

IMPROVEMENT IN THE FUNCTIONAL PROPERTIES OF COTTON PROTEIN  
FOR NUTRIENT PURPOSES

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It has been shown that with an increase in the concentration of hydrochloric acid the solubility of the food protein obtained by extraction in a weakly acid medium falls, and it is practically completely absent at HCl concentrations greater than 2%. Food protein containing 0.05-0.07% of bound gossypol has been obtained. It has been established that when phytic acid is present in the protein, the bound gossypol can exist not only in the form of a compound with the  $\epsilon$ -NH<sub>2</sub> groups of the protein but also in the form of a complex with the phytic acid.

In addition to the traditional methods of solving the problem of nutrition — increasing the productivity of agriculture, animal husbandry, etc. — the full-value utilization of plant raw material is important. Effective sources of isolated protein are oil seeds — especially cotton seeds — and their extracted meals [1-3].

Protein isolates of cottonseed meal differ from the widely used soyabean food protein by poorer functional properties, which considerably decreases the sphere of utilization of the protein. For the effective use of cotton protein for food purposes its quality must be improved, namely the amount of essential amino acids, the digestability, the assimilability, and the solubility under various conditions must be raised, the amounts of toxic and coloring matters must be lowered, the taste and smell must be improved. The solution of the complex of these properties is a necessary part of the research work of chemists, technologists, and other specialists working in the field of food proteins.

Cottonseed protein, particularly if it contains a high amount of gossypol, possesses a low solubility in a neutral medium, which is due to a number of factors but above all to the composition of the food protein obtained. However, in this case it is impossible to draw a direct analogy with proteins according to the existing classification (albumins, globulins, glutelins, etc.), since the conditions of obtaining cottonseed meal on the industrial scale are extremely severe and may lead to substantial changes in the proteins which include not only their complete denaturation but also the possibility of the formation of complexes of the proteins with various accompanying components [4, 5]. Thus, the presence in the proteins of pigments, even if bound to come to the former only by a noncovalent bond, can sharply decrease their solubility. In cotton seeds such pigments include, in the first place, gossypol, which may be present both in the bound and in the free state [6-9].

Another no less important reason for the low solubility of the protein obtained may be the presence of phytic acid in it.

One of the promising methods for improving the solubility of a protein is its acetylation [10]. When the protein under investigation was acetylated, its properties did not change. This is connected with the fact that the  $\epsilon$ -NH<sub>2</sub> groups in the protein are mainly blocked, which prevents the acetylation of the cottonseed protein.

It is known that the  $\epsilon$ -NH<sub>2</sub> groups of lysine in a protein can be blocked by gossypol which, in general, decreases its solubility and also makes it difficult to increase the solubility of the protein by acetylation.

Analysis of the protein under investigation showed the presence of phosphorus compounds in it. These may be represented by phytic acid, which is present in small amount.

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## STRUCTURE OF THE PEPTIDE MOIETY OF VERTICILLIN

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The amino acid sequence of the peptide moiety of verticillin (I) — a phytotoxic metabolite of the causative agent of verticillium wilt of the cotton plant — has been determined. On the basis of the determination of the amino acid sequences of five short peptides isolated from the products of incomplete acid hydrolysis of oxidized desferriverticillin, the structure cyclo[L-glycyl-L-seryl-L-glycyl-(L- $\delta$ -N-hydroxyornithyl)<sub>3</sub>] has been established for the peptide moiety of (I).

We have previously reported the presence in a culture liquid of the fungus *Verticillium dahliae* Kleb., the causative agent of wilt in the cotton plant, of phytotoxic metabolites causing the characteristic symptoms of the disease when they are introduced into the cotton plant [1, 2]. In the present paper we give the results of a study of the structure of the peptide moiety of verticillin (I) — one of these substances.

On the basis of information obtained previously concerning the elementary composition, qualitative reaction, physicochemical properties, and some chemical transformations, (I) has been assigned to the siderophores of the ferrichrome type — cyclic hexapeptides forming strong complexes with trivalent iron and containing in their molecule three residues of  $\delta$ -N-acylated  $\delta$ -N-hydroxyornithine and three residues of other small amino acids [3]. Analysis of the products of the complete acid hydrolysis of (I) and of desferriverticillin with 7 N hydrochloric acid and reductive hydrolysis with 50% hydriodic acid showed that the peptide moiety of (I) consists of serine, glycine, and  $\delta$ -N-hydroxyornithine in a ratio of 1:2:3 [1]. Among the known siderophores, such an amino acid composition is possessed by ferricrocine [4]. However, the question of the sequence of amino acids in (I) and consequently its identity with the latter has remained unanswered.

To isolate (I) we used a method developed previously [5]. Desferriverticillin (II) was obtained by removing iron ions from (I) with the aid of 8-hydroxyquinoline [6]. To obtain peptide fragments we used incomplete acid hydrolysis, since the treatment of siderophores of the ferrichrome type with trypsin, chymotrypsin, or pepsin does not lead to the cleavage of this cyclopeptide [7]. The hydrolysis of (II) formed a complex mixture of ninhydrin-positive substances, probably because of the lability of the  $\delta$ -N-hydroxyornithine. On the other hand, the known method for the catalytic conversion of the latter into ornithine [8] is inapplicable, since it is accompanied by the reduction of serine to glycine. In order to eliminate these difficulties, the oxidation of (II) was carried out with performic acid, leading to the quantitative conversion of the  $\delta$ -N-hydroxyornithine into glutamic acid [6].

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