SOME CHARACTERISTICS OF THE ALKALI-SOLUBLE PROTEIN FROM THE RED ALGA Furcellaria fastigiata

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The Baltic agar-bearing red alga furcellaria (Furcellaria fastigiata), like other red algae, contains a considerable amount of nitrogenous substances [1]. A large part of them consists of soluble protein substances. In a consideration of the fractional composition of the proteins it was found that in the red Black sea alga phyllophora (Phyllophora nervosa) [2] the bulk of them consists of an alkali-soluble fraction. The ratio of the protein fractions in the red agar-bearing algae (% of total nitrogen) is as follows:

Protein Substances	Furcellaria	Phyllophora
Water-soluble	20.5	34.0
Salt-soluble	20.5	24.2
Alkali-soluble	54.8	41.8

The overwhelming amount of the alkali-soluble fraction among the soluble proteins of the alga furcellaria induced us to investigate it.

The alkali-soluble protein was isolated by a method similar to that described previously for phyllophora [3]. The alkali-soluble protein first isolated was highly contaminated. Its subsequent purification and careful fractionation by methods of repeated reprecipitation to constancy of composition, preparative gel filtration, and paper electrophoresis enabled us to obtain a purified preparation suitable for further investigation. The characteristics of the alkali-soluble protein of furcellaria during its purification and fractionation are shown in Table 1 and in Fig. 1.

The results obtained show that the alkali-soluble protein of furcellaria, like the analogous protein of phyllophora, contains carbohydrates strongly bound to it which cannot be separated either by reprecipitation, by gel filtration through Sephadex, or by electrophoresis.

In characterizing the isolated furcellaria protein we determined its amino-acid composition (% on the dry matter) and the N-terminal amino acids.

TABLE 1. Characteristics of the Alkali-Soluble Protein of Furcellaria (% on the dry matter)

Index	Initial extracted protein	Reprecipitate protein	Fractionated protein homogeneous on gel filtration and electrophoresis
Total nitrogen	6,91	12,68	15,61
Carbohydrates	58,72	19,68	11,15
Galactose	11,71	6,78	4,65
Glucose	35,45	10,10	6,50
Arabinose	6,00	0,66	—
Xylose	5,56	2,14	—

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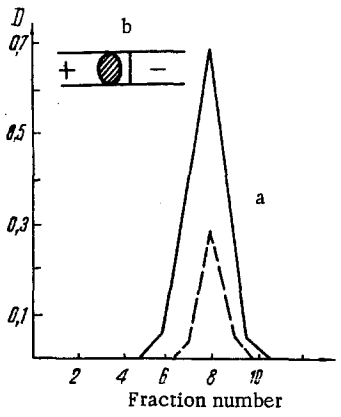

Fig. 1

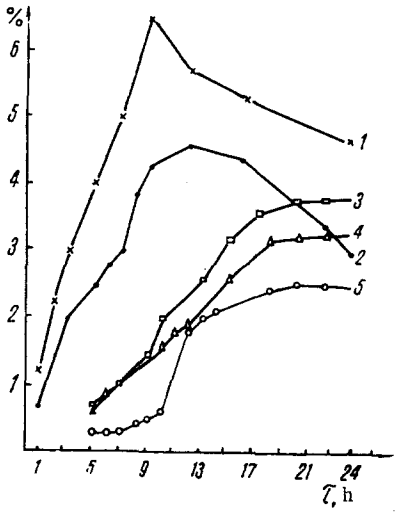

Fig. 2

Fig. 1. Preparative isolation of a high-molecular-weight fraction from the purified furcellaria protein: a) gel filtration through Sephadex G-100, rechromatography (full line – protein according to Lowry; dashed line – carbohydrates by the anthrone method); b) electrophoresis in borate buffer, pH 10.5.

Fig. 2. Kinetic curves of amino acids and carbohydrates in the weak acid hydrolysis with 0.5 N HCl of a high-molecular-weight fraction of the alkali-soluble protein of furcellaria: 1) glucose; 2) galactose; 3) tyrosine; 4) glutamic acid; 5) serine.

Amino Acids	Amount, %	Amino Acids	Amount, %
Cystine + cysteine	7.28	Alanine	5.04
Lysine	3.98	Proline	+
Histidine	3.78	Tyrosine	3.67
Arginine	9.06	Tryptophan	2.38
Aspartic acid	10.95	Methionine	1.33
Serine	4.36	Valine	3.69
Glycine	4.46	Phenylalanine	7.72
Glutamic acid	9.00	Leucine	2.57
Threonine	3.54	Isoleucine	4.38

As the figures given above show, the alkali-soluble protein of furcellaria is of full value with respect to its set of amino acids and their amounts. Attention is attracted by the high content of essential amino acids (with a full set of them) and the predominance of aspartic acid over glutamic. The sum of the basic amino acids is close to the sum of the acidic amino acids, amounting in total to 36.77%. The contents of hydroxy-containing amino acids (serine, threonine, and tyrosine: 11.57%) and of sulfur-containing amino acids (cystine + cysteine and methionine: 8.61%) are also relatively high. With such a peculiar ratio of basic and acid amino acids (16.82% and 19.95%, respectively), the N-terminal amino acid proved to be arginine.

The precipitation of the alkali-soluble protein at pH 4.5, the presence of arginine as the N-terminus, and the similar proportions of basic and acidic amino acids in its composition show the peculiar nature of this protein and its fundamental difference from the analogous protein of phyllophora investigated previously [4].

The presence in the furcellaria protein studied of strongly bound carbohydrates led to the necessity for using chemical methods, one of which is hydrolysis. As the catalyst we used a 0.5 N solution of hydrochloric acid. We followed the process simultaneously from the accumulation both of carbohydrates and of amino acids in the hydrolyzate. The kinetic curves characterizing acid hydrolysis are shown in Fig. 2.

An analysis of the kinetic curves showed a fact which we have observed previously in the study of other proteins of the alga phyllophora [3, 5], namely: At the beginning of hydrolysis it is mainly carbohy-

drates that pass into the solution and only a very small amount of amino acids. However, as soon as the yield of glucose in the hydrolyzate reaches a maximum, there is a sharp jump in the accumulation of certain amino acids (serine, tyrosine, glutamic acid). It may be assumed that the peptide chain of the alkali-soluble protein of furcellaria is stable as slong as it is "protected" by the carbohydrate bound to it. With a disturbance of the bond of the carbohydrates with the peptide chain, it is destroyed. Then precisely those amino acids which, as is known from the literature [6, 7], may because of their structure, participate in the destruction of peptide chains appear in the hydrolyzate. Thus, the appearance of the hydroxyl-containing amino acids serine and tyrosine and the dibasic glutamic acid in the hydrolyzate only after the elimination of the carbohydrates shows that, apparently, the carbohydrates and, primarily, the glucose ensured the preservation of the peptide chain by bonds with the amino acids mentioned.

A comparison of these facts and of the nature of the kinetic curve with V. A. Derevitskaya's results [8, 9] for other proteins has permitted a series of hypotheses concerning the nature of the hypothetical carbohydrate-protein bond in the alkali-soluble protein of furcellaria. The appearance of serine in the hydrolyzate after the liberation of glucose has given grounds for the suggestion of a possible O-glycosidic bond.

To confirm this hypothesis, we started from known facts concerning the β-elimination of serine in alkaline hydrolysis and the production of unsaturated compounds (aminoacrylic acid derivatives) which, on subsequent reduction with sodium tetrahydroborate, are converted into alanine [8, 9].

To determine the nature of the changes taking place on alkaline hydrolysis we used IR spectroscopy. A comparison of the IR spectra of the initial and the hydrolyzed proteins showed the following facts. In an alkaline hydrolyzate well-defined bands appear in the 880 cm^{-1} region that are characteristic for unsaturated compounds ($CH_2=C<$ deformation vibrations) and also the absorption of the valence vibration characteristic for a $-C=C-$ double bond in the 1585-1680 cm^{-1} region. In the subsequent reduction of the products of alkaline hydrolysis, the changes in the amounts of serine and alanine taking place as a result of the alkaline hydrolysis and subsequent reduction with tetrahydroborate were taken into account (% on the dry matter):

Amino Acid	Initial Protein	After Alkaline Hydrolysis
Serine	4.36	2.89
Alanine	5.04	6.75

The figures give grounds for the assumption that at least one of the types of carbohydrate-protein bond in the alkali-soluble protein of furcellaria is a O-glycosidic bond.

EXPERIMENTAL METHOD

The amino-acid composition was determined by paper chromatography [3] in the butan-1-ol–acetic acid–water (4 : 1 : 1 and 4 : 1 : 5) systems. The leucine and isoleucine were determined by using pyridine–isoamyl alcohol–water (35 : 35 : 30) as solvent.

To determine the amino acids, hydrolysis was performed for various times (1-48 h) at 100° C with 6 N HCl [5].

The monosaccharide composition was determined after hydrolysis with 0.5 N HCl (1 : 200) at 100° C for various times (1-24 h) followed by paper chromatography [5].

The uniformity of the fraction was shown by rechromatography on Sephadex-100 and electrophoresis in polyacrylamide gel [10]. The carbohydrate moiety was determined by the method of Coive and Grunwald in Kostyukovskaya's modification [3]. To determine the N-terminal amino acids we used the method of V. M. Stepanov [11] and S. V. Firfarova [12] with some additions [5]. As markers we used DNP-(amino acids) from British Drug Houses, Ltd.

Alkaline hydrolysis was performed by the method of N. K. Kochetkov and V. A. Derevitskaya [8, 9]. The IR spectra were taken on a IKS-14 spectrograph in carbon tetrachloride as solvent [13].

SUMMARY

From the red agar-bearing alga furcellaria we have isolated an alkali-soluble protein. Its amino-acid composition and N-terminal acid – arginine – have been determined. It has been shown to belong to

the group of glycoproteins. One of the possible types of carbohydrate–protein bonds is a O-glycosidic bond.

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