CONFORMATIONAL CHANGES IN THE RESERVE PROTEINS OF THE COTTON PLANT

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A quantitative study of the secondary structures of the 11S and 7S globulins from cotton seeds has been made by the circular dichroism (CD) method. It has been established that the 11S and 7S globulins contain, respectively: 16 and 14% of α-helices; 15% each of β-structures; 18 and 20% of β-bends; and 51% each of irregular sections.

The 11S and 7S globulins are the main reserve proteins of cotton seeds and the main components of the food protein obtained from cotton seeds. These two globulins differ in their amino acid compositions [1, 2], their N-terminal amino acids, and their properties. The N-terminal amino acid of the 11S globulin is histidine and that of the 7S globulin is arginine.

The secondary structures of the 11S and 7S globulins have been established from their circular dichroism (CD) spectra using a method described in [3-5]. It has been found that the 11S and 7S globulins contain, respectively: 16 and 14% of α-helices; 15% each of β-structures; 18 and 20% of β-bends; and 51% each of irregular sections. (The globulins were isolated from seeds of the cotton plant of variety 108-F.) The calculations were based on the assumption that the molar ellipticity of a protein [θ] is described by the sum of the contributions from five components (α-helix, antiparallel and parallel β-structures, β-bends, and irregular sections):

$$[\theta]_\lambda = \sum_{i=1}^{5} f_i [\theta]_{\lambda_i},$$

where f_i represents the molar fractions of the residues in the corresponding formations; and $[\theta]_{\lambda_i}$ represents the reference spectra of the corresponding formations.

It has been shown [5] that the separation of the parallel and antiparallel β-structures is not very accurate, and therefore in these figures we give the total amounts for the β-structure.

It has also been shown [6, 7] that the secondary structures of plant proteins change as the seeds grow and during their ripening. Some properties of the globulins change when they are isolated from the seeds of different varieties of cotton plant and from seeds stored under different conditions. Since a change in the properties of a protein is connected with a change in its structure, we have studied by the CD method the elements of the secondary structure of the 11S and 7S globulins isolated from the seeds of different varieties stored under different conditions. The 11S and 7S globulins were isolated from fresh seeds of the variety Tashkent 1 and from the seeds of the same variety stored for two months in the air at +20°C, +4°C, and −15°C. The results of measurements of the elements of the secondary structures of the histidine and arginine proteins are given below:

Plant	α-Helix, %	β-Structure, %	β-Bends, %	Irregular sections, %
Histidine globulins from the fresh seeds	27	22	24	27
" stored for 2 months at				
+20°C	17	17	18	48
+4°C	9	16	17	58
−15°C	4	18	18	60

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Arginine globulins from seeds stored for 2 months at				
+20°C	13	17	21	49
+4°C	13	17	20	50
−15°C	9	17	19	55

Analysis of the results obtained showed that the secondary structure was most ordered in the 11S globulin isolated from the fresh seeds. When the seeds were stored, the orderedness of the secondary structures fell, the most labile section being the α-helical sections of the polypeptide chain, while the conditions of storage had a substantial influence on this denaturation. Thus, the storage of the seeds under conditions of increased humidity even at −15°C led to a marked change in the conformation of the protein.

The change in secondary structure led to a change in the properties of the 11S globulin such as its solubility. Thus, the 11S globulin isolated from fresh cotton seeds possesses a good solubility which fell sharply after the seeds had been stored for only two months. The arginine globulin was more stable than the histidine globulin.

It must be mentioned that both globulins from fresh cotton seeds are fairly stable. Thus, when they were stored in sealed tubes in the refrigerator their secondary structures had scarcely changed even after two years, as can be seen from the results presented below:

Sample	α-Helix, %	β-Structure, %	β-Bends, %	Irregular section, %
Histidine globulin from fresh seeds of variety 108-F	16	15	18	51
" stored in sealed tubes for 2 years	14	18	18	50
Arginine globulin from fresh seeds of variety 108-F	14	15	20	51
" stored in sealed tubes for 2 years	12	16	19	53

For the case of the histidine globulin we also found a dependence of the secondary structure on the variety of the seeds:

Histidine globulin from the seeds of variety	α-Helix, %	β-Structure, %	β-Bends, %	Irregular sections, %
Tashkent 1	27	22	24	27
108-F	16	15	18	52

Thus, the secondary structure and the properties of the globulins from cotton seeds depend on the conditions under which they are stored and the variety from which they have been obtained, which must be taken into account in the development for the conditions for obtaining food protein.

EXPERIMENTAL

The histidine and arginine globulins were isolated from graded cotton seeds by the procedures described in [8, 9].

The CD spectra were recorded on a JASCO J-20 spectropolarimeter at protein concentrations of ∿0.5 mg/ml in cells 0.05 cm long. The sensitivity of the instrument was 0.002° per 1 cm and the constant time of the instrument 4 sec. The concentrations of the solutions were determined by the biuret method [10]. The results obtained were expressed in the form of molar ellipticities calculated to mean amino acid residue [θ]. The mean molecular weight of a residue was calculated from the amino acid composition of the protein and was 129.

The secondary structures of the proteins were determined from the CD spectra by the method of least squares as a result of the minimization of the functional

$$S = \sum_{\lambda} \left(\sum_{i} f_i [\theta]_{i\lambda} - [\theta]^{\exp} \right)^2$$

under the conditions $\sum_i f_i = 1$ and $1 \geqslant f_i \geqslant 0$, where f_i is the molar fraction of the i-th secondary structure, $[\theta]_\lambda^{exp}$ is the experimental CD spectrum and $[\theta]_{i\lambda}$ is the reference CD spectrum for the i-th structure. The values of $[\theta]_\lambda^{exp}$ and $[\theta]_{i\lambda}$ were taken every 1 nm in the wavelength range from 205 to 240 nm. The calculations were performed on a Hewlett-Packard 9830 A computer.

CONCLUSION

1. The secondary structures of the reserve proteins from cotton seeds have been determined by the CD method.

2. It has been established that the conformations of the main globulin components of cotton seeds change according to the conditions of their storage.

3. It has been shown that the secondary structure of the 11S globulin differs for seeds from the cotton plant varieties Tashkent 1 and 108-F.

LITERATURE CITED

1. S. I. Asatov, É. G. Yadgarov, T. S. Yunusov, and P. Kh. Yuldashev, Khim. Prir. Soedin., 54 (1978).
2. M. A. Kuchenkova, É. F. Redina, N. L. Ovchinnikova, and P. Kh. Yuldashev, Khim. Prir. Soedin., 687 (1977).
3. I. A. Bolotina, V. O. Chekhov, V. Yu. Lugauskas, A. V. Finkel'shtein, and O. B. Ptitsyn, Mol. Biol., 14, 891 (1980).
4. I. A. Bolotina, V. O. Chekov, V. Yu. Lugauskas, and O. B. Ptitsyn, Mol. Biol., 14, 902 (1980).
5. I. A. Bolotina, V. O. Chekhov, V. Yu. Lugauskas, and O. B. Ptitsyn, Mol. Biol., 15, 167 (1981).
6. K. G. Kumar, L. V. Venkataraman, and A. G. A. Rao, J. Agric. Food. Chem., 28, 518 (1980).
7. A. L. Li, R. A. Rafikov, Z. S. Yunusova, I. A. Bolotina, T. S. Yunusov, and G. P. Moiseeva, Khim. Prir. Soedin., 349 (1984) [in this issue].
8. S. I. Asatov, T. S. Yunusov, and P. Kh. Yuldashev, Khim. Prir. Soedin., 791 (1977).
9. S. S. Yunusova and T. S. Yunusov, Khim. Prir. Soedin., 770 (1981).
10. T. Devenyi and J. Gergely, Amino Acids, Peptides, and Proteins, Elsevier, New York (1974).

ACTIVATION OF A CARBOXY GROUP BY DIALKYL PYROCARBONATES. SYNTHESIS OF SYMMETRICAL ANHYDRIDES AND ARYL ESTERS OF N-PROTECTED AMINO ACIDS USING DI-tert-BUTYL PYROCARBONATE AS CONDENSING REAGENT

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It has been shown that di-tert-butyl pyrocarbonate can be used as a condensing reagent in the production of anhydrides and some aryl esters of carboxylic acids. The synthesis of anhydrides and of phenyl, p-nitrophenyl, β-naphthyl, and quinolin-8-yl esters of N-protected amino acids is described.

Di-tert-butyl pyrocarbonate (DBPC) which we have proposed as a reagent for the introduction of the tert-butoxycarbonyl (BOC) amino-protective grouping into amino acids [1, 2] has recently found wide use not only for obtaining N-BOC derivatives of amino acids [3, 4] but also for the tert-butoxycarbonylation of compounds of various classes. DBPC comparatively readily acylates hydrazides [5, 6] and, in the presence of alkali, the mercapto group of cysteine [2] and the phenolic hydroxyl of tyrosine [7].

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