PRELIMINARY NOTES

BBA 61216

Isolation of a cytochrome peroxidase from *Thiobacillus novellus*

Yeast cytochrome *c* peroxidase (ferrocytochrome *c*: hydrogen-peroxide oxidoreductase, EC 1.11.1.5) was first isolated and partially purified by ALTSCHUL, *et al.*[1] and has been studied extensively by YONETANI and his co-workers[2,3]. So far as it is known, the peroxidase has not been found in any organism other than yeast. Recently, we have succeeded in isolation and purification of a haemoprotein from *Thiobacillus novellus*, and found that it shows a cytochrome *c* peroxidase activity.

Strain of *T. novellus* (Starkey) was kindly supplied by Dr. A. Asano(Institute for Protein Research, Osaka Univ., Japan). The cells (1 kg) of the organism which were cultivated in a bouillon–peptone medium at pH 7.2, were disrupted by sonication (Blackstone, 20Kc, 500W) for 5 min in 10 mM phosphate buffer, pH 7.0. The resulting sonicate was centrifuged at 13 500 × *g* for 25 min, and the supernatant thus obtained was fractionated by $(NH_4)_2SO_4$. The precipitate obtained at 60–90% saturation of $(NH_4)_2SO_4$ was collected by centrifugation and dissolved in a minimum volume of 10 mM Tris–HCl buffer, pH 8.5. The resulting solution was dialysed against 10 mM Tris–HCl buffer, pH 8.5, for 2 days, and the dialysate thus obtained was charged on

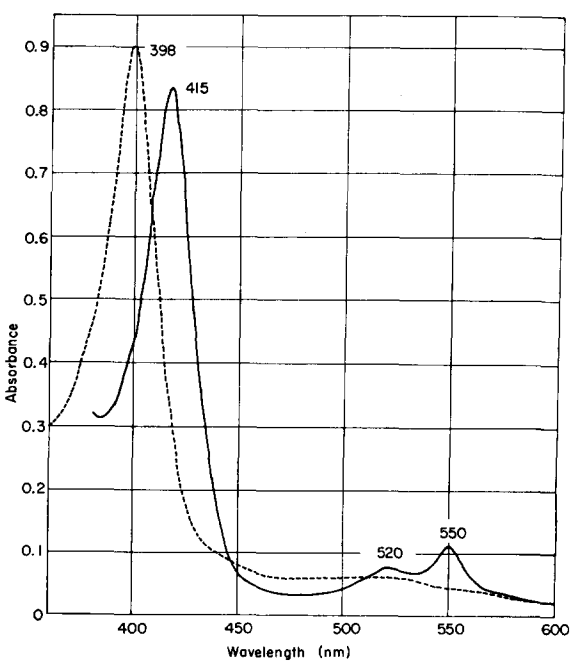

Fig. 1. Absorption spectrum of *T. novellus* haemoprotein. The haemoprotein was dissolved in 50 mM phosphate buffer. pH 7.0. – – – –, oxidized; ———, reduced with $Na_2S_2O_4$.

the DEAE-cellulose column which had been equilibrated with the same buffer as that used for the dialysis. On the cellulose column two bands appeared; one was brownish and at the top of the column, the other red and spreading below the brownish band to make a broad band. When the column was washed with 10 mM Tris–HCl buffer, pH 8.5, containing 0.2 M NaCl, the broad red band was eluted, while the brownish band remained on the column. The red eluate thus obtained contained cytochrome *c*-550. (This was quite similar in spectral properties to the cytochrome reported by CHARLES AND SUZUKI[4], with the organism cultivated in an inorganic medium.) The brownish band was eluted with 10 mM Tris–HCl buffer, pH 8.5, containing 1 M NaCl, and this eluate possessed a cytochrome *c* peroxidase activity.

As shown in Fig. 1, the fraction which has cytochrome *c* peroxidase activity showed an absorption peak at 398 nm in the oxidized form and peaks at 415, 520 and 550 nm in the reduced form. Although the absorption spectrum of the reduced form was very similar to that of C-type cytochromes, $A\gamma$ (reduced) was smaller than $A\gamma$ (oxidized). It was also noted that the absorption spectrum of the oxidized form showed a little larger absorbance around 480 nm than usual C-type cytochrome did, resulting in no peak around 530 nm. The haemoprotein in the reduced form combined with CO resulting in a shift of γ-band from 415 to 410 nm and sharpening of the band. Pyridine ferrohaemochrome of the preparation had absorption peaks at 413, 520 and 550 nm. This shows that the haemoprotein obtained here possesses haem *c* as the prosthetic group. From the absorption spectrum of the pyridine ferrohaemochrome, ε_{mM} at 550 nm of the haemoprotein was determined to be 16.8.

The haemoprotein preparation showed a cytochrome *c* peroxidase activity (Table I). The molecular activity (moles of reoxidized cytochrome *c*/mole of the haemoprotein) was 344 per min at 23° and pH 7.5 with tuna cytochrome *c*. The enzymatic activity was 39% inhibited in the presence of 1 mM KCN. The reaction was also

TABLE I

CYTOCHROME *c* PEROXIDASE ACTIVITY OF *T. novellus* HAEMOPROTEIN

The standard reaction mixture was composed of 0.05 ml of 245 μM tuna cytochrome *c* (ref. 5), 0.95 ml of 25 mM Tris–HCl buffer, pH 7.5, 0.01 ml of 3.7–49.5 mM H_2O_2 and 0.02 ml of 3 μM haemoprotein. The reduced form of tuna cytochrome *c* was actually not reoxidized on addition of H_2O_2 without the haemoprotein. In the experiment with yeast cytochrome *c* peroxidase, 0.02 ml of 0.1 μM the enzyme was added in place of the *T. novellus* haemoprotein. The reaction was performed at 23°, and the absorbance change was measured in a Cary spectrophotometer, Model 15.

Enzyme	*Salt added*	*H_2O_2 (μM(*	*$-\Delta A$ (550 nm/ min)*	*Molecular activity (moles of reoxidized cytochrome c/mole of enzyme per min)*
T. novellus	—	36	0.085	73
haemoprotein	—	180	0.296	255
haemoprotein	—	480	0.398	344
haemoprotein	0.1 M NaCl	180	0.218	188
haemoprotein	50 mM phosphate* buffer (pH 7.4)	180	0.122	105
Yeast cytochrome *c* peroxidase	—	180	0.323	8350

* The phosphate buffer was used in place of Tris–HCl buffer.

fairly depressed by salts; the depressions were 26% with 0.1 M NaCl and 59% with 50 mM phosphate. Although the inhibitory effect of the salts on the sulphite: cytochrome *c* oxidoreductase (EC 1.8.3 group) of the organism (cultivated in an inorganic medium) has been reported[4], it seems very interesting that the enzymatic activity, which does not directly relate to inorganic salt, is also affected appreciably by the salts. The cytochrome *c* peroxidase reaction of the haemoprotein was also affected by the kind of cytochrome *c* used as the electron denor. Namely, tuna and yeast cytochromes *c* reacted quite rapidly with the haemoprotein, while cytochrome *c* (551, *Pseudomonas aeruginosa*)[6] did not react with it. Reactivity with the haemoprotein of cytochrome *c* (551, *Rhodospirillum rubrum*)[7] and cytochrome *c* (555, *Chlorobium thiosulphatophilum*)[8] was 24% and 6%, respectively, of that of tuna cytochrome *c*. However, it is very curious that cytochrome *c* (550, *T. novellus*)[4,9] has reacted with the haemoprotein very poorly.

Although the cytochrome *c* peroxidase activity of the haemoprotein isolated from *T. novellus* was considerably low as compared with that of yeast cytochrome *c* peroxidase[2], it was 14 times as high as that of horseradish peroxidase (EC 1.11.1.7) (purchased from Worthington Biochem. Co., U.S.A.) on the basis of molecular activity. Further, the ratios of cytochrome *c* peroxidase activity to (guaiacol) peroxidase activity were 3.91 with the *T. novellus* haemoprotein and 0.0024 with horseradish peroxidase. As judged from these facts, it seems that the haemoprotein isolated from *T. novellus* is a cytochrome *c* peroxidase although it possesses haem *c* as the prosthetic group, unlike other peroxidases[10]. It seems quite interesting, in relation with the evolutionary position of *T. novellus*[11], that the haemoprotein is very similar to yeast cytochrome *c* peroxidase in the specificity for C-type cytochromes (T. YAMANAKA, unpublished results).

This research has been partly supported by a grant-in-aid from the U.S. National Institute of Health (GM 05871-12). We wish to thank Dr. T. Yonetani (Johnson Research Foundation, Univ. of Pennsylvania, U.S.A.), Dr. T. Horio (Institute for Protein Research, Osaka Univ., Japan) and Dr. K. Wada of this laboratory for their generosity in supplying yeast cytochrome *c* peroxidase, *R. rubrum* cytochrome *c*-551 and tuna cytochrome *c*, respectively.

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