No appreciable differences have been observed in the absorption spectra of the two peptide fragments.

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IODINE-CONTAINING COMPLEXES OF THE BLACK SEA ALGA *Phyllophora nervosa*

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Iodine-containing complexes have been isolated from a Black Sea red alga. Information is given showing their carbohydrate nature. The causes of the hydrophobic peptide–peptide interaction in the complex are discussed.

The Black Sea red alga *Phyllophora nervosa* (DC) Grev. accumulates considerable amounts of iodine from seawater (up to 0.5% on the dry weight) and contains it in the form of organoiodine compounds which are not extracted by water, weak solutions of acids and alkalis, solutions of salts, or organic solvents [1, 2]. On the isolation from this alga of albumin, globulin, glutelin, and other soluble nitrogenous compounds by known methods [3], the iodine again remains in the insoluble residue. It is logical to assume that the soluble nitrogenous compounds of the alga do not contain iodine. At the same time, attention is attracted by the high amount of insoluble nitrogenous substances in the alga [3], which can be converted into soluble forms as the result of the alkaline degradation of its biomass [4, 5]. Under these conditions, the iodine compounds also pass into a water-soluble state and are detected in the form of iodinated peptides. This suggests that the alga contains insoluble and stable iodine–protein complexes.

We give the results of investigations of the iodine-containing fragments (peptides) obtained by the incomplete alkaline hydrolysis of insoluble iodine–protein complexes of *P. nervosa* which, in our opinion, permit definite information to be obtained about their structure and properties. The iodinated fragments were isolated by means of the following scheme (see scheme on following page.)

The water-soluble nitrogenous compounds were eliminated from the initial air-dry raw material by successive extraction with acetone, 70% ethanol, 7% NaCl solution, and 0.2% NaOH at 4°C and then the water-soluble polysaccharides (the bulk of the mass) were eliminated by treatment with hot water (90°C). The seaweed residue was treated with 1% caustic soda at 90-95°C for 4 h. As a result of the partial hydrolysis of the insoluble iron-protein complexes, alkali-soluble, iodine-containing compounds were obtained which were isolated by precipitation at pH 3.5. The precipitate was purified by repeated reprecipitation and was studied. The presence in the IR spectrum of the iodine compounds obtained of absorption bands (1660,

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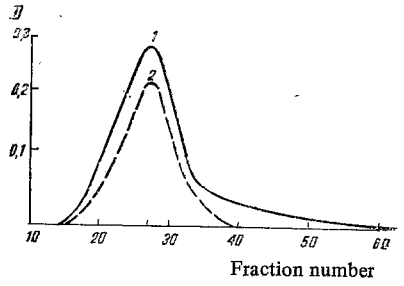


Fig. 1

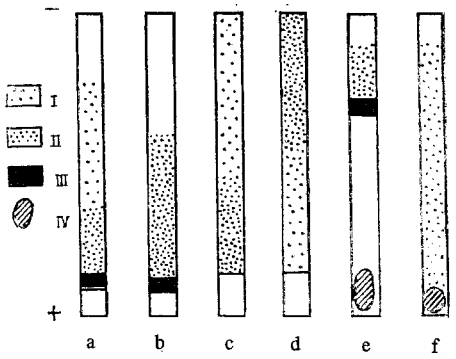
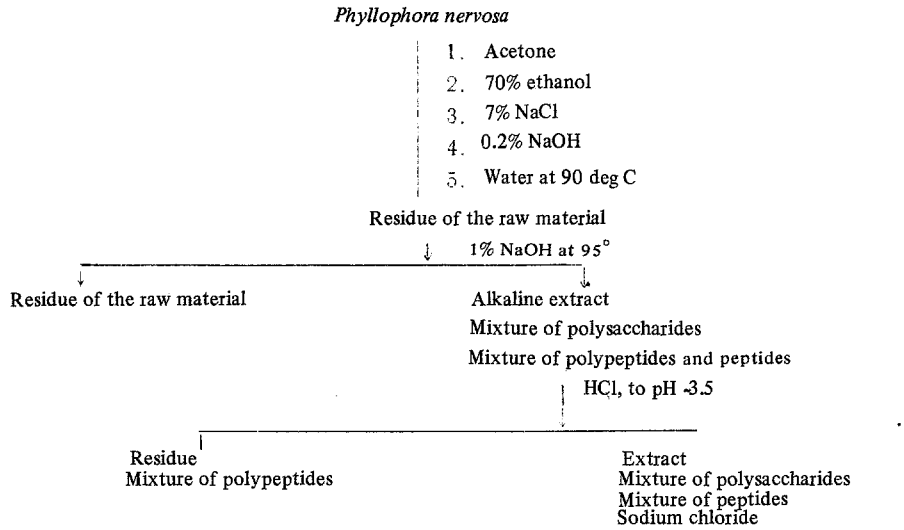


Fig. 2

Fig. 1. Gel chromatography of the iodine-containing compounds of *Phyllophora nervosa* on Sephadex G-75: column 1.8 × 6 cm: 1) protein according to Lowry; 2) carbohydrates.

Fig. 2. Electrophoretogram of the iodinated compounds of the alga in 7% PAAG in the presence of detergents: a) 0.2% Triton X-100; b) 8 M urea; c) 0.1% SDS; d) without detergent; e) 0.2% Triton X-100 after dansylation; f) without detergent after dansylation. The intensities of the coloration of the protein bands are indicated as follows: I) weak; II) strong; III) very strong; IV) DNS-OH.



1540, 1450 cm^{-1}) characteristic for proteins gives grounds for judging the structure of the natural iodine-protein complexes, as well. By comparing the IR spectra of the iodine-protein under investigation and of another protein of *Phyllophora* — a glycoprotein — likewise subjected to partial alkaline hydrolysis [6], it is possible to see that these proteins have a number of common absorption bands (at about 1450, 1250, 1080, 940, and 850 cm^{-1}) and reveal common properties. At the same time, in the IR spectrum of the iodine-protein complexes investigated a number of new bands are observed, as compared with the glycoprotein, mainly in the low-frequency region which apparently characterize a more complex structure of their side chains.

Thus, the results of gel filtration (Fig. 1) show that like the glycoprotein, the compounds under investigation probably contain bound carbohydrate residues. As can be seen from Fig. 1, in the process of gel filtration through a column of Sephadex G-75, the compounds under investigation are not separated into fractions but elute completely with the free (zero) volume of the column. Similar results were obtained on filtration through a column of Sephadex G-200. This can be explained by the presence of particles with masses exceeding 200 thousand. However, the formation of individual compounds with such a molecular mass under

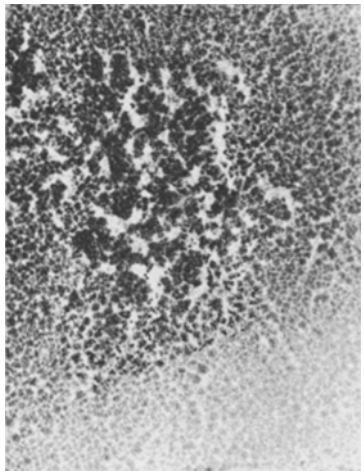


Fig. 3. Electron photomicrograph of a solution of the iodine–protein compounds of the alga (magnification 40,000).

these conditions is unlikely. It is more likely that the compounds under investigation are capable of mutual aggregation into larger formations. This is confirmed by the results of investigations using disk electrophoresis in polyacrylamide gel by Davis's method [7] in the presence of urea and of detergents — sodium dodecyl sulfate (SDS) and Triton X-100 — and also by electron microscopy. On electrophoresis in the presence of urea and of Triton X-100, the bulk of the iodine-containing compounds were concentrated in the form of a narrow band which moved towards the anode immediately after the marker (Fig. 2a, b). This shows that the iodine compounds under investigation are low-molecular-weight peptides with pronounced aggregating properties. The formation of high-molecular-weight associates is due mainly to hydrophobic interactions, as is shown by an increase in disaggregation (dissociation) on the addition of a nonionic detergent and of urea.

The presence of high-molecular-weight associates in solution containing the polypeptide compound of the phyllophora was also confirmed by electron microscopy. In Figure 3 small formations of rounded shape with a diameter of about 200 Å which are joined into larger aggregates — shapeless fragments of a size of about 1500 Å — can be clearly seen. In our case, with a rise in the pH the degree of disaggregation increased, which demonstrated the hydrophobic nature of the interactions in solution [8].

To confirm the results of electrophoresis in PAAG in the presence of a mixture of the peptides isolated, we determined their N-terminal amino acids and investigated them by isoelectric focusing with ampholines. Figure 4 shows isoelectrophoretograms of the preparation obtained, from which it can be seen that it consists of a complex mixture containing more than 20 fractions. After treatment with mercaptoethanol, additional zones were formed on the isoelectrophoretogram (Fig. 4b), since some of the peptides in the preparation consisted not of one but of several subunits linked, as usual, by disulfide bonds. The following amino acids were identified as N-terminal: Gly, Tyr, Ala, Phe, Leu, Val, Ile, Asp, Glu, and Ser. The amount of N-Gly considerably exceeded the amounts of the other amino acids. Thus, when a dansylated preparation was subjected to electrophoresis in a gel containing 0.2% of Triton X-100, a sharp fluorescing zone was formed (Fig. 2e) which was represented by peptides containing only glycine as their N-terminal amino acid residue. These results show that in the process of acidifying an alkaline hydrolysate at pH 3.5 there is a selective precipitation of peptides containing N-glycine. Furthermore, since the amount of precipitated peptides was about 70% (in terms of iodine) of the initial amount of iodine-containing complexes in the alga, in this case, obviously, there was a selective cleavage of peptide bonds with the glycine residue.

We turned our attention to the fact that the total amount of nitrogen by the Dumas method in the preparation (10.3-10.5%) was higher than the amount of protein nitrogen (9.4-9.6%) determined by the Kjeldahl method. This indicates that the polypeptides obtained also contain nitrogen groupings of nonprotein nature, which can be assigned to the chromophores, since the absorption spectra of the solutions investigated has intense absorption maxima in the visible region.

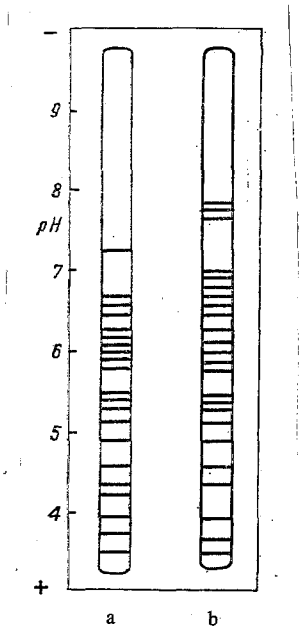


Fig. 4. Isoelectrophoretogram of the iodine-protein compounds of the alga: a) without treatment; b) after treatment with mercaptoethanol.

Using a method which is employed for the isolation of native chromophoric groups from phycobiliproteins (PBPs) [9], we treated the preparation with boiling methanol and passed the resulting extract through a column of silica gel. All the fractions collected had an absorption spectrum identical with the electronic spectrum of chlorophyll a. An intense band at about 405 nm (Soret band) appears in all cyclic tetrapyrrole compounds with a closed conjugated system [10]. An absorption band at 670 nm is characteristic both for chlorophyll a and also for its transformation products. It is known [11] that compounds of the chlorophyll type possess the capacity for effective association and the formation of adducts with extremely high molecular masses. This is due to the presence in them of nitrogen and oxygen atoms with unshared pairs of electrons, and also of keto groups and ester groups which form coordination bonds, in this case, with water molecules. The considerable amount (~10%) of compounds of the chlorophyll type in the preparation obtained is apparently the main reason for the formation in solutions of the polypeptides of the high-molecular-weight aggregates which we detected by gel filtration through Sephadexes, by disk electrophoresis, and by electron microscopy. Since these porphyrin derivatives can be isolated from the preparation obtained only by treatment with such strong reagents as methanol and concentrated solutions of HCl, it is obvious that in the alga they are present in the form of stable natural iodine–protein complexes.

EXPERIMENTAL

Isolation of the Polypeptides. The air-dry alga (50 g) was comminuted in a mill and was ground in a mortar with the addition of liquid nitrogen, and then the nitrogen compounds were eliminated successively with acetone, 70% ethanol, 7% NaCl, and 0.2% NaOH at 4°C. In all cases, extraction was continued until the nitrogen compounds soluble in the corresponding solvent had been completely eliminated. The residue of the alga was extracted with water (S:L ratio = 1:10) at 90°C for 10 h, the water being changed every 2 h. This eliminated the bulk of the gelling polysaccharides from the algal mass. The residue of the alga (with a moisture content of 87%) was treated with a 1% solution of NaOH at 95°C for 4 h (S:L ratio = 1:2). The iodine compounds were isolated from the resulting extract by acidification with hydrochloric acid to pH ~3.5, and filtered off and washed with water. Gel filtration was carried out on a column of Sephadex G-75 (fine) equilibrated with an ammonia buffer, pH 10. The rate of elution was 20 ml/h.

Fractions with a volume of 2 ml were collected in a LKB automatic collector (Sweden), and the protein fraction was monitored from its absorption at 280 nm and the carbohydrate fraction by means of anthrone. Disk electrophoresis in polyacrylamide gel was carried out

by the method of Ornstein and Davies [7], and isoelectric focusing in polyacrylamide gel with ampholines in the apparatus for disk electrophoresis. A 1% solution of ampholines (pH 8-10) was used as catholyte. For electron microscopy we used a Hitachi H-500 instrument. The iodopeptides were dissolved in an ammoniacal buffer at 40°C, and the solution obtained was included in a supporting film of 3% of collodion in amyl acetate, dried, and fixed with carbon in a Hitachi HUS-50 CB vacuum evaporating apparatus. The materials prepared in this way were dewatered with absolute ethanol and isopentane and were then examined under the microscope.

The free NH_2 groups of the amino acids were determined by the dansyl method of Gray and Hartley [12]. IR spectra were taken on a UR-20 instrument (tablets with KBr), and the electronic spectrum on a Specord spectrophotometer.

SUMMARY

From the insoluble iodine-protein compound of the red alga *Phyllophora nervosa* (DC) Grev., after degradation with alkali, iodonated peptides containing a bound carbohydrate component and nitrogen groupings of nonprotein nature with glycine as the N-terminal amino acid have been isolated.

The compounds isolated exhibit aggregation properties in weakly alkaline media which leads to the formation of high-molecular-weight associates as the result of hydrophobic interactions of the peptides.

The hypothesis has been expressed that the main reason for the formation of high-molecular-weight aggregates in solutions of the polypeptides of *P. nervosa* is the presence in them of a considerable amount of the compounds of the chlorophyll type.

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