AMINO ACID COMPOSITION OF THE CRYSTALLIZED RNA-CONTAINING PROTEIN FROM PIG-EYE LENSES

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1. Introduction

In 1971 one of us reported the crystallization of a protein from pig-eye lenses containing 2–3% RNA [1]. Later, we found this nucleoprotein to dissociate into its protein and RNA part during gel-filtration in diluted hydrochloric acid and the protein part to consist of polymers of a 33 000 mol. wt monomer [2]. In this communication we shall report on the isolation of the 33 000 mol. wt subunit and its amino acid composition.

2. Materials and methods

The crystallized nucleoprotein was obtained as described earlier [2]. The crystals were dissolved either in 0.01 N HCl for gel-filtration on a Sephadex G-100 column (0.9 X 60 cm) or an 8 M urea solution containing 1% SDS for electrophoresis on 7.5% polyacrylamide gels with 8 M urea and 0.1% SDS [3]. To recover the monomers after the 7 h electrophoresis the gels were removed from the glass tubes (18 mm in diameter) and extracted by incubating them in the same solvent that was used to dissolve the crystals. The extracts then were dialyzed against distilled water. The monomers thus obtained and the protein peak of the Sephadex G-100 fractionation of the nucleoprotein were hydrolyzed in 5.6 N HCl at 110°C for 24 h and the amino acid analysis was carried out with the two column system of a Beckman Unichrom amino acid analyser.

3. Results

On polyacrylamide gels the nucleoprotein separated into a 33 000 mol. wt subunit and several oligomers (fig.1). The recovered monomers were homogeneous and – as shown by analytical disc electrophoresis –

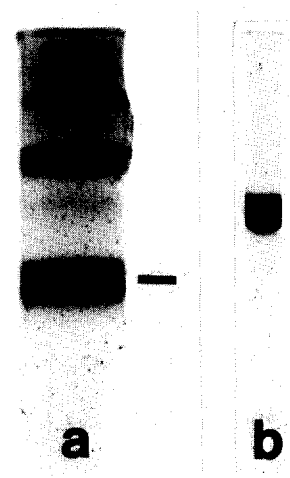

Fig.1. Purification of the nucleoprotein by preparative gel electrophoresis. (a) A Coomassie Brilliant Blue-stained vertical strip cut from the center of a 7.5% polyacrylamide gel column after electrophoresis. The position of the monomer is indicated. Such stained strips were used as a guide to cut out horizontal slices of the unstained rest of the gel columns which contained the monomer. The monomer was then extracted by incubating the gel-slices with an 8 M urea solution containing 1% S D S. (b) Re-electrophoresis of the purified nucleoprotein monomers obtained as described in (a) on 7.5% polyacrylamide gels.

without any impurities even though large amounts of protein were applied to the gels. The extracted monomers were extensively dialyzed against distilled water and stored at 4°C. After a few days long crystals formed in these extracts and the shape of these crystals was very similar to the shape of the crystals of the whole protein [1,2]. The amino acid compositions of the highly purified monomers and the protein peak of the Sephadex G-100 fractionation of the nucleoprotein are given in table 1.

Table 1
Amino acid composition of the protein peak of the Sephadex G-100 fractionation and the highly purified subunit of the crystallized nucleoprotein (mol./100 mol. amino acid recovered)

	Protein peak[a]	Subunit
Lysine	6.3	6.7
Histidine	2.3	2.5
Arginine	3.5	3.6
Cys–SO_3	1.1	1.5
Aspartic acid	9.0	8.0
Threonine	3.8	4.6
Serine	4.9	8.5
Glutamic acid	10.7	12.0
Proline	4.6	3.5
Glycine	11.4	13.3
Alanine	10.5	9.7
Half-cystine	–	–
Valine	8.7	7.8
Methionine	2.1	–
Met–SO_2	–	1.6
Isoleucine	7.1	5.8
Leucine	9.5	8.0
Tyrosine	2.2	0.7
Phenylalanine	3.0	2.3
Ratio $\frac{\text{Asp + Glu}}{\text{His + Lys + Arg}}$	1.64	1.59

[a] Taken from Frenzel, J. (1969) Thesis, Martin-Luther-University, Halle
(–) Not found

4. Discussion

Because of its tendency to aggregate and the consequent difficulty in isolating and fractionating it, the RNA-containing protein from pig-eye lenses presented certain problems in its investigation. Its first amino acid analysis (table 1, column 1) was carried out a few years ago when the only possible way to further purify the crystallized protein was its fractionation on Sephadex G-100. At that time we could not be sure that the protein we analyzed was absolutely pure and homogeneous. With the introduction of polyacrylamide gel electrophoresis it turned out that all preparations of the nucleoprotein separated into several bands (fig.1). As the addition of mercaptoethanol caused the slowly migrating bands to disappear in favour of the leading zone [2] and the amino acid composition of the highly purified monomer is very similar to the one we got with the unfractionated protein (table 1), we assume that the protein consists of only one type of monomer.

The ratio of acidic to basic amino acids is 1.6. Glutamic (12 mol. percent) and aspartic (8 mol. percent) acids together amount to 20% of the total amino acids. The protein contains a high amount of glycine and almost 55% of the amino acids present are non-polar amino acids. It also contains cysteine and cysteine seems to be essential in covalently binding the monomers to form the polymers.

Acknowledgements

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References

[1] Glässer, D. (1971) FEBS Lett. 17, 185–186.
[2] Glässer, D., Weber, E., Frenzel, J. and Hanson, H. (1975) FEBS Lett. 56, 124–128.
[3] Swank, R. T. and Munkres, K. D. (1971) Analyt. Biochem. 39, 462–477.