LITERATURE CITED

1. A. L. Li, G. A. Piyakina, E. G. Yadgarov, T. Yu. Shadrina, S. I. Asatov, T. S. Yunusov, and P. Kh. Yuldashev, Khim. Prir. Soedin., 680 (1979).
2. W. R. Gray, Methods Enzymol., 25, 333 (1972).

PRIMARY STRUCTURE OF SUBUNIT B OF THE 11S GLOBULIN OF COTTON SEEDS OF VARIETY 108-F. VI. RECONSTRUCTION OF THE POLYPEPTIDE CHAIN

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UDC 547.962.5

Subunit B of the 11S globulin of cotton seeds, the polypeptide chain of which contains about 190 amino acid residues, has been reconstructed on the basis of large fragments from three types of tryptic hydrolyses.

The study of proteins from thermophilic and mesophilic sources has attracted a broad group of research workers in recent years [1]. The elucidation of the interrelationship of these proteins with their properties is of both theoretical and practical interest. It is primarily of practical importance for the reserve proteins of plant seeds.

The production of the proteins of food isolates is usually connected with the treatment of the raw material under various conditions, including different temperatures. As a rule, for the reserve proteins, a correlation is made between composition and thermal stability [2]. Thus, Biglow [3] assigned proteins with a high value of the mean hydrophobicity to the thermophilic proteins. The mean hydrophobicity of the main globulin of cotton seeds (11S) is about 900 cal/res. At the same time, the protein has a comparatively low solubility in solvents of high ionic strength and exhibits a high thermolability [4].

It must be mentioned that the hydrophobicity of protein globules is undoubtedly connected with the total number of hydrophilic residues in the molecule. However, the thermophilicity of proteins is, in our view, determined also by the serial arrangement of the hydrophobic residues along the polypeptide chain and, in particular, by the distribution of the hydrophobic radicals between the hydrophobic core of the molecule and the hydrophobic clusters on the surface of the protein globule. For proteins with a complex quaternary structure, this factor is of fundamental importance. In the protein that we are studying there are no disulfide bonds, and in the intersubunit interactions, in addition to ionic forces, hydrophobic forces are definitely involved [5] — all this, and also the generally low mean hydrophobicity, imparts a highthermolability to the molecule.

In a study of the interconnection of the structure of a protein with its properties, in addition to chemical modification and enzymatic cleavage of the native molecule with various enzymes, we are determining the primary structures of the individual subunits in order to elucidate the features of each of them. The complete amino acid sequence of subunit C has been shown [6].

In the present paper we give results on the reconstruction of the polypeptide chain of subunit B. The strategy of the study included chymotryptic hydrolysis, complete tryptic hydrolysis, tryptic hydrolysis at arginine residues, and tryptic hydrolysis at lysine residues, and also the chemical cleavage of the chain at methionine and tryptophan residues. The study of all types of cleavage has been described in individual publications. Basic information on the polypeptide chain was given by a study of large fragments obtained with different types of cleavage of the protein by tyrpsin. Chymotryptic hydrolysis led to the formation of a large number of short peptides, part of which were lost on the ion-exchange column.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 230-233, March-April, 1984. Original article submitted February 18, 1983.

 0009-3130/84/2002-0212$08.50 © 1984 Plenum Publishing Corporation

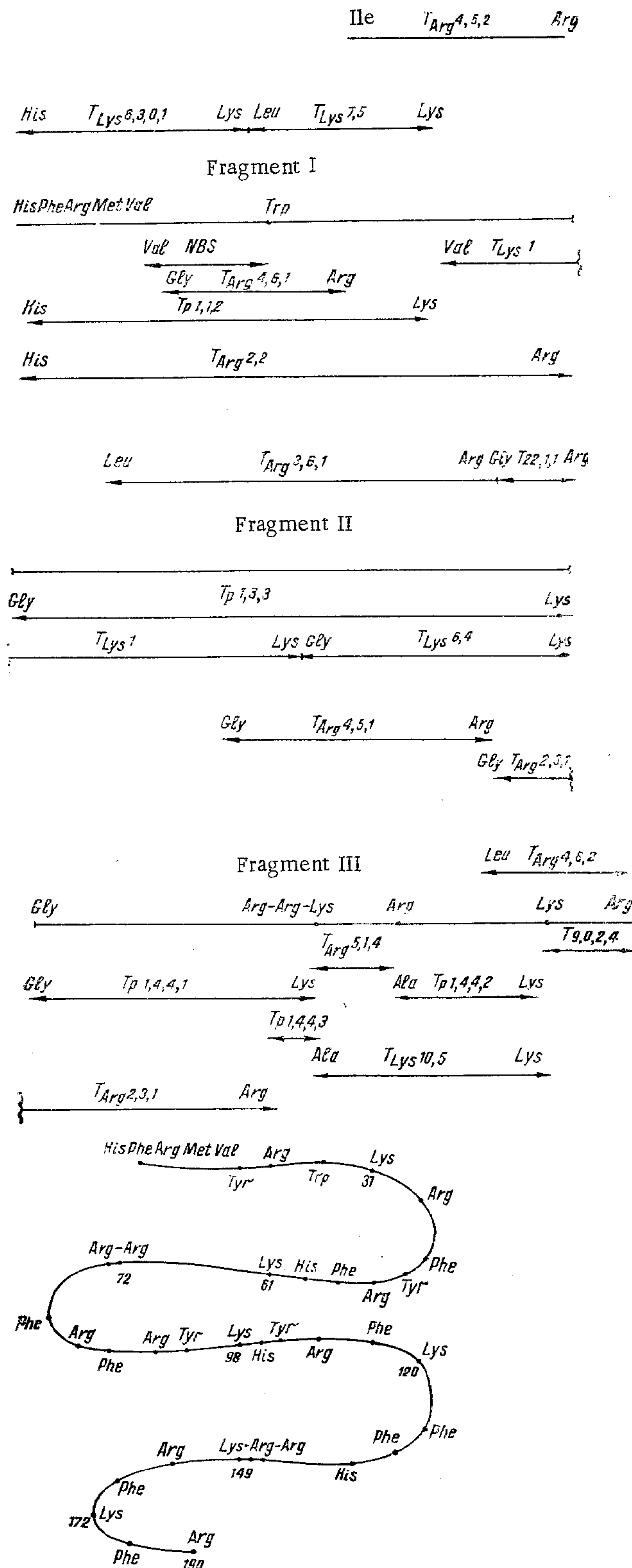

Illustration of the polypeptide chain of subunit B.

On complete tryptic hydrolysis, in addition to short peptides, three long ones were isolated: Tp1,4,4, Tp1,1,2, and Tp1,3,3, covering 165-170 amino acid residues of the 185 in subunit B. In the peptides mentioned there were uncleaved bonds at arginine and lysine residues. The stability of peptide bonds formed by arginine residues also appeared in the cleavage of the maleylated subunit B. On the other hand, in the cleavage of subunit B modified by cyclohexanedione complete cleavage took place at all the lysine residues. It must be mentioned that on modification at arginine residues the substrate was not soluble in the buffer while

on modification at lysine residues it was soluble. Thus, the solubility factor does not play an important role in this case. The stability of the peptide bonds of arginine and lysine in the protein under investigation may be a consequence of at least two factors:

the presence in the protein of some nonprotein substances (for example, phytic acid) blocking the arginine and lysine residues; and

the aggregation properties of subunit B or its partial hydrolysates, which are responsible for their resistance to the action of trupsin. It is possible that in the cyclohexanedione-modified protein such a change in the conformation of the substrate takes place and makes the lysine residues accessible to the action of tryptin in spite of the fact that the protein is insoluble under the conditions of cleavage.

The hypothesis of the possibility of the participation of phytic acid in the manifestation of the properties described above is not an arbitrary one. Cotton seeds contain an appreciable amount of phytin which, under certain conditions, may be bound to protein through arginine, lysine, and histidine residues [7]. In view of the fact that our protein had an isoelectric point of about 6.0-6.5, the binding of phytic acid to the protein could take place at pH values below 6.0 — all the more since traces of phosphorus have been detected in subunit B. To prove this, glucose oxidase and egg albumin were treated with phytic acid at pH 4.0. We then studied the capacity of these protein for being digested by trypsin. It was found that the untreated proteins were cleaved considerably more slowly than the proteins treated with phytic acid. This is apparently connected with the fact that phytic acid denatures the proteins at comparatively high pH values but is not strongly bound to them. This means that the resistance of the polypeptide chain of subunit B to trypsin is a consequence of features of the structure of the protein. A similar resistance to trypsin has been exhibited by subunit C from the same protein. Thus, the resistance of the subunits of the main globulin of cotton seeds to trypsin is a property of them and, moreover, the native 11S globulin also exhibits resistance to trypsin.

An analysis of the fragments obtained in various types of cleavage has permitted the reconstruction of the whole polypeptide chain of subunit B to be carried out. The basis of reconstruction consisted of the peptides of the tryptic hydrolysis of subunit B at lysine residues. In some cases, the large fragments were subjected to additional cleavage. Thus, in order to establish the position of the -Arg-Arg- bond, the peptide $T_{Lys}1$ was cleavage by trypsin, and the position of the -Arg-Arg-Lys- bond was determined by the trypsin cleavage of peptide Tp1,4,4 modified with cyclohexanedione at the arginine residues. In the majority of cases only the N-terminal sequences were established for the large peptides. On the whole, the reconstruction of subunit B, together with subunit C, which has been studied, has permitted an analysis of the distribution of the arginine and lysine residues and also of the hydrophobic amino acid residues along the chain, and this has enabled a number of properties of the native 11S globulin of cotton seeds to be explained. The results obtained are being used to investigate the process of obtaining food cottonseed protein.

EXPERIMENTAL

Treatment with phytic acid was carried out by dissolving a weighed amount of protein in a 0.1% solution of phytic acid at pH 4.0. The solution was dialyzed first against distilled water at pH 4.0 to eliminate the excess of phytic acid and then against water. After dialysis the solution was lyophilized, and the dry residue was used for cleavage with trypsin. The degree of cleavage was monitored by the TLC method. As a control we used the same protein kept at pH 4.0 for the same time as in the experiment.

SUMMARY

1. The reconstruction of subunit B has been carried out. A partial amino acid sequence of the polypeptide chain has been established.

2. The factors of the resistance of subunit B to trypsin have been ascertained.

3. It has been shown that the treatment of proteins with phytic acid can lead to substantial conformational changes in them which favors their digestion by proteases.

LITERATURE CITED

1. G. D. Kutuzova, N. N. Ugarova, and I. V. Berezin, Bioorg. Khim., 1445 (1982).
2. K. Shimada and S. Matsushita, J. Agr. Food Chem., 413 (1980).

3. C. C. Biglow, J. Theoret. Biol., 187 (1967).
4. S. I. Asatov, T. S. Yunusov, and P. Kh. Yuldashev, Khim. Prir. Soedin., 809 (1980).
5. T. Yu. Shadrina, T. S. Yunusov, and P. Kh. Yuldashev, Khim. Prir. Soedin., 554 (1979).
6. S. I. Asatov, É. G. Yadgarov, T. S. Yunusov, and P. Kh. Yuldashev, Khim. Prir. Soedin., 54 (1978).
7. K. Okubo, D. V. Myers, and G. A. Jacobucci, Cereal Chem., 513 (1976).

ARACHIDONIC ACID AND METHODS FOR ITS ISOLATION FROM NATURAL MATERIALS

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UDC 547.39:577.115.3:665.127.4

Methods that have been developed for isolating arachidonic or, in full, cis-eicosa-5,8,11,14-tetraenoic, acid from the wastes of the endocrine industry are described. The acid isolated has been characterized by physicochemical and spectral features. Prostaglandin E_2 has been obtained by enzymatic synthesis from arachidonic acid.

Arachidonic acid, together with other polyunsaturated acids, is a structural component of the lipoproteins of cell membranes and participates in the performance of a number of very important biochemical processes in the cell, which ensure the vital activity of the organism. The rising interest in arachidonic acid and methods for its isolation in recent times is due to the transformations of this acid into a whole series of biologically important metabolites — prostaglandins (PGs), thromboxanes (TXs), leukotrienes (LTs), etc. The processes for forming these metabolites in the living cell are based on the enzymatic oxidation of arachidonic acid with subsequent biotransformation into the final compounds.

Thromboxanes are closely interlinked with the processes of thromogenesis and hemopoiesis, and the leukotrienes participate in allergic (anaphylactic) reactions of the organisms. The prostaglandins, which are known as intracellular bioregulators of many physiologically important processes, exert an influence on the cardiovascular, respiratory, reproductive, and other systems.

The prostaglandins, thromboxanes, and leukotrienes are finding wide use in human and veterinary medicine. Already today, the use of prostaglandins has been described for the treatment of hypertonia, bronchial asthma, vascular thrombosis, and gastric ulcers, and in gynecology, etc. [1].

One of the promising methods of obtaining prostaglandins E_2 and $F_{2\alpha}$, thromboxanes A_2 and B_2, and prostacyclin I_2 is their enzymatic synthesis from arachidonic acid with the aid of specific multienzyme complexes isolated from various sources of animal origin.

Arachidonic acid (Δ^4Ach) is widely distributed in animal tissues. However, its isolation is complicated by its high sensitivity to oxidation and by its low percentage in the materials mentioned, and also by the fact that it is present together with other polyenoic acids with similar physicochemical properties. Arachidonic acid in the form of its methyl ester with different degrees of purity (87-96%) has been obtained from cattle adrenal glands [2, 3] and from porcine liver [4].

In the present paper we describe methods which we have developed [5] for isolating arachidonic acid from the lipids of the cattle pancreas, which are wastes of the endocrine industry. The proposed methods for isolating arachidonic acid from lipid wastes of the currently existing manufacture of endocrine preparations makes it possible to have not only the desired product but also to create a wastefree production for a number of preparations of the endocrine industry.

M. V. Lomonosov Moscow Institute of Fine Chemical Technology. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 233-241, March-April, 1984. Original article submitted March 25, 1983.

0009-3130/84/2002-0215$08.50 © 1984 Plenum Publishing Corporation