THE NATURE OF THE GLOBULIN OF THE ALGA PHYLLOPHORA NERVOSA

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The alga Phyllophora nervosa (a red alga of the Black Sea) contains a considerable amount of protein substances [1, 2]. We have investigated the globulin isolated from Phyllophora and purified it by fractional precipitation at the isoelectric point [2].

The total globulin was fractionated by gel filtration through a column of Sephadex G-100. This gave fractions A, B_1, and B_2, the homogeneity of which were checked by rechromatography and electrophoresis (Fig. 1). The fractions were dialyzed and then freeze-dried. The content of amino acids was determined in the hydrolysate (Table 1), tryptophan being determined in a separate sample [3].

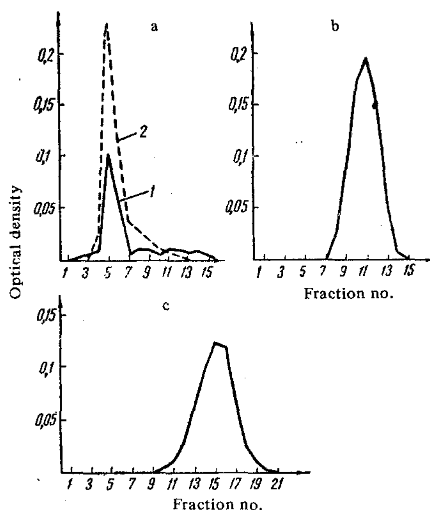

Fig. 1. Rechromatograms of fractions of the globulin of Phyllophora on Sephadex G-100. (Column 28 × 1.7 cm, buffering and elution with a 7% solution of common salt at pH 7.0 with a rate of elution of 0.5 ml/min). 1) Protein according to Lowry, 2) carbohydrates by the anthrone method, a) fraction A, b) fraction B_1, c) fraction B_2.

As the figures in Table 1 show, the qualitative amino acid composition of fractions A and B of the Phyllophora globulin is identical. However, there are considerable quantitative differences. Thus, a characteristic feature of fraction A is the high content of aspartic acid, threonine, proline, and phenylalanine, while fraction B has a considerably higher content of the basic amino acids, lycine, histidine, and arginine, and also tyrosine.

The results of a determination of the N-terminal amino acids in the fractions of the Phyllophora globulin studied are given in Table 2.

We see from Table 2 that the N-terminal amino acids of fraction A of Phyllophora globulin are lysine, of fraction B_1, lysine and glutamic acid, and of fraction B_2, glutamic acid.

The Phyllophora globulin fractions isolated preparatively were characterized by their content of carbohydrates,

[4]. On considering the rechromatography curves, we see as before [2], that the bulk of the carbohydrates is found in fraction A. Fraction B_1 also contains carbohydrate, but in a considerably smaller amount. Fraction B_2 does not contain any carbohydrate. The composition and amount of carbohydrates in fractions A and B_1 are different not only with respect to the total but also with respect to the particular monosaccharides present. Thus, fraction A contains 10.29% galactose, 6.40% glucose, and traces of arabinose and xylose. In contrast, only glucose is found in fraction B_1. The time necessary for the hydrolysis of these fractions shows that they can be classed among the easily-hydrolyzed protein substances. However, amino sugars were not found in any of the fractions.

Table 1. Amino Acid Content in the Fractions of Phyllophora Globulin (% on the Dry Weight of the Samples)

Amino acid	Fractions of the Phyllophora globulin		Amino acid	Fractions of the Phyllophora globulin	
	A	B		A	B
Lysine	3,36	8,85	Valine	4,02	3,45
Histidine	0,74	1,77	Methionine	0,42	0,35
Arginine	4,00	8,39	Isoleucine	2,75	1,41
Aspartic acid	11,06	8,63	Leucine	5,41	4,77
Threonine	6,14	3,78	Tyrosine	2,42	4,44
Serine	5,62	4,47	Phenylalanine	6,63	5,45
Glutamic acid	9,70	10,20	Tryptophan	1,40	+
Proline	8,02	6,96	Norleucine	+	+
Glycine	6,02	5,87	Cysteic acid	+	+
Alanine	5,28	4,50			

Thus, the differences in the molecular weight, in the amino acid content, and in the N-terminal amino acids, and also the qualitatively different carbohydrate composition and the difference in total amount of carbohydrates emphasize the substantial differences between fractions A and group B.

Table 2. Results of the Identification of the DNP-Amino Acids of the Fractions of Phyllophora Globulin

Fraction	R_f of the DNP-amino acids and other products of dinitrophenylation in the following solvents						N-terminal amino acid
	toluene		citrate buffer		borate buffer		
	exp	marker	exp	marker	exp	marker	
A	0,93	0,94	—	—	0,66	0,65	Lysine
	0,70	0,70	—	—	0,70	0,70	Dinitroaniline
B_1	0,054	0,054	0,62	0,63	—	—	Glutamic acid
	0,70	0,70	0,43	0,44	—	—	Dinitroaniline
	0,95	0,94	0,37	0,36	—	—	Lysine
B_2	0,049	0,051	—	—	—	—	Glutamic acid
	0,70	0,70	—	—	—	—	Dinitroaniline

On analyzing the nature of the kinetic curves of the liberation of carbohydrates in the acid hydrolysis of fraction A (Fig. 2), we see that the strength of the bonds of the glucose and galactose with the protein part of the molecule is different. Thus, at the beginning of hydrolysis with 0.5 N HCl glucose is liberated, and this continues for 6 hr. This period of degradation of the protein molecule is characterized by a very small accumulation of amino acids in the hydrolysate. After hydrolysis for 12–15 hr, the galactose has been liberated completely, after which the amount of aspartic acid, lysine, and tyrosine in the solution increases sharply. This is apparently due to the fact that galactose is a binding link between the carbohydrate and peptide moieties of fraction A. Consequently, the liberation of galactose is reflected in the degradation of the peptide chain. Thus, on the basis of the results obtained it may be assumed the carbohydrate part of the molecule is attached to the peptide chain through galactose. The part of the polypeptide chain to which the carbohydrates are attached is made up of the amino acids aspartic acid, lysine, and tyrosine.

The results obtained allow us to assume the existence of several types of bonds between the carbohydrate and protein parts of the molecules. In the present case, these may be ester, ether, amide or similar bonds [5].

An independent proof of the presence of an ester bond between the carbohydrate part and the peptide chain in fraction A, apparently through the β-carboxyl group of aspartic acid, is the appearance of aspartic acid in the hydrolysate immediately after the liberation of the carbohydrates. If the β-carboxyl group of aspartic acid were not

bound, the predominant cleavage of the peptide chain would take place in the region of aspartic acid, according to literature data.

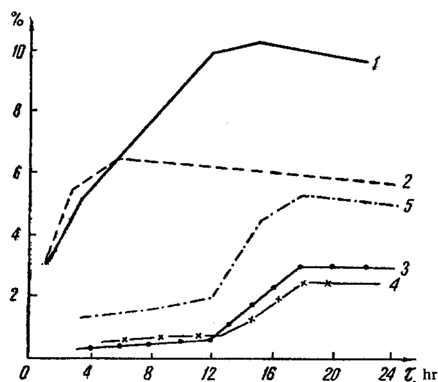

Fig. 2. Kinetic curves of the liberation of carbohydrates and amino acids in the hydrolysis of fraction of A of Phyllophora globulin with 0.5 N HCl: 1) galactose, 2) glucose, 3) lysine, 4) tyrosine, 5) aspartic acid.

The participation of the phenyl hydroxyl in the formation of a bond with the carbohydrate can be studied by several methods, including UV spectroscopy. The absorption curves of fractions A, B_1, and B_2 in the UV, which are given in Fig. 3, show that the absorption maximum for fractions B_1 and B_2 is at 278–279 mμ. This corresponds to conventional ideas concerning protein molecules.

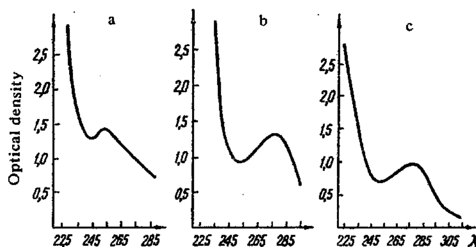

Fig. 3. UV spectra of fractions of Phyllophora globulin: a) fraction A, b) fraction B_1; c) fraction B_2.

The absorption maximum of fraction A is shifted to 255–260 mμ, which is generally associated with a decrease in the polarizability of the phenyl ring [7, 8] and may show the presence of a bound phenyl hydroxyl.

By comparing these results with the nature of the kinetic curves of the liberation of amino acids and carbohydrates, we may assume that an ether linkage exists between the carbohydrate moiety and the hydroxyl group of the tyrosine of the peptide chain of fraction A of Phyllophora globulin.

The results obtained enable fractions A and B_1 of Phyllophora globulin to be classed with the glycoproteins, although they differ from one another in their amino acid and carbohydrate content and in their N-terminal amino acids.

EXPERIMENTAL

Separation of the total globulin. The crude globulin was subjected to gel filtration through a column of Sephadex G-100, being eluted with a 7% solution of common salt at pH 7.0 at the rate of 0.5–0.8 ml/min. Fractionation was monitored for the protein fraction by Lowry's method and for the carbohydrate fraction with anthrone [2]. The

fractions obtained were salted out with ammonium sulfate to 45% saturation at pH 5.0, and then subjected to accelerated dialysis and freeze-dried.

Determination of the homogeneity of the fractions was carried out by moving-boundary electrophoresis on an EMIB instrument for microphoresis. Borate buffer with pH 10.8 and an ionic strength 0.05 was used to separate the globulin fractions. In contrast to the buffer solutions recommended in the instructions for the instrument, the solution which we used ensured a better separation and the production of a better defined curve.

Determination of the N-terminal amino acids was carried out by dinitrophenylation using the method of Stepanov [9] and Firfarova [10] with some additions. The dinitrophenylated amino acids were separated by chromatography after hydrolysis of the material under study in two solvents, toluene and citrate or borate buffer. The combination of these solvents gave the best separation and the most reliable identification. The markers were DNP-amino acids from the firm British Drughouses Ltd.

Determination of the carbohydrate content was carried out after the hydrolysis of the fractions in the catalytic presence of 0.5 N HCl (ratio 1 : 200) at 100° C for 1–18 hr. Samples of these hydrolysates were taken for the chromatographic determination of the carbohydrates and amino acids [11].

Determination of the amino sugars was carried out by the Elson-Morgan method [12] using as markers amino sugars from the firm California Corporation of Biochemical Research, Los Angeles.

Determination of the total amino acids content was carried out on the hydrolysate obtained by the action of 6 N HCl on an analyzer from the firm Evans Electroselenium Ltd. (England).

The UV spectra were taken on an SF-4a spectrophotometer in a 7% solution of common salt at pH 7.0.

CONCLUSIONS

The globulin of the alga Phyllophora is heterogeneous and consists of a number of fractions, some of which we assign to the glycoproteins. These fractions differ in their molecular weights, N-terminal amino acids, and the amount and composition of the amino acids and carbohydrates.

The main difference between the fractions of Phyllophora globulin is the nature of the carbohydrate bond with the true protein part of the molecule.

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