CYTOCHROME *c* (553, *CHLOROBIUM THIOSULFATOPHILUM*) IS A SULPHIDE-CYTOCHROME *c* REDUCTASE

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1. Introduction

Three kinds of *c*-type cytochromes, cytochromes *c*-551, *c*-553 and *c*-555 have been isolated from the green sulphur bacterium, *Chlorobium thiosulfatophilum* [1, 2]. Recently, we have succeeded in the purification of a thiosulphate-cytochrome *c* reductase derived from *C. thiosulfatophilum* and found that cytochrome *c*-551 functions as the direct electron acceptor for the enzyme [3]. Molecular features of cytochrome *c*-553 have been well studied [1, 4]; it has flavin (probably FMN) as well as haem *c*. However, its function was obscure.

In the present investigation, we have found that cytochrome *c*-553 is reduced more rapidly with sulphide than other cytochromes *c*, and it catalyses reduction of cytochromes *c* with sulphide. Therefore, cytochrome *c*-553 seems to act as a sulphide-cytochrome *c* reductase. Further, we have found that the cytochrome combines with cyanide very strongly and as a result the absorption spectrum attributable to the bound flavin of the cytochrome is considerably changed.

2. 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 and Okunuki [2], respectively. Yeast (*Saccharomyces oviformis*) cytochrome *c* was kindly supplied by Sankyo Co., Ltd. (Tokyo, Japan).

Isoelectric focusing was performed at 0°C, using a 1% (v/v) ampholyte solution (LKB-Produkter AB, Stockholm) [5]. Spectral measurement was performed with a Cary spectrophotometer, model 15, using cuvettes with 1 cm light path.

Table 1

Reduction of several kinds of cytochromes *c* by sodium sulphide and effect on their reduction rates of added cytochrome *c*-553.

Cytochromes	Addition of cytochrome *c*-553	(ΔA_{α}/min)
Cytochrome *c*-551	–	0.18
	+	0.18
Cytochrome *c*-553		> 4.0
Cytochrome *c*-555	–	0.02
	+	0.20
Yeast cytochrome *c*	–	0.15
	+	0.45

The reaction mixture contained: 0.1 M Tris–HCl buffer (pH 7.4), 50 μM sodium sulphide and 50 μM each cytochrome *c* with (+) or without (–) 34 nM cytochrome *c*-553. The total volume of the reaction mixture was 1.0 ml. The reaction was started by adding sodium sulphide.

3. Results and discussion

As table 1 shows, cytochrome *c*-553 was reduced by sodium sulphide considerably more rapidly than cytochrome *c*-551, cytochrome *c*-555 and yeast cytochrome *c*, and the reduction rates of cytochrome *c*-555 and yeast cytochrome *c* by sodium sulphide were

Table 2
Reduction of yeast cytochrome *c* by cytochrome *c*-553 with sodium sulphide.

Conditions for determination of activity	(ΔA_{550}/min)	Net increase in ΔA_{550}/min by cytochrome *c*-553
Complete	0.45	0.30
− Cytochrome *c*-553	0.15	0.00
Cytochrome *c*-553 treated with heat	0.15	0.00
+ KCN (1 μM)	0.21	0.06
+ KCN (0.1 μM)	0.30	0.15
Cytochrome *c*-553 treated with 1 μM cyanide and then with Sephadex G-25	0.25	0.10
Cytochrome *c*-553 treated with cyanide, then with $HgCl_2$ and Sephadex G-25	0.45	0.30

The complete reaction mixture contained 0.1 M Tris–HCl (pH 7.4), 50 μM sodium sulphide, 50 μM yeast cytochrome *c* and 34 nM cytochrome *c*-553. The total volume of the reaction mixture was 1.0 ml. The final concentration of added cyanide was shown in the parentheses. The reaction was started by adding sodium sulphide.

greatly accelerated on addition of a small amount of cytochrome *c*-553 (final concn., 34 μM). The reduction rate of cytochrome *c*-551 with sulphide was not affected on addition of cytochrome *c*-553 even at the concentration of 7 μM. As the acceleration effect of cytochrome *c*-553 on the reduction of cytochrome *c* with sulphide was observed with yeast cytochrome *c* as well as with cytochrome *c*-555, the yeast protein was used in the following experiments. The acceleration effect of cytochrome *c*-553 on the reduction of cytochromes *c* with sulphide was proportional to its concentration between 0 and 50 nM. When cytochrome *c*-553 was heated (80°C, for 2 min), it completely lost the accelerating effect (table 2). Further, the accelerating effect of cytochrome *c*-553 was strongly inhibited by cyanide at a very low concentration (table 2). Therefore, it may be concluded that cytochrome *c*-553 is an enzyme acting as a sulphide-cytochrome *c* reductase. Since the bacterium utilizes sulphide as the electron donor in addition to thiosulphate, the sulphide-cytochrome *c* reductase activity of cytochrome *c*-553 seems physiologically significant for the organism.

Addition of cyanide (at a concentration almost equal to the cytochrome) caused the colour of ferricytochrome *c*-553 to change rapidly from brownish red to dark brown: the absorbance at the peaks or shoulders at 480 and 450 nm (which are thought to be attributable to the bound flavin [1]) decreased, while the absorbance between 600 and 700 nm increased (fig. 1). The spectral change was very rapid and remained even after addition of $K_3Fe(CN)_6$ to the cyanide-treated cytochrome solution, overnight dialysis or treatment with a Sephadex G-25 column of the cyanide-treated cytochrome, or the cyanide-treated cytochrome being subjected to isoelectric focusing. These facts indicate that the spectral change caused by addition of cyanide is not attributable to reduction by this compound of the flavin in the cytochrome but to its binding with the cytochrome. When the cyanide-treated ferricytochrome *c*-553 was used in the sulphide-cytochrome *c* reductase reaction in place of the intact cytochrome *c*-553 after treatment with the Sephadex G-25 column, the reaction rate reduced to one third of that obtained with the intact cytochrome *c*-553 (table 2). When a small amount of $HgCl_2$ (final concn., 1 mM) was added to the cyanide-treated ferricytochrome *c*-553, the colour of the cytochrome solution returned to brownish red from dark brown. The Sephadex G-25 column chromatography of the brownish red solution obtained above yielded cytochrome *c*-553 which had the same absorption spectrum and enzymatic activity as the untreated cytochrome *c*-553 (table 2).

Although the reduction rate with sulphide of the cyanide-treated ferricytochrome *c*-553 was very slow, the absorption spectrum of the resulting reduced form was the same as that of the intact ferrocytochrome *c*-553. When the reduced form of the cyanide-treated cytochrome *c*-553 was passed through the Sephadex G-25 column, it showed the spectrum of the oxidized form of the untreated cytochrome *c*-553. This fact implies that cyanide, which has been bound to ferricytochrome *c*-553, is dissociated on reduction of the cytochrome, and the original ferricytochrome *c*-553 is recovered by the treatment with the Sephadex G-25 column as the ferrocytochrome *c*-553 is autoxidizable.

Carbon monoxide and azide neither caused any change in the absorption spectrum of the ferri- or ferrocytochrome *c*-553, nor affected the sulphide-cytochrome *c* reductase activity of the cytochrome.

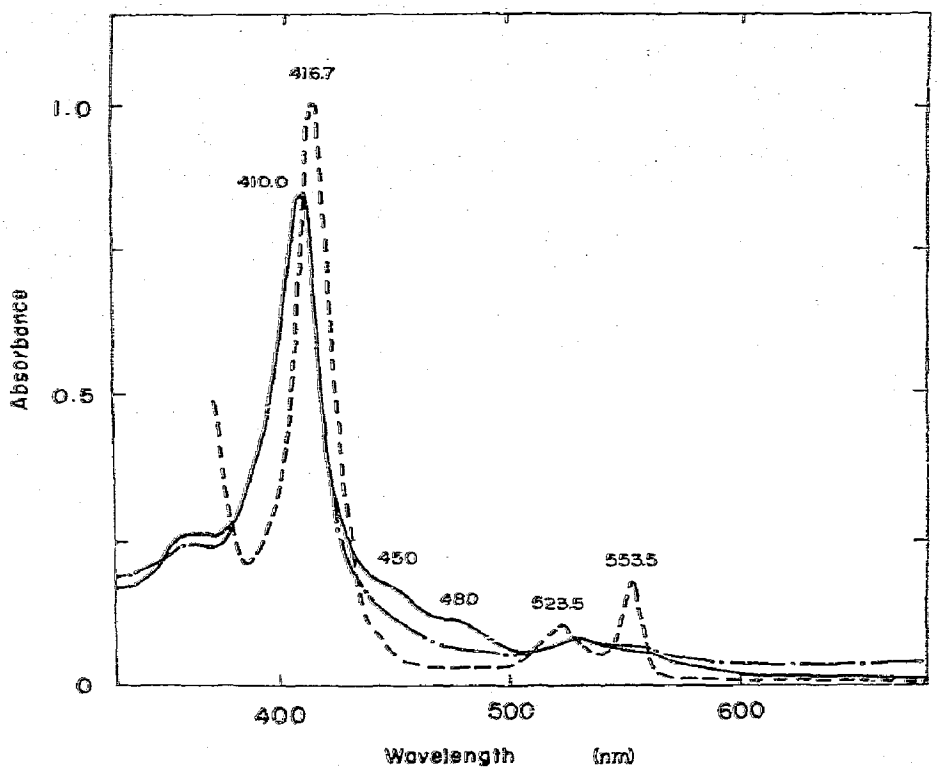

Fig. 1. Effect of cyanide on absorption spectrum of cytochrome *c*-553. Cytochrome *c*-553 was dissolved in 0.1 M Tris–HCl buffer, pH 7.4, at a concn. of 6.5 μM. (——) Oxidized form: (– · –) oxidized form *plus* 8.0 μM KCN; (– – –) a small amount of dithionite was added to the cyanide-treated or untreated cytochrome *c*-553.

References

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