the mono-, di-, and tetramers, respectively. When mercaptoethanol was added to the protein under the same electrophoretic conditions, it resulted in the disappearance of the oligomers in favour of the monomer and the occurrence of a weak double band in the position of the dimer. The weak band ahead of the monomer is then clearly stronger than that without mercaptoethanol (fig.6).

The monomer can apparently disintegrate into several smaller components. If the protein peak of

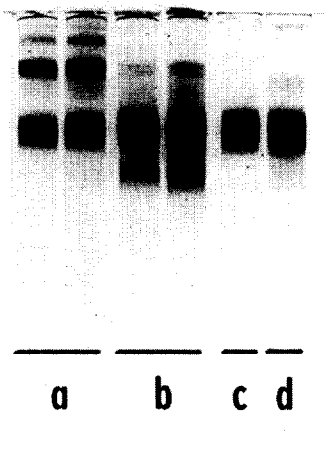

Fig.6. SDS electrophoresis pattern of nucleoprotein II on 7.5% polyacrylamide gels containing 8 M urea at pH 6.8 [8]. (a and c) In the absence of reducing agent. (b and d) In the presence of 2-mercaptoethanol. (c and d) Re-electrophoresis of monomers cut out of acrylamide gels. The anode is at the bottom. The gels were stained with Coomassie.

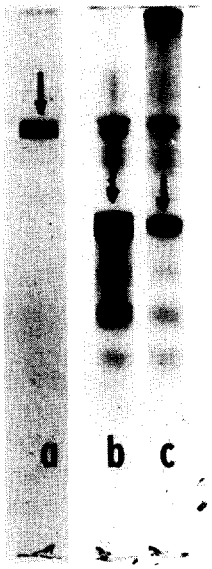

Fig.7. Disc electrophoresis of the protein peak of nucleoprotein II after storage at 4°C for 12 months. (a) Nucleoprotein II before separation on Sephadex G-100; protein peak after 12 months: (b) with, and (c) without mercaptoethanol and SDS. (b) and (c) were run at the same time. The monomer is marked with an arrow. Electrophoresis was carried out at pH 6.8 on 7.5% gels.

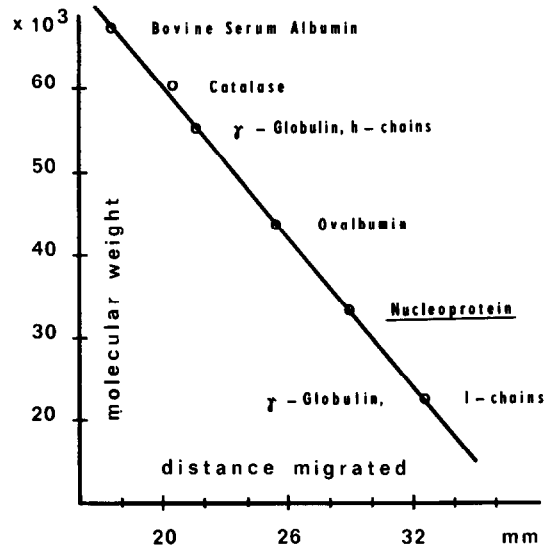

Fig.8. Molecular weight determination of nucleoprotein II on 7.5% acrylamide gels according to the method described by Swank and Munkres [8].

the Sephadex G-100 fractionation is kept at 4°C for several months, three faster moving bands are observed in addition to the monomer. Much of the material of this peak remains at the start or is trailing behind the monomer if it is not treated with mercaptoethanol (fig.7). The mol. wt of the main fraction was estimated to be 33 000 in polyacrylamide gel (fig.8).

4. Discussion

The zinc-heat method is a simple and effective procedure to precipitate proteins. Up to 99% of the proteins in homogenates of eye lenses and more than 95% of the proteins from bovine kidneys are precipitated [9,13]. This coincides with the purification of several enzymes. LAP, e.g., the activity of which is fully preserved during the procedure, can then be crystallized. Another group of proteins is also purified to the point of crystallization. This group is characterized by low solubility at neutral pH, good solubility in dilute HCl and above pH 9.0 with a tendency to fall out again, and by the binding of about 3% RNA or nucleotides. Some preparations of the crystallized nucleoprotein reacted with indol indicating a content of less than 0.4% DNA. But this could also be attributed to unspecific reactions of the protein itself. A more exact analysis of the nucleic acids bound to the proteins is in progress.

Judging from observations with the light microscope nucleoprotein I consists of small particles of a homogeneous shape. These were found to be composed of 6–7 different parts in gel electrophoresis, one of them being of the same mobility as the main fraction of the crystallized nucleoprotein. This monomer with an estimated mol. wt of 33 000 is not only able to form polymers but can apparently decompose into smaller units, which are capable of aggregating again. A preliminary amino acid analysis gave a relation of basic to acidic amino acidic amino acids of 0.61.

From their RNA-binding capacity, their acidic nature and molecular weight, and the tendency to form insoluble aggregates it could be suggested that the proteins described in this paper and the RNA-binding nuclear proteins from rat liver isolated by Voronina [14] and Schweiger and Schmidt [15] are related proteins.

Acknowledgements

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