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## Reactivity of *Anacystis nidulans* cytochrome *c*-554 with cytochrome oxidases

As has been established by previous work[1–3], the reactivity of cytochromes C with Pseudomonas and cow cytochrome oxidases reflects surprisingly well the taxonomic character of the organisms from which the cytochromes C were isolated. In general, cytochromes C from primitive organisms react very rapidly with Pseudomonas cytochrome oxidase but do not react with cow cytochrome oxidase, while those from higher organisms react rapidly with cow enzyme but react very poorly with Pseudomonas enzyme.

Among cytochromes C of the photosynthetic organisms, algal cytochromes C and Chlorobium cytochrome *c*-555 (*f*-type cytochromes) react fairly rapidly with Pseudomonas cytochrome oxidase[4] but do not react with cow enzyme, while cytochromes C of non-sulphur purple bacteria ($c_2$-type cytochromes) react very poorly with both the cytochrome oxidases[5]. During investigation of C-type cytochromes derived from the photosynthetic organisms, it has been found that cytochrome *c* (554, *Anacystis nidulans*) differs greatly from the other algal cytochromes C in reactivity with Pseudomonas cytochrome oxidase. In the present investigation, we describe briefly the reactivity of Anacystis cytochrome *c*-554 with Pseudomonas and cow cytochrome oxidases as compared with that of cytochromes C isolated from the other algae.

The strain of *Anacystis nidulans* Richt. (IAM M-6) used in the present study was kindly supplied by the Institute of Applied Microbiology, University of Tokyo. The organism was cultivated in KRATZ–MYERS'[6] medium for a week at 35° using about 300 Roux bottles (each bottle had a volume of 1.5 l). Each bottle was filled with 800 ml of the medium, and illuminated by incandescent lamps after the medium was inoculated with the organism.

The cells thus cultivated were collected by centrifugation and treated with cold acetone. The acetone-treated cells (about 50 g) were suspended in 750 ml of 10 mM phosphate buffer (pH 7.0). After standing overnight at 5°, the suspension was centrifuged at 10000 × *g* for 10 min, and the resulting supernatant was fractionated with $(NH_4)_2SO_4$. The precipitate appearing between 40 and 90 % saturation was collected by centrifugation at 10000 × *g* for 10 min, dissolved in 10 mM Tris–HCl buffer (pH 8.0) and the solution thus obtained was dialysed against the same buffer. The dialysed solution was subjected to chromatography by the DEAE-cellulose column which had been equilibrated with the same buffer as used for the dialysis. Cytochrome *c*-554 was adsorbed on the column together with cytochrome *c*-549, phycocyanin and ferredoxin. Cytochrome *c*-554 was eluted by 10 mM Tris-HCl buffer (pH 8.0), containing 10 mM NaCl, while cytochrome *c*-549, phycocyanin and ferredoxin remained on the column. The resulting eluate was again subjected to DEAE-cellulose column chromatography after being dialysed against 10 mM Tris–HCl buffer (pH 8.0), and the cytochrome adsorbed was eluted as mentioned above.

Highly purified brown algal cytochromes C, cytochrome *c* (553 *Endarachne binghamiae*)[7] and cytochrome *c* (553, *Petalonia fascia*)[7], were kindly supplied by Drs. E. Yakushiji and Y. Sugimura, and Pseudomonas and cow cytochrome oxidases were highly purified according to the methods established in our laboratory[8,9].

The Anacystis cytochrome *c*-554 obtained here showed the same spectral properties as those reported by HOLTON AND MYERS[10,11]; its absorption spectrum had peaks

at 417, 522 and 554 mμ in the reduced form, the ratio of $A_\gamma$ (reduced)/$A_\alpha$(reduced) was 6.9, and the α-peak at 554 mμ was asymmetric with a shoulder around 550 mμ.

The cytochrome reacted slowly with Pseudomonas cytochrome oxidase and not at all with cow cytochrome oxidase.

As shown in Fig. 1 and Table I, the reactivity of Anacystis cytochrome *c*-554 with Pseudomonas cytochrome oxidase was considerably lower than that of red algal, brown algal and diatom cytochromes C. Anacystis cytochrome *c*-554 is quite similar

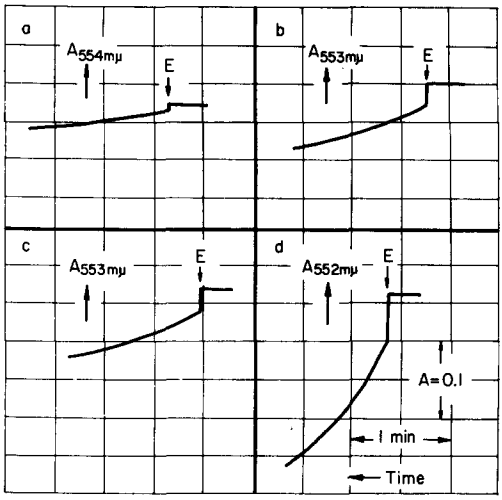


Fig. 1. Reactions with Pseudomonas cytochrome oxidase of Anacystis cytochrome *c*-554, Endarachne cytochrome *c*-553 and Petalonia cytochrome *c*-553. Reactions were performed in 40 mM phosphate buffer (pH 6.5) at 23°. At point E, 0.05 ml of 3.22 μM Pseudomonas cytochrome oxidase was added to 1.0 ml of each cytochrome *c* solution. (a) *Anacystis nidulans* (12 μM). (b) *Endarachne binghamiae* (22 μM). (c) *Petalonia fascia* (25 μM). (d) *Pseudomonas stutzeri* (15 μM). As *P. stutzeri* cytochrome *c*-552 has been known to react rapidly with the enzyme[1–3], it was included as the standard. The reactivity of Anacystis cytochrome *c*-554 tabulated in Table I was corrected for concentration in order to compare it with those of the other cytochromes.

TABLE I

SPECTRAL AND ENZYMATIC PROPERTIES OF ANACYSTIS CYTOCHROME *c*-554 AS COMPARED WITH THOSE OF THE OTHER ALGAL CYTOCHROMES C

| *Organism* | *α-Peak (mμ)* | $\varepsilon_{mM}$ *at α-peak* | $A_\gamma/A_\alpha$ | *Reactivity (%)** | |
|---|---|---|---|---|---|
| | | | | *Pseudomonas cytochrome oxidase* | *Cow cytochrome oxidase* |
| *Anacystis nidulans* | 554 | 24.6 | 6.9 | 7.6 | 0 |
| *Endarachne binghamiae*[7] | 553 | | 6.6 | 26 | 0 |
| *Petalonia fascia*[7] | 553 | | 6.9 | 33 | 0 |
| *Porphyra tenera*[12] | 553 | 21.7 | 6.9 | 21 | 0 |
| *Navicula pelliculosa*[13] | 554 | 23.8 | 6.8 | 36 | 0 |

* The reactivity was expressed as a relative value; the turnover number obtained for the reaction of Pseudomonas cytochrome oxidase with *Pseudomonas aeruginosa* cytochrome *c*-551 was taken to be 100% to estimate the relative rates of the reaction of the bacterial enzyme with other cytochromes C.

to the other algal cytochromes C in spectral properties, redox potential and isoelectric point[10,11]; namely it is the *f*-type cytochrome. Nevertheless it differs considerably from the other *f*-type cytochromes in its reactivity with Pseudomonas cytochrome oxidase. According to current taxonomy, blue-green algae are known to be considerably different from the other algae and to resemble the photosynthetic bacteria, mainly on the basis of electron microscopic studies[14]. The results obtained in the present study also support this idea.

As mentioned previously, the organisms whose cytochromes C react rapidly with Pseudomonas cytochrome oxidase seem to be more primitive than those whose cytochromes C react poorly with the enzyme. As Anacystis cytochrome *c*-554 reacted with Pseudomonas cytochrome oxidase considerably more slowly than the other algal cytochromes C did, the blue-green alga may be said to be a more recent organism than the other algae tested here on the basis of our hypothesis. Although this conclusion seems to conflict with current taxonomy, future investigations should clarify whether blue-green algae are more recent organisms than the other algae.

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