A SOLUBLE CYTOCHROME OF THE b-TYPE FROM A GREEN ALGA, MONOSTROMA sp.*

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During a previous study of c-type cytochromes in various algae, the presence of a soluble cytochrome of the b-type in a water-extract of a green seaweed, Monostroma nitidum, was noted. This b-type cytochrome exhibited an absorption maximum for the α-band at approximately 560 mμ and occurred in an amount comparable to that of Monostroma cytochrome 553 (Katoh, 1959). Inasmuch as there has been no report concerned with a soluble cytochrome of the b-type from algal material, the isolation and characterization of this new heme protein was undertaken. This report describes the purification and some of the properties of this b-type cytochrome.

MATERIALS AND METHODS

Monostroma sp. was collected from the Chesapeake Bay at

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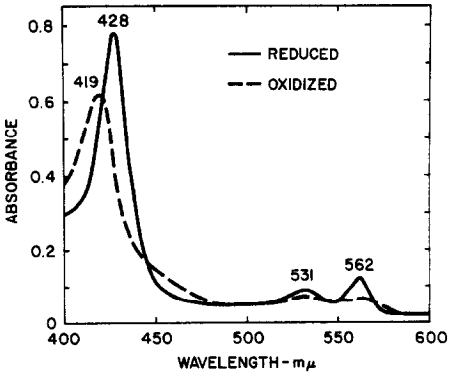
Solomon's Island, Md. Thalli were washed with distilled water and homogenized with 0.01 M phosphate buffer, pH 7.5, in a Waring blendor for 2 minutes. The homogenate was left at 4°C overnight and then strained through cheese cloth. Visual inspection of the filtrate with a hand spectroscope revealed the presence of distinct absorption bands at 553 and 562 mμ, respectively, which were intensified somewhat by the addition of sodium hydrosulfite. To one liter of the filtrate was added 400 g of ammonium sulfate and the resulting precipitate removed by centrifugation. An additional 36 grams of ammonium sulfate was added to the supernatant solution and the heavy precipitate, which appeared after standing for 2 hours at 4°C, was collected by centrifugation. During the ammonium sulfate fractionation, the pH of the solution was maintained at 7.5 with 2 N sodium hydroxide. The precipitate thus obtained was dissolved in 0.01 M phosphate buffer, pH 7.5, dialyzed extensively against a large amount of the same buffer, and placed on a column (5 x 15 cm) of diethylaminoethyl (DEAE) cellulose previously equilibrated against the same buffer. The charged column was washed with 0.05 M phosphate buffer, pH 7.5, and the cytochromes were eluted with 0.1 M phosphate buffer, pH 7.5, containing 0.2 M sodium chloride. A large amount of viscous material, brownish yellow in color, was removed from the cytochrome solution by this procedure. The cytochrome containing eluate was dialyzed against 0.01 M phosphate buffer, pH 7.5, and rechromatographed on a column (2 x 30 cm) of DEAE cellulose. After washing the charged column with 0.1 M phosphate buffer, pH 7.5, cytochrome 553 and a heme protein having an absorption maximum at 562 mμ were eluted separately with 0.2 M and

0.4 M phosphate buffer, pH 7.5, respectively. The latter protein was purified further by a similar chromatographic procedure.

RESULTS AND DISCUSSION

Absorption spectra of the purified heme protein are shown in Fig. 1. As isolated the heme protein was in the partially reduced form. The oxidized form of the heme protein was prepared by the addition of ferricyanide and dialysis against dilute phosphate buffer, pH 7.5. The protein exhibits absorption maxima at 419, 530 and 564 mμ in the oxidized form, and at 428, 531 and 562 mμ when reduced with sodium hydrosulfite.

The oxidized heme protein is readily reduced by ascorbic acid. Oxidation of the reduced protein is observed on addition of ferricyanide or oxidized horse heart cytochrome c. When the reduced protein was aerated for 10 minutes, there was only a slight decrease in absorbance at the absorption maxima of the α-and Soret-bands, indicating that the reduced protein is very poorly autooxidizable. Carbon monoxide is completely without effect on the absorption spectrum of the reduced protein.



Absorption spectra of cytochrome b (562 Monostroma).

The heme protein seems to have an oxidation-reduction potential higher than zero volt, since it was almost completely reduced by a mixture ferric and ferrous oxalate in a ratio of ferric to ferrous ion of 10; E_h of the mixture is 0.06 volt (Hill, 1954). On the other hand, the interaction between cytochrome c and the protein, when each was present in equivalent amount and each in the half reduced state, resulted in the complete oxidation of the algal protein and the concomitant reduction of the cytochrome c. This observation indicates that the heme protein has an oxidation-reduction potential lower than 0.25 volt (Rodkey and Ball, 1951).

The heme moiety of the protein was determined to be protoheme as judged from the absorption spectra of the corresponding hemochromogens. The absorption maxima of the reduced forms occurred at 419, 526 and 558 $m\mu$ for the pyridine hemochromogen and at 434, 539 and 569 $m\mu$ for the cyanide hemochromogen.

On the basis of these properties, the heme protein reported here is considered to be a new cytochrome of the b-type and is tentatively named cytochrome b (562 Monostroma). It is present in this alga in quite high concentration, when compared with the concentration of cytochrome 553. Assuming the millimolar extinction coefficients of cytochrome 553 and 562 to be 21.7 (Katoh, 1960) and 25.6 (Strittmatter and Velick, 1956) respectively, at the position of the α-band, the amounts of the cytochrome 553 and 562 isolated from 2.4 kg of thalli (fresh weight) are 15.7 and 14.5 $m\mu$moles, respectively. The molar ratio of cytochrome 553 to 562 is very near unity.

The presence of a similar cytochrome has only been observed in two green algae, Ulva sp. and Chlorococcum wimmeri. Thus far,

there is no indication of the presence of a water-soluble cytochrome of the b-type among other sea or fresh water algae including green, brown, red and blue-green algae.

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