A NOVEL FUNCTION OF CYTOCHROME C (555, CHLOROBIUM THIOSULFATOPHILUM) IN OXIDATION OF THIOSULFATE

A. Kusai and T. Yamanaka

Department of Biology, Faculty of Science, Osaka University
Toyonaka, Osaka, Japan

Received January 23,1973

Summary: Thiosulfate-cytochrome c-551 reductase derived from Chlorobium thiosulfatophilum has been highly purified. The enzyme reduces cytochrome c-551 of C. thiosulfatophilum in the presence of thiosulfate while cytochrome c-555 of the organism is not reduced by the enzyme. Cytochrome c-555 reacts with the enzyme at an appreciable rate only in the presence of cytochrome c-551. However, the reduction rate of cytochrome c-551 by the enzyme is greatly enhanced on addition of a catalytic amount of cytochrome c-555. Therefore, cytochrome c-555 seems to function as an effector on thiosulfate-cytochrome c-551 reductase as well as it acts as the electron donor to the light-excited chlorobium chlorophylls.

Although Chlorobium thiosulfatophilum acquires energy for its life processes by photosynthetic oxidation of thiosulfate, very little is known about the enzymatic mechanisms in the oxidation of thiosulfate in the organism.

The green sulphur bacterium possesses three kinds of C-type cytochromes, cytochrome c-551, cytochrome c-553 and cytochrome c-555 (1). As cytochrome c-555 shows many properties similar to those of the f-type cytochrome (2,3), it seems to function in the organism in the similar way as the f-type cytochrome does in algae and higher plants. The function of the other two kinds of cytochromes is still obscure. Recently, Mathewson et al. (4) have claimed that cytochrome c-551 is related to the oxidation of thiosulfate, as it is easily reduced on addition of thiosulfate with a crude extract of the organism, while the other two C-type cytochromes are not. However, they have not reported the detail

mechanisms in the enzymatic reduction of cytochrome c-551 by thiosulfate. In the present investigation, we have succeeded in purification of a thiosulfate-oxidizing enzyme of C. thiosulfatophilum, thiosulfate-cytochrome c-551 reductase and found that the reduction of cytochrome c-551 by the enzyme in the presence of thiosulfate is greatly accelerated by addition of a catalytic amount of cytochrome c-555 although the latter cytochrome is not directly reduced by the enzyme.

MATERIALS AND METHODS

Cultivation of C. thiosulfatophilum (NCIB 8346) was performed according to the method as previously described (2). Cytochrome c-551 and cytochrome c-553, and cytochrome c-555 were highly purified by the methods of Meyer et al. (1) and of Yamanaka et al. (2), respectively.

Purification of thiosulfate-cytochrome c-551 reductase was performed as follows: The acetone-dried cells (about 10 g) were suspended in 400 ml of 10 mM Tris-HCl buffer, pH 8.5 which had been 10% saturated with $(NH_4)_2SO_4$. The resulting suspension was centrifuged at 12,000 x g for 20 min after allowed to stand overnight with a continuous stirring. The supernatant thus obtained was fractionated with $(NH_4)_2SO_4$, the precipitate formed between 45% and 65% saturation collected by centrifugation and dissolved in 0.1 M Tris-HCl buffer, pH 8.5. The solution thus obtained was charged on the DEAE-cellulose column (4 cm x 8 cm) which had been equilibrated with 0.1 M Tris-HCl buffer, pH 8.5, after overnight dialysis against the same buffer as used for chromatography. After the column on which the enzyme was adsorbed was washed with 200 ml of the same buffer, the enzyme was eluted by the linear gradient which was produced from 200 ml each of 0.1 M Tris-HCl

buffer, pH 8.5 and 0.1 M Tris-HCl buffer, pH 8.5 containing 0.2 M NaCl. Fractions of 4 ml were collected. The enzyme was eluted at the NaCl concentration of 0.05-0.07 M. This chromatography step was once repeated and the main fraction thus obtained was used as the enzyme preparation.

Spectral measurement was performed with a Cary spectrophotometer, model 14 or 15, using cuvettes with 1 cm light path. Protein content was determined according to Lowry et al. (5).

RESULTS

As Table 1 shows, when the enzyme preparation was added to the reaction mixture of the oxidized form of cytochrome c-551 and thiosulfate, the cytochrome was gradually reduced. The reduction rate of cytochrome c-551 was greatly accelerated on addition of a catalytic amount or cytochrome c-555, while cytochrome c-555 itself was hardly reduced by the enzyme unless cytochrome c-551 was added (Table 2). Therefore, it is evident that cytochrome c-551 can accept electrons at an appreciable rate from the enzyme, whereas cytochrome c-555 does not react directly with the enzyme. However, cytochrome c-555 was fairly rapidly reduced by the enzyme in the presence of a small amount of cytochrome c-551. Cytochrome c-553 was not reduced by the enzyme even in the presence of cytochrome c-555. The acceleration effect on the reduction of cytochrome c-551 by the enzyme was also observed with horse cytochrome c in place of cytochrome c-555 although less effective.

As acetylated cytochrome c-555 was not so effective when used in place of cytochrome c-555, the basicity of the protein seemed bo be required for the activation effect on the enzyme. However, cytochrome c (554, Nitrosomonas europaea) (pI = 10.7) (6), cytochrome c_3 of Desulfovibrio vulgaris Miyazak (pI = 10.6) (7),

Table 1. Effect of cytochrome c-555 on the reduction of cytochrome c-551 by thiosulfate-cytochrome c-551 reductase with thiosulfate

Standard reaction mixture contained 100 mM sodium phosphate (pH 6.0), 10 mM sodium thiosulfate, 40 mM cytochrome c-551 and the enzyme (87 µg) in a total volume of 1.0 ml. The final concentration of added cytochrome c-555 was shown in the parentheses. The reaction was started by adding the enzyme and the increase in the absorbance at 551 nm was followed spectrophotometrically with time.

Conditions for activity determination	$\Delta A_{551\ nm}$ / min
Standard	0.035
- Enzyme	0.010
- Thiosulfate	0.000
- Cytochrome c-551	0.000
+ Cytochrome c-555 (0.5 µM)	0.100
+ Cytochrome c-555 (2.0 µM)	0.150

Table 2. Reduction of cytochrome c-555 by thiosulfate-cytochrome c-551 reductase with thiosulfate in the presence of cytochrome c-551

Complete reaction mixture contained 100 mM sodium phosphate (pH 6.0), 10 mM sodium thiosulfate, 40 mM cytochrome c-555, 4.5 µM cytochrome c-551 and the enzyme (87 µg) in a total volume of 1.0 ml. The reaction was started by adding the enzyme and the increase in the absorbance at 555 nm was followed spectrophotometrically with time.

Conditions for activity determination	$\Delta A_{555\ nm}$ / min*
Complete	0.05
- Enzyme	0.006
- Thiosulfate	0.000
- Cytochrome c-551	0.006
- Cytochrome c-555	0.003

* Calculated from the absorbance difference per 10 min.

lysozyme or polylysine was ineffective. Therefore, the activation effect of cytochrome c-555 on the thiosulfate-cytochrome c-551 reduction seems to be specific for the cytochrome although it seems not to undergo oxidation-reduction during its functioning as the activator.

The thiosulfate-cytochrome c-551 reductase preparation of C. thiosulfatophilum was colourless; i.e. there was only a peak at 280 nm when the spectrum was measured in the wavelength region from 260 nm to 600 nm. The enzyme was not activated on addition of FMN or FAD, or ions such as Cu^{++}, Zn^{++}, Co^{++} or Mn^{++}. It was strongly inhibited by cyanide and sulfite. Molecular weight of the enzyme was determined to be about 80,000 by acrylamide gel electrophoresis in the presence of SDS and mercaptoethanol (8).

DISCUSSION

A thiosulfate-cytochrome c-551 reductase has been highly purified from C. thiosulfatophilum. The enzyme reduces slowly C. thiosulfatophilum cytochrome c-551 in the presence of thiosulfate, and the reduction rate is greatly accelerated by addition of a catalytic amount of cytochrome c-555 of the organism. However, cytochrome c-555 itself does not react with the enzyme unless cytochrome c-551 is present. Namely, cytochrome c-551 reacts with the enzyme even in the absence of cytochrome c-555 while cytochrome c-555 does not react with the enzyme without addition of cytochrome c-551. This seems to mean that although cytochrome c-555 accelerates the thiosulfate-cytochrome c-551 reduction greatly, the cytochrome does not function simply as the electron carrier between the enzyme and cytochrome c-551. In any case, the fact that cytochrome c-555 which can not accept electrons directly from the enzyme accelerates the electron trans-

fer from the enzyme to cytochrome c-551 seems to mean that cytochrome c-555 may function not only as the electron carrier but as an effector on the enzyme.

C. thiosulfatophilum possesses three kinds of C-type cytochromes as already mentioned. From the facts mentioned above, cytochrome c-551 seems to function as the direct electron acceptor of the thiosulfate-cytochrome c-551 reductase. This is in good agreement with the observations reported by Mathewson et al. (4). However, the reduction mechanisms of cytochrome c-551 by the enzyme are not so simple as they have reported. As cytochrome c-555 has many properties similar to those of the f-type cytochrome (2,3), it seems to function as the electron donor to the light excited chlorobium chlorophylls. Therefore, the major part of the cytochrome will function as the electron donor to the light excited chlorophylls, while the remaining part of the protein will function as the effector on the thiosulfate-cytochrome c-551 reductase.

REFERENCES

1. Meyer, T. E., Bartsch, R. G., Cusanovich, M. A. and Mathewson, J. H. (1968), Biochim. Biophys. Acta, 153, 854-861.
2. Yamanaka, T. and Okunuki, K. (1968), J. Biochem. Tokyo, 63, 341-346.
3. Kusai, A. and Yamanaka, T. (1973), Biochim. Biophys. Acta, (in press).
4. Mathewson, J. H., Burger, L. J. and Millstone, H. G. (1968), Fedn. Proc., 27, 774.
5. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951), J. Biol. Chem., 193, 265-275.
6. Yamanaka, T. and Shinra, M. (1971), Seikagaku, 43, 647.
7. Yagi, T. and Maruyama, K. (1971), Biochim. Biophys. Acta, 243, 214-224.
8. Weber, K. and Osborn, M. (1969), J. Biol. Chem., 244, 4406-4412.