A COBALT-FREE CORRINOID COMPOUND IN STREPTOMYCES OLIVACEUS

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SUMMARY

An unknown orange-red pigment was obtained from the cells of Streptomyces olivaceus 605 grown on a cobalt-free medium. This compound showed similar behaviors to those of the cobalt-free corrinoids of Chromatium isolated by Toohey in UV, CD and fluorescence spectra. However it exhibited a more acidic character in paper electrophoresis. Although an attempted insertion of cobalt atom has been unsuccessful, available evidence indicates that this pigment is the first cobalt-free corrinoid found in nonphotosynthetic bacteria, having carboxylic acid side-chains on its corrin nucleus.

Corrinoid compounds containing no cobalt were first discovered in some photosynthetic bacteria by Toohey (1) (2). Despite the fact that this stimulating work has aroused interest widely, such kinds of compounds have not been obtained from nonphotosynthetic bacteria. In this communication we wish to describe the isolation of a new cobalt-free corrinoid from the cells of a strain of Streptomyces olivaceus grown under cobalt-deficient conditions.

MATERIALS AND METHODS

Streptomyces olivaceus 605 used in this study was supplied from Kyowa Hakko Kogyo Co., Tokyo, Japan. This organism has been known to produce a large amount of corrinoids, both complete and incomplete forms (3), in cobalt-sufficient media. For the present study, this organism was cultivated in the same glycerol medium as mentioned in our previous paper (3) except that $CoCl_2$ was omitted. The cells were harvested at the middle exponential phase of growth. The isolation procedure of the pigment from the cells was similar to those of ordinary corrinoids reported earlier (4) : Ethanol extraction, purification by the usual phenol treatment and P-cellulose column chromatography. The same pig-

ment was also obtained from a protein fraction (0 to 70 % $(NH_4)_2SO_4$-precipitate) of cell-free extracts. Since the latter method had an advantage in decreasing other contaminating pigments, the following procedure was employed : The wet cells, suspended in the same amount of KPO_4 buffer (0.1 M, pH 7.5), were sonicated for 10 min at 20 KC. The resulting homogenate was centrifuged at 13,000 X g for 30 min. To the supernatant was added solid $(NH_4)_2SO_4$ and the fraction precipitating to 70 % of saturation was resuspended in ethanol (20 ml per g of protein). This suspension was boiled for 20 min and centrifuged at 13,000 X g for 30 min. The supernatant was concentrated to dryness *in vacuo* and the resulting residue was, after dissolved in a small amount of distilled water, purified by the usual phenol treatment as described in our previous report (4). The pigment thus obtained was further purified by column chromatography on P-cellulose equilibrated previously with 0.1 N acetic acid. When eluted with water, the compound moved in one orange-red band and the eluate gave one spot in the paper electrophoresis performed at 25 volts per cm using Toyo filter paper No. 50 (20 X 400 mm) at various pH. Lyophillization of the eluate gave an amorphous orange-red powder having a reddish fluorescence. Absorption and CD spectra were measured with Jasco ORD/UV/CD-5 spectrophotometer. Fluorescence spectrophotometry was carried out using Hitachi spectrophotometer MPF-2.

RESULTS

The yield of the pigment was *ca.* 25 µg per 100 g of the cells grown in the cobalt-deficient glycerol medium. It was increased about two-fold by the simultaneous addition of glycine, succinate and methionine to the medium. On the other hand, such a compound was not formed in cobalt-sufficient media. Figure 1 shows the absorption, CD and fluorescence spectra of the puried *S. olivaceus* pigment. The absorption spectrum indicates its close similarity to that of *Chromatium* Co-free corrinoids reported by Toohey (1) (2). Although the wavelengths of absorption maxima for the *S. olivaceus* compound coincide with those of the *Chromatium* compounds, the absorption ratio are different, especially in the ultraviolet region. It appears likely that this discrepancy may be due to a delicate difference between the structures of these compounds in minor details. By lacking the absorption around 400 mµ, the *S. olivaceus* compound can be clearly distinguished from porphyrins.

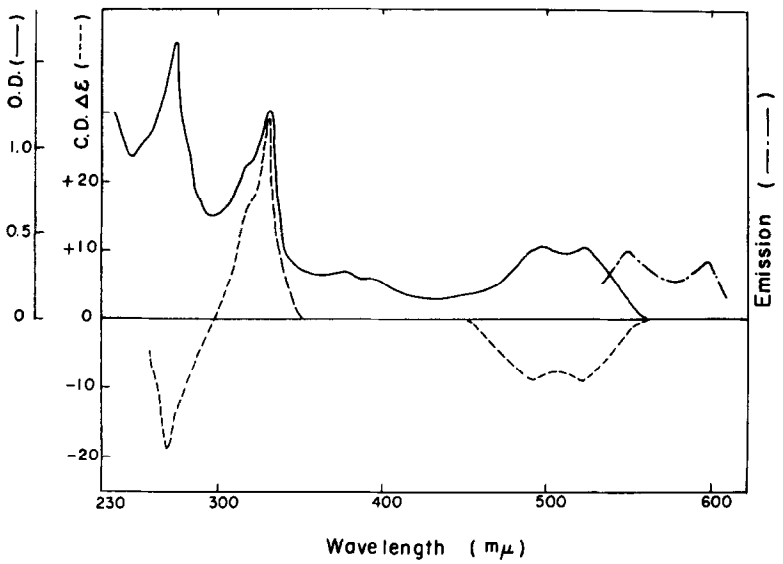

Fig. 1 Absorption, emission and CD spectra of the S. olivaceus compound.

The emission spectrum was measured relative to excitation at 500 mμ. The concentration of the compound was approximately 3.4×10^{-5} M.

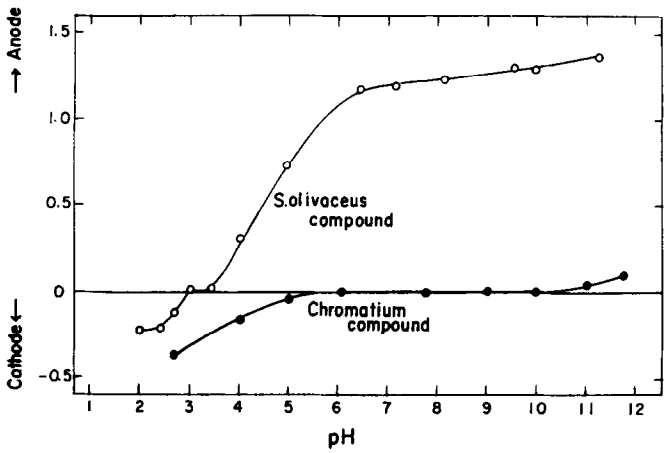

Fig. 2 Paper electrophoretic behavior of the S. olivaceus compound at varied pH.

The experimental conditions are described in the text. The Chromatium compound cited as a reference is a Toohey's "positive" Co-free corrinoid (2).

The CD and fluorescence spectra of this pigment are also identical with those of the Co-free corrinoids of Chromatium (5) and obviously

different from those of porphyrins (6) and Co-containing corrinoids (7). These facts offer additional evidence for the possibility that S. olivaceus pigment is a kind of Co-free corrinoid. The striking feature of this compound was the behavior in paper electrophoresis. Figure 2 shows the results obtained by the method of Ladd et al. (8). The S. olivaceus compound exhibits a more acidic behavior than the Chromatium compound. Judged from the degree of dissociation at neutral and alkaline pHs, it seems probable that the carboxylic acid side-chains of its corrin nucleus are not amidated yet unlike the Chromatium compounds.

DISCUSSION

The pigment produced by S. olivaceus 605 gave the spectral behaviors characteristic of the Co-free corrinoids of photosynthetic bacteria (1) (5). This compounds could not be detected when the organism was cultivated in cobalt-sufficient media. Addition of glycine, succinate and methionine, the precursors of corrin nucleus, enhanced the yield of the pigment in the cobalt-free medium used. Paper electrophoretic studies performed at various pH indicated a more acidic character of this compound than the Chromatium compounds. From these results it seems reasonable to conclude that the S. olivaceus compound is a Co-free corrinoid having free carboxylic acid side-chains on its corrin nucleus and, hence, located at an earlier stage of corrinoid biosynthesis than the Chromatium compounds. This compound was more unstable than the Chromatium compounds. In alkaline conditions its reddish color turned into yellowish like the Chromatium compound, but the attempt to insert cobalt atom into the yellowish compound performed as mentioned by Toohey (1) was unsuccessful. The reason was considered that the conditions, such as boiling, employed for the insertion of cobalt would not be appropriate for this unstable compound.

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