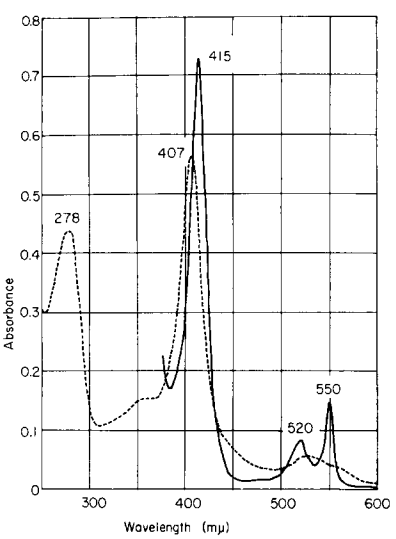
SHORT COMMUNICATIONS

BBA 43169

Purification and some properties of cytochrome *c* derived from the marine worm, *Dendrostomum zostericolum*

We have isolated cytochrome *c* in a state of high purity from the marine worm, *Dendrostomum zostericolum*, and studied some of its properties.

The marine worms (1500 g) were cut into pieces of approx. 1 cm length, homogenized with 0.01 M phosphate buffer, pH 7.0, and the resultant suspension was allowed to stand overnight at 5°. It was then filtered through celite and the filtrate centrifuged at 35000 × *g* for 20 min. The supernatant fluid obtained was dialyzed against 0.01 M phosphate buffer, pH 7.0, and the dialysate was charged on an Amberlite CG-50 column which had been equilibrated with the same buffer as used for the dialysis. The cytochrome *c* was adsorbed on the column. After the column had been washed with 0.05 M phosphate buffer, pH 7.0, the cytochrome *c* was eluted with 0.1 M phosphate buffer, pH 7.0, containing 10% saturated $(NH_4)_2SO_4$. To the eluate obtained (70 ml, $A_{550\,m\mu}$ (reduced) = 0.130) we added $(NH_4)_2SO_4$ to the saturation. The solution was centrifuged at 35000 × *g* for 10 min. The supernatant fluid



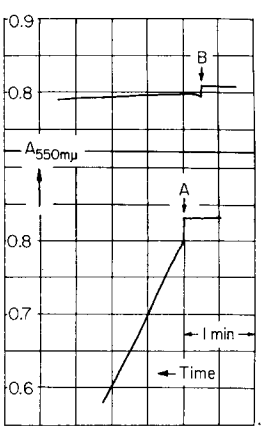


Fig. 1. Absorption spectrum of *D. zostericolum* cytochrome *c*. The protein was dissolved in 0.2 M phosphate buffer, pH 7.0. -----, oxidized; ———, reduced with $Na_2S_2O_4$.

Fig. 2. Reactivities of *D. zostericolum* cytochrome *c* with Pseudomonas and cow cytochrome oxidases. The cytochrome *c* was reduced by addition of a small amount of $Na_2S_2O_4$ and dialyzed for several hours against 0.04 M phosphate buffer, pH 6.5. The reactions were carried out in 0.04 M phosphate buffer at pH 6.5 and at 21°. At points B and A, 0.05 ml of 5.6 μM Pseudomonas cytochrome oxidase and 0.05 ml of 5.2 μM cow cytochrome oxidase were added, respectively, to 1.0 ml of the cytochrome *c* solution.

was charged on an Amberlite CG-50 column (described above) after dialysis against 0.01 M phosphate buffer, pH 7.0. The cytochrome *c* was adsorbed on the column. After the column had been washed with 0.05 M phosphate buffer, pH 7.0, the cytochrome was eluted with 0.05 M phosphate buffer (pH 7.0) containing 20 % saturated $(NH_4)_2SO_4$. The eluate obtained was dialyzed against 0.04 M phosphate buffer, pH 6.5, overnight, and the resulting dialysate was used as the purified *D. zostericolum* cytochrome *c* preparation. From 15 g of worms about 3 mg of cytochrome *c* was obtained, assuming that the ε_{mM} at 550 mμ and the mol. wt. were 28000 and 12000, respectively.

The cytochrome *c* possessed absorption maxima at 407 and 525 mμ in the oxidized form, at 415, 520 and 550 mμ in the reduced form, and its pyridine hemochrome showed absorption maxima at 414, 518 and 549 mμ.

The cytochrome *c* reacted very poorly with Pseudomonas cytochrome oxidase[1], and very rapidly with cow cytochrome oxidase[2]. Thus, the molecular activities (moles of cytochrome *c* oxidized per mole of cytochrome oxidase per min) were 1.0 and 105 in the reactions with Pseudomonas and cow cytochrome oxidase, respectively, at 21° and pH 6.5. These molecular activities were relative values; that is, in the reaction with Pseudomonas cytochrome oxidase, the molecular activity obtained when *Pseudomonas aeruginosa* cytochrome *c* reacted with the bacterial enzyme was taken as 100 %, and in the reaction with cow cytochrome oxidase the molecular activity when *Saccharomyces ovidormis* cytochrome *c* reacted with the animal enzyme was taken as 100 % (ref. 3). From the facts given above, it is apparent that *D. zostericolum* cytochrome *c* is a mammalian-type cytochrome *c*.

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