EUKARYOTIC CYTOCHROME *C*-LIKE PROPERTIES OF CYTOCHROME *C*-550 (*THIOBACILLUS NOVELLUS*)

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1. Introduction

In the previous studies [1], it has been established that there is a strict biological specificity in the reactions of cytochrome *c* with redox enzymes such as cytochrome oxidase and cytochrome *c* peroxidase; cytochromes *c* derived from most eukaryotes react rapidly both with cow cytochrome oixdase (EC 1.9.3.1) and with yeast cytochrome *c* peroxidase (EC 1.11.1.5), whereas those from prokaryotes in general do not react or react very poorly with both the enzymes but some of them react rapidly with *Pseudomonas aeruginosa* nitrite reductase (EC 1.9.3.2) (which acts also as a cytochrome oxidase). Indeed, eukaryotic cytochromes *c* are found to be homologous to one another [2], and their primary structures differ greatly from those of prokaryotic cytochromes *c* [3] although a few of the prokaryotic cytochromes *c* are found which appear homologous to the eukaryotic proteins to some extent [4]. However, we have found that cytochrome *c* isolated from *Thiobacillus novellus* reacts with the cow oxidase more rapidly than with the bacterial nitrite reductase and it reacts with yeast cytochrome *c* peroxidase as rapidly as cytochromes *c* derived from eukaryotes in spite of its prokaryotic origin [5]. From these facts, we have extended the study on the enzymatic properties of *T. novellus* cytochrome *c*. The experimental results obtained here suggest to us that cytochrome *c*-550 of *T. novellus* may belong to the eukaryotic cytochrome *c*.

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2. Materials and methods

2.1 *Purification of enzymes and cytochromes*

P. aeruginosa nitrite reductase (EC 1.9.3.2) (= *Pseudomonas* cytochrome oxidase) [6], *T. Novellus* sulphite-cytochrome *c* reductase (EC 1.8.2.1) [5,7], and *N. europaea* hydroxylamine-cytochrome *c* reductase (EC 1.7.3.4) [8,9] were highly purified as described previously. Cow cytochrome oxidase (EC 1.9.3.1) purified by the method of Okunuki [10] was kindly supplied by Drs Y. Orii and S. Yoshikawa, and yeast cytochrome *c* peroxidase (EC 1.11.1.5) [11] by Dr T. Yonetani (University of Pennsylvania, USA).

Highly purified cytochrome *c*-550 (*T. novellus*) [5], cytochrome *c*-552 (*N. europaea*) [9], cytochrome *c*-551 (*P.aeruginosa*) [12], tuna cytochrome *c* [13], and cow cytochrome *c* [14] were obtained by the methods previously established. Cytochrome *c*-551 (*A. vinelandii*) was purified according to Swank and Burris [15]. Cytochrome *c*-550 (*Rhodospirillum rubrum*) [16] and cytochrome *c* (*Saccharomyces oviformis*) [17] were kindly supplied by Dr T. Horio (Institute for Protein Research, Osaka University, Osaka, Japan) and Sankyo Co. Ltd. (Tokyo, Japan), respectively. Horse cytochrome *c* (type VI) was purchased from Sigma Chemical Company (USA).

2.2 *Assay of enzyme activities*

Reactivities of cytochromes *c* with *P. aeruginosa* nitrite reductase [1], cow cytochrome oxidase [1], yeast cytochrome *c* peroxidase [18], *T. novellus* sulphite-cytochrome *c* reductase [5], and *N. europaea* hydroxylamine-cytochrome *c* reductase [9] were determined by the respective methods as reported

previously. The concentrations of cytochromes *c* were made substantially the same through a series of the assay experiments for each enzyme in order to make sure of differences in the reactivity between cytochromes *c*. The spectrophotometric determinations were performed in a Cary spectrophotometer, model 15.

3. Results

As table 1 shows, *P. aeruginosa* nitrite reductase reacted rapidly with cytochromes *c* derived from *P. aeruginosa, N. europaea,* and *A. vinelandii* but reacted very poorly with cytochromes *c* isolated from eukaryotes, whereas cow cytochrome oxidase and yeast cytochrome *c* peroxidase reacted rapidly with the latter proteins but did not react with the former proteins. However, it is obvious from the data shown in the table, cytochrome *c*-550 (*T. novellus*) is greatly different from the other bacterial cytochromes *c*; in spite of its bacterial origin it reacted with cow cytochrome oxidase more rapidly than with *P. aeruginosa* nitrite reductase, and further it reacted with yeast cytochrome *c* peroxidase as rapidly as cytochromes *c* of eukaryotes. In the reaction with the peroxidase, the K_m values observed were 8 μM and 6 μM for cytochrome *c*-550 (*T. novellus*) and tuna cytochrome *c*, respectively.

The specificities for cytochrome *c* of *T. novellus* sulphitecytochrome *c* reductase and *N. europaea* hydroxylamine-cytochrome *c* reductase were quite similar to those of the cow oxidase and the yaest peroxidase. Also with the two reductases, cytochrome *c*-550 (*T. novellus*) reacted as rapidly as did eukaryotic cytochromes *c*.

Table 1
Reactivities of various cytochromes *c* with *P. aeruginosa* nitrite reductase, cow cytochrome oxidase, yeast cytochrome *c* peroxidase, *T. novellus* sulphite-cytochrome *c* reductase and *N. europaea* hydroxylamine-cytochrome *c* reductase

Organism	α Peak	Relative reactivity				
	(nm)	Nitrite reductase	Cytochrome oxidase	Cytochrome *c* peroxidase	Sulphite-cytochrome *c* reductase	Hydroxylamine-cytochrome *c* reductase
Pseudomonas aeruginosa	551	100	0	0	0	0
Nitrosomonas europaea	552	56	0	0	0	0*
Azotobacter vinelandii	551	25	0	0	ND**	ND**
Rhodospirillum rubrum	550	2.0	8.5	17	36	27
Thiobacillus novellus	550	6.0	23	106	100	91
Saccharomyces oviformis	550	4.9	100	100	104	40
Tuna	550	8.7	93	120	117	128
Cow	550	0.53	73	ND**	106	70
Horse	550	2.5	124	88	97	100

The reactions were performed in 40 mM phosphate buffer at pH 6.5 for nitrite reductase, cytochrome oxidase and cytochrome *c* peroxidase, in 25 mM Tris–HCl buffer at pH 7.5 for sulphite-cytochrome *c* reductase, and in 0.1 M glycine–NaOH buffer at pH 9.6 for hydroxylamine cytochrome *c* reductase. To 1.0 ml of cytochrome *c* solution (15–20 μM) was added 0.05 ml of 5 μM nitrite reductase, 0.05 ml of 2.5 μM cytochrome oxidase, 0.02 ml of 0.1 μM peroxidase, 0.03 ml of sulphite-cytochrome *c* reductase (27 μg/ml), or 0.05 ml of 2.8 μM hydroxylamine-cytochrome reductase, and the decrease or increase in the absorbance at the α peak of each cytochrome was spectrophotometrically followed with time. The reaction with cow cytochrome oxidase was performed without addition of Emasol for reasons as described previously [1]. The reactivities of cytochromes *c* were expressed as relative values; the molecular activity (moles of cytochrome *c* reduced or oxidized per mole of enzyme) or specific activity was taken as 100% which was observed when each enzyme reacted with its native cytochrome *c* except for the cases of cow cytochrome oxidase and *N. europaea* hydroxylamine-cytochrome *c* reductase where yeast and horse cytochromes *c* were used, respectively, in place of their native cytochromes *c*.

* Native electron acceptor of the enzyme is cytochrome *c*-554 (*N. europaea*) but not cytochrome *c*-552 (*N. europaea*) [9].
** Not determined.

4. Discussion

The primary structures of cytochromes *c* derived from eukaryotes are extensively studied, and shown to be similar to one another, *i.e.* they are homologous [2]. However, the structures of cytochromes *c* derived from most prokaryotes are distinctly different from those of the eukaryotic proteins so far determined [3], although a few prokaryotic cytochromes *c* are found which appear more or less homologous to the eukaryotic proteins [4]. These differences in the primary structure of cytochromes *c* seem to be reflected quite well on their reactivities with redox enzymes.

The primary structure of cytochrome *c*-550 (*R. rubrum)* resembles those of the eukaryotic cytochromes *c* to some extent [4]. Indeed, this bacterial cytochrome reacts fairly rapidly with yeast cytochrome *c* peroxidase, although it reacts poorly with cow cytochrome oxidase.

The ractivities with these enzymes of cytochrome *c*-550 (*T. novellus*) are considerably larger than respective reactivities of cytochrome *c*-550 (*R. rubrum*). Therefore, it will be expected that homology to the eukaryotic cytochromes *c* may be greater with the *T. novellus* cytochrome than with the *R. rubrum* protein. Indeed, K_m value of the yeast peroxidase for cytochrome *c* (*T. novellus*) is similar to that for the eukaryotic cytochromes *c* [19], and the bacterial cytochrome *c* reacts also with sulphite-cytochrome *c* reductase and hydroxylamine-cytochrome *c* reductase as rapidly as do the eukaryotic proteins. Therefore, the bacterial cytochrome *c* is expected to be considerably homologous to the eukaryotic cytochromes *c*, although the determination of its primary structure is a future problem.

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