A STUDY OF THE GLOBULINS OF COTTON SEEDS

XX. STABILITY OF THE QUATERNARY STRUCTURE OF THE 11S-GLOBULIN

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The 11S-globulin has a complex stable quaternary structure in the formation of which three types of subunits participate. The aim of the work was to determine what forces form and stabilize the quaternary structure of this protein. The products of the maleylation and acetylation of the 11S-globulin and also the stability of the native 11S-globulin in the presence of ionic and nonionic detergents were investigated by disk electrophoresis. It was shown that hydrophobic interactions play a deciding role in the formation and stabilization of the quaternary structure of the 11S-globulin.

It was shown previously [1] that 11S-globulin (mol. wt. 260,000) isolated from cotton seeds contains three subunits, A, B, and C, with molecular weights of 28,000, 24,000 and 17,000, respectively. It obviously has a complex quaternary structure, since three types of subunits participate in its formation. The native 11S-globulin molecule has a low solubility. It does not dissociate in 8 M urea and SDS under the conditions of gel filtration. This is probably connected with features of the topography of the functional groups of each subunit, thanks to which the forces forming and stabilizing the quaternary structure are very strong. The quaternary structure may be formed by noncovalent bonds such as ionic, hydrogen, and hydrophobic interactions.

The aim of the present work was to establish what forces form and stabilize the quaternary structure of the 11S-globulin. To solve this problem it was necessary first of all to force the molecule of the 11S-globulin to dissociate into subunits, i.e., to disrupt the quaternary structure. There is information according to which the acylation of the proteins with anhydrides of dicarboxylic acids leads to the dissociation of molecules consisting of more than one polypeptide chain [2, 3]. We decided to use this method to determine whether the ε-NM_2 group of lysine plays any role whatever in the stabilization of the quaternary structure of the 11S-globulin.

We first modified the lysine ε-NH_2 group of the 11S-globulin with maleic anhydride in the presence of various amounts of acylating agent. It was found that the solubility of the maleylated 11S-globulin had considerably increased. It dissolved well in aqueous solutions with pH > 8 (on storage the solubility fell). The maleylated 11S-globulin dissociated into subunits under the conditions of disk electrophoresis and gel filtration not only in the presence of a detergent but also without it (Fig. 1). This showed the breakdown of the quaternary structure, since the native molecule did not dissociate under the same conditions (Fig. 1). In view of this, the question arose as to the reason for the destabilization of the 11S-globulin molecule — because of the blockage of the lysine ε-NH_2 groups, which make some contribution to the stabilization of the quaternary structure, or because of the electrostatic repulsion arising as the result of the appearance of additional carboxy groups on maleylation. In order to answer this, we decided to modify the lysine ε-NH_2 groups with acetic anhydride under the same conditions.

The solubility of the acetylated 11S-globulin, like that of the maleylated globulin, had increased. In dissolved well in aqueous solutions with pH > 8 (on storage, again, the solubility fell). The acetylated 11S-globulin also dissociated into subunits under the conditions of disk electrophoresis in the presence of 0.1% of SDS. It is true that in this case no sharp division between the subunits was observed, but the native 11S-globulin was completely absent (Fig. 1). Under the conditions of disk electrophoresis, the acetylated

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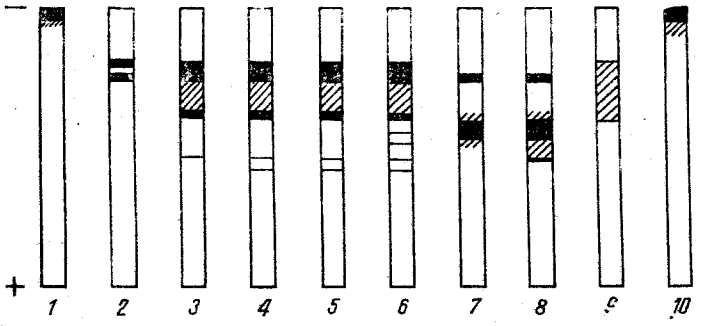

Fig. 1. Electrophoretogram of proteins in 15% polyacrylamide gel: 1) Native 11S-globulin molecule (0.1% SDS); 2) mixture of subunits A and B (0.1% SDS). Maleylated 11S-golbulin (0.1% SDS); 3) tenfold excess of maleic anhydride; 4) 20-fold excess; 5) 50-fold excess; 6) 100-fold excess. Maleylated 11S-globulin (without detergent); 7) 100-fold excess of maleic anhydride; 8) 300-fold excess. Acetylated 11S-globulin: 1) 300-fold excess of acetic anhydride (0.1% SDS); 10) 300-fold excess of acetic anhydride (without detergent).

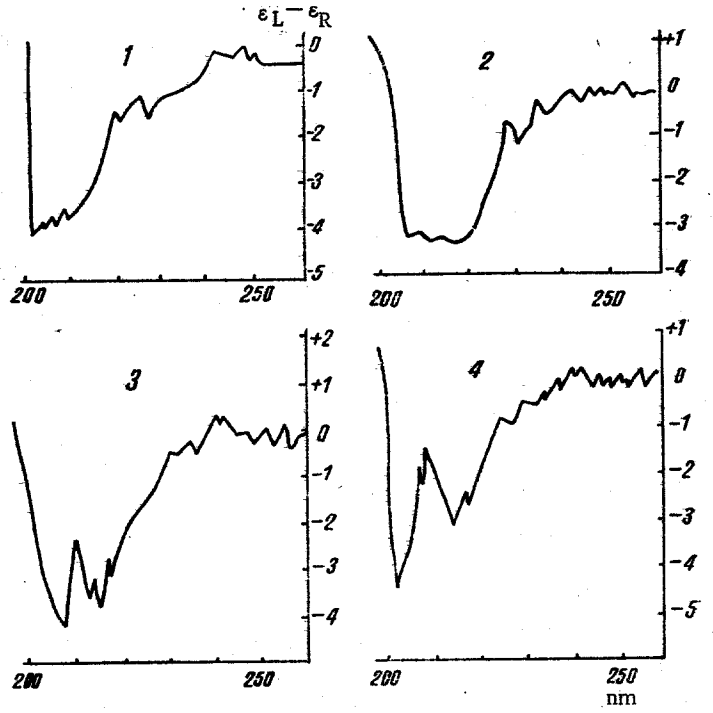

Fig. 2. CD spectra: 1) Native 11S-globulin molecule. Maleylated 11S-globulin: 2) 100-fold excess of maleic anhydride; 3) 200-fold excess; 4) 300-fold excess.

11S-globulin did not dissociate into subunits in the absence of a detergent (Fig. 1).

Since on acetylation no additional carboxy groups are formed, the dissociation of the acetylated 11S-globulin under the conditions of disk electrophoresis in the presence of a detergent shows the participation of the ε-NH_2 groups of the lysine residues in the stabilization of the quaternary structure. However, the contribution of the lysine ε-NH_2 groups is probably small, since in the absence of detergent the acetylated 11S-globulin did not dissociate on disk electrophoresis. The maleylated 11S-globulin dissociates into subunits under the conditions of disk electrophoresis and gel filtration even in the absence of a detergent.

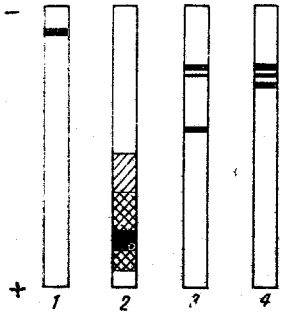

Fig. 3. Electrophoretogram of native 11S-globulin in 7% polyacrylamide gel in the presence of ionic (SDS) and nonionic (Triton X100) detergents: 1) 0.2% SDS; 2) 2% SDS; 3) 0.2% Triton X100; 4) 2% Triton X100.

Consequently, the additional carboxy groups formed on maleylation promote the destabilization of the 11S-globulin molecule.

With a 300-fold excess of acylating agent the reaction took place practically quantitatively. However, dissociation of the modified 11S-globulin into subunits under the conditions of disk electrophoresis was observed even with a 10-fold excess of anhydride, although in this case a very large number of freee lysine ε-NH_2 groups remained. Thus, it may be assumed that on the surface of the native molecule there are a comparatively small number of lysine ε-NH_2 groups, some of which, as we have seen, take part in the stabilization of the quaternary structure of the 11S-globulin.

The acylation of the surface glycine ε-NH_2 groups with a small excess does not lead to fundamental conformational changes, as can be seen from the CD spectra (Fig. 2).

We have found that the lysine ε-NH_2 groups play some part in the stabilization of the ternary structure of the 11S-globulin, but their contribution is small. This means that the stability of the molecule is due to some other forces. A decisive role in the formation of the quaternary structure is possibly due to hydrophobic interaction. In order to check this hypothesis, we performed the disk electrophoresis of the native 11S-globulin molecule in the presence of ionic (SDS) and nonionic (Triton X100) detergents. It was found that native 11S-globulin molecule dissociates into subunits under the conditions of disk electrophoresis in the presence of even low concentrations of nonionic detergent (Fig. 3). It was found that the mobility of the subunits decreased with an increase in the concentration of Triton X100.

A change in the concentration of SDS did not lead to any changes whatever (Fig. 3).

The dissociation of the native 11S-globulin under the conditions of disk electrophoresis in the presence of low concentrations of nonionic detergent shows the decisive importance of hydrophobic interactions in the formation and stabilization of the quaternary structure of the 11S-globulin. Ionic and hydrogen bonds probably do not play an important role here.

EXPERIMENTAL

The 11S-globulin was isolated by the method described previously [1].

Maleylation was carried out in the following way. The 11S-globulin was suspended in borate-alkaline buffer, pH 8.8. With stirring, maleic anhydride in 10-, 20-, 50-, 100-, 200-, and 300-fold excess with respect to the lysine was added to the protein suspension cooled to 0°C. The reaction was performed at 0°C for 1 h at pH 8.8-9.5, the pH of the medium being maintained by the addition of 5 N NaOH. After the end of the reaction, the excess of reagent was eliminated by dialysis against distilled water alkalinized to pH 8 at 4°C. Then the protein solution was freeze-dried.

The 11S-globulin was acetylated under the same conditions as were used for maleylation. Acetic anhydride (freshly distilled) was added in 10-, 100-, and 300-fold excess with respect to lysine.

Disk electrophoresis in polyacrylamide gel was carried out by the method of Ornstein and Davis [4].

The free lysine ε-NH_2 groups were determined by the dansyl method of Gray and Hartley [5].

Gel filtration was carried out on a columm of Sephadex G-75 (fine) equilibrated with borate-alkali buffer, pH 9, in the absence of detergent. On a 1.8 × 200 cm column was de-

posited 45-50 mg of maleylated 11S-globulin. The rate of elution was 15 ml/h and the volume of the fractions was 1.6 ml.

The CD spectra were obtained on a Jasco J = 20 spectropolarimeter. The thickness of the cell was 0.01 cm. Protein was dissolved in 0.1 M phosphate buffer containing 7.2% of NaCl, pH 7.75. The protein concentration was 1 mg/ml.

SUMMARY

1. It has been shown that a decisive role in the formation and stabilization of the quaternary structure of the native 11S-globulin is that of hydrophobic interactions.

2. It has been established that the ε-NH_2 groups of the lysine residues take part in the stabilization of the quaternary structure of the 11S-globulin.

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A STUDY OF THE PROPERTIES OF SYNTHETIC ANALOGS OF THE TRYPTOPHAN-CONTAINING FRAGMENT 113-121 OF THE BASIC PROTEIN OF MYELIN

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New analogs of fragment 113-121 of the basic protein of myelin were synthesized: Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Arg, in which the phenylalanine in the first position has been replaced by L-alanine (V) and by D-phenylalanine (IX), and also a shortened analog with the deletion of the serine in the second position (XIII) and a lengthened analog with the insertion of glycine between the phenylalanine and tryptophan (XVII). In experiments on guinea pigs, one of the compounds obtained exhibited encephalitogenic activity. The circular dichroism spectra of compounds (I) and (IX), and also of some model compounds, have been studied in order to analyze the contribution of the aromatic amino acid residues to the dichroic absorption.

In preceding papers [1-3] devoted to the synthesis and study of the physicochemical and encephalitogenic properties of analogs of the tryptophan-containing fragment 113-121, Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Arg (I), of the basic protein of human myelin, we have shown that replacement of the glutamic acid residue in the sixth position of glycine and also the acetylation of the N-terminal amino groups of the nonapeptide (I) have no effect on encephalitogenic activity. However, the introduction of glycine in place of the alanine-5 or phenylalanine-1 residue leads to the complete loss of EAE activity; the same effect is observed with an increase or decrease by one amino acid residue of the length of the encephalitogenic determinant.

We have continued the study of the relationship between the biological properties of analogs of the peptides (I) and their primary structure and conformation. In particular, we have investigated the role of the N-terminal phenylalanine residue and its position relative to the tryptophan residue for the induction of experimental allergic encephalomyelitis

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