Hajra, A. K., and Radin, N. S. (1962), *J. Lipid Res. 3*, 131.
Lynen, F. (1961), *Fed. Proc. 20*, 941.
Lynen, F., Kessel, I., and Eggerer, H. (1960), *Bayerische Academie der Wissenschaften*, Sitzung vom March 4.
Martin, D. B., Horning, N. G., and Vagelos, P. R. (1961), *J. Biol. Chem. 236*, 663.
Robinson, J. D., Bradley, R. M., and Brady, R. O. (1962a), *J. Biol. Chem.* in press.
Robinson, J. D., Brady, R. O., and Maxwell, C. R. (1962b), *J. Lipid Res. 3*, 243.
Stern, J. R. (1958), *in* Methods in Enzymology, Colowick, S. P., and Kaplan, N. O., editors, New York, Academic Press, Inc., p. 573.

The Occurrence of Cytochrome and Coenzyme Q in *Thiobacillus thiooxidans**

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Received August 13, 1962

The existence of a cytochrome system and coenzyme Q_8 has been demonstrated in *Thiobacillus thiooxidans* and *Thiobacillus thioparus*. Cytochrome 550 of *T. thiooxidans* has an absorption spectrum resembling that of cytochrome *c*. The coenzyme Q_8 content of *T. thiooxidans* was estimated to be 4.3 μmoles (3.2 mg) per gram of dry weight.

Thiobacillus thiooxidans is a chemosynthetic autotrophic bacterium which derives its energy from the oxidation of elemental sulfur. This oxidation, and consequently the growth of the organism, is aerobic and proceeds only in the presence of molecular oxygen. Earlier studies on the effects of inhibitors (Vogler *et al.*, 1942) showed sulfur oxidation to be blocked by low concentrations of sodium cyanide, sodium azide, and carbon monoxide. All of these were known to be potent inhibitors of the cytochrome system. The aerobic nature of the process and its sensitivity to these inhibitors naturally led to the assumption that the cytochrome system was involved in sulfur oxidation by *T. thiooxidans*.

Subsequently, the presence of cytochromes was demonstrated in various species of the genus *Thiobacillus*. Klimek *et al.* (1956) showed the presence of a respiratory pigment resembling cytochrome *c*, which they termed cytochrome *s*, in *Thiobacillus thioparus*.

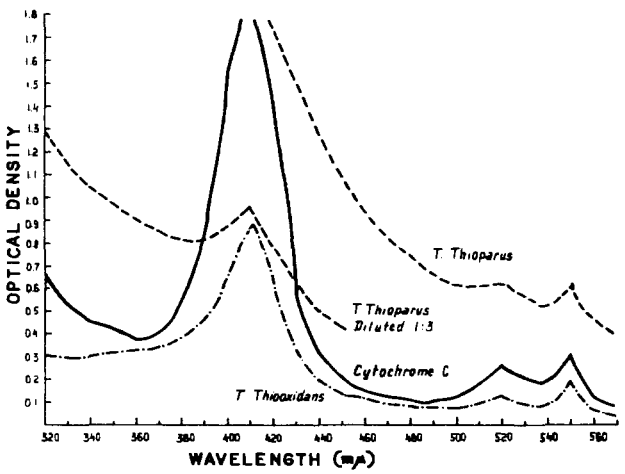

FIG. 1.—Absorption spectrum of reduced bacterial cytochromes compared with reduced mammalian cytochrome *c*. The *T. thioparus* preparation was a crude sonic extract containing 10 mg protein per ml. The *T. thiooxidans* material was a partially purified preparation (calcium phosphate gel-treated acid extract) containing 1.5 mg protein per ml.

* This work was supported by National Science Foundation Grant G-9085.

A cytochrome of the *c* type was also shown in *Thiobacillus denitrificans* by Aubert *et al.* (1958). In the thiosulfate-oxidizing organism, *Thiobacillus* X, Trudinger (1961a) found at least three separate cytochrome *c* type pigments.

On the other hand, Szczepkowski and Skarzynski (1952) were unable to demonstrate typical cytochrome absorption spectra in extracts of *T. thiooxidans*, although cyanide and azide were inhibitory to growth. As pointed out in a recent review (Peck, 1962), this finding casts some doubt on a role for cytochrome and oxidative phosphorylation in sulfur oxidation. We would therefore like to report the demonstration of a cytochrome *c* type pigment and a coenzyme Q compound in *T. thiooxidans*, as well as *T. thioparus*.

EXPERIMENTAL PROCEDURES

Cultures.—The strain of *T. thiooxidans* used in these studies was originally obtained from Dr. R. L. Starkey. A culture of *T. thioparus* was generously supplied by Dr. H. D. Peck, Jr., Oak Ridge National Laboratory, Tenn.

Growth Media.—*T. thiooxidans* was grown on a mineral medium described by Vogler and Umbreit (1941) plus elemental sulfur. Growth of *T. thioparus* was made on the thiosulfate medium of Starkey (1935).

Extraction of Cytochromes.—Washed cell suspensions, freed of sulfur, were prepared in *p*H 8 Tris buffer (0.05 M) and disrupted for 15 minutes in the Raytheon 10KC sonic oscillator. Unbroken cells and debris were removed by centrifugation at 10,000 × *g* for 15 minutes. Acid extracts of such sonicates were made by adjusting to *p*H 3 and centrifuging down the precipitated protein. After neutralization of the acid extract to *p*H 7.5, the cytochromes could be absorbed on calcium phosphate gel prepared by the method of Dixon and Webb (1958), washed with distilled water, and then eluted with *p*H 5 ammonium acetate (1.0 M). The absorption spectra of these fractions (adjusted to *p*H 7.5) were determined with and without reduction by sodium hydrosulfite with the Beckman DU spectrophotometer.

Cytochrome Oxidase Assay.—The cytochrome oxidase activity of the sonic extracts was determined in the colorimetric assay using the nadi reagent as described by Straus (1954). The development of color was followed with a Beckman DU spectrophotometer equipped with a standard Beckman recorder.

Extraction of Coenzyme Q.—The presence of coenzyme Q (ubiquinone) was shown by exhaustive acetone extraction of lyophilized cells, as well as by the extraction of alkali-pyrogallol digests made according to the method of Page *et al.* (1960). Extracts were examined by thin-layer chromatography using silica gel plates and the solvent systems described by Wagner *et al.* (1962).

Although crude extracts showed broad absorption peaks in the region of 270–275 mμ which disappeared upon borohydride reduction, it was necessary to remove interfering material to obtain sharp maxima. This could be done conveniently by scraping off and eluting the coenzyme Q spots from thin-layer chromatograms on silica gel. Alternatively, the extracts were fractionated by column chromatography on silicic acid plus Celite as described by Crane *et al.* (1959). Absorption spectra of ethanolic solutions were determined before and after reduction with sodium borohydride.

Chemicals.—Mammalian cytochrome *c* was purchased from Nutritional Biochemicals Company. The sample of authentic coenzyme Q_{10} was a gift from Dr. Karl Folkers of Merck, Sharp and Dohme Research Laboratories, Rahway, N. J.

Results

Examination of the absorption spectra of crude sonic extracts of *T. thioparus* showed the presence of a large Soret peak with a maximum at 410 mμ for both oxidized and reduced preparations. The reduced spectrum showed also a peak at 550 mμ and a shoulder at 510–520 mμ. Figure 1 presents the comparison of the absorption spectra of the sonic extract of *T. thioparus* and mammalian cytochrome *c*. Since this spectrum resembles that of the cytochrome previously reported for *T. thioparus* (Szczepkowski and Skarzynski, 1952), no attempt was made at purification. Crude sonic extracts of *T. thiooxidans* showed strong absorption due to the Soret band, with maxima at 410 mμ and 415 mμ for the oxidized and reduced extracts, respectively. Although only small peaks were found at 550 mμ in crude neutral extracts, they were more pronounced in acid extracts. Figure 1 also shows the absorption spectrum of the cytochrome fraction of *T. thiooxidans* after calcium phosphate gel treatment. The oxidized preparation showed a single peak at 410 mμ. After reduction, peaks were found at 412, 520, and 550 mμ. From this it can be concluded that *T. thiooxidans* contains a cytochrome component resembling cytochrome *c*, which we may term cytochrome 550.

Since the extracts were found to contain cytochromes, they were next examined for cytochrome oxidase activity. The results obtained in these tests are illustrated in Figure 2. Extracts of *T. thioparus* showed a very rapid formation of blue color with the nadi reagent, which was not dependent upon addition of exogenous cytochrome *c*. This oxidation was immediately abolished by the addition of cyanide. Although less active, *T. thiooxidans* extracts also showed a cyanide-sensitive oxidation of the nadi reagent. Thus both organisms exhibit cytochrome oxidase activity as well as typical cytochrome absorption spectra.

Solvent extraction provided evidence for the presence in both organisms of an additional component of the respiratory chain, coenzyme Q. Figure 3 presents the absorption spectra of partially purified coenzyme Q fractions of *T. thiooxidans* and *T. thioparus* compared with authentic coenzyme Q_{10}. The coenzyme Q fractions were golden yellow in color and possessed the characteristic absorption peak at 272–274 mμ for the oxidized material. Upon reduction of these fractions

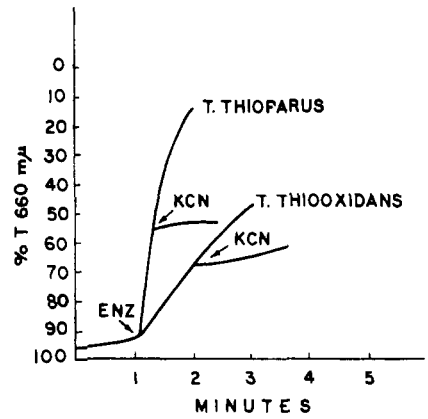

FIG. 2.—Cytochrome oxidase activity (nadi reagent) of *Thiobacillus* sonic extracts. Curves represent actual tracings from the recorder. Reaction mixture consisted of 100 μmoles of phosphate buffer, *p*H 7.3, 0.1 mg α-naphthol, and 0.3 mg *N,N*-dimethyl-phenylenediamine in 3.0 ml. Reaction was started by addition of *T. thioparus* (2 mg protein) or *T. thiooxidans* (20 mg protein) enzyme, and inhibited by 1 μmole KCN.

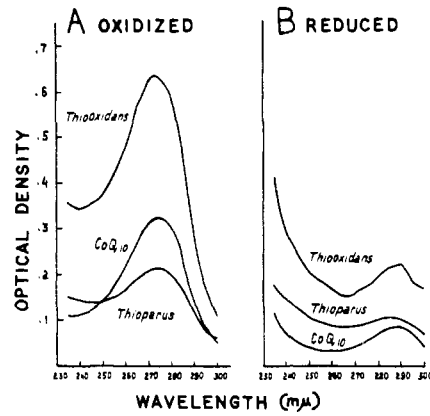

FIG. 3.—Absorption spectrum of *Thiobacillus* coenzyme Q fractions in ethanol compared with coenzyme Q_{10} (33 μg/ml solution in ethanol). *A*, oxidized; *B*, reduced with sodium borohydride.

with sodium borohydride, this peak disappeared and a new peak appeared at 290 mμ.

Thin-layer chromatography on paraffin-impregnated silica gel plates with acetone-water (9:1) as solvent (Wagner *et al.*, 1962) was then employed to determine the length of the side-chain. The average R_F values for the *T. thiooxidans* and *T. thioparus* coenzyme Q fractions were 0.53 and 0.54, respectively. This is very close to the value of 0.55 reported for coenzyme Q_8 (Wagner *et al.*, 1962). Although authentic coenzyme Q_8 was not available for comparison, there was good agreement between the experimental R_F for coenzyme Q_{10} (0.32) and that reported in the literature (0.31). This supports the designation of the *Thiobacillus* material as coenzyme Q_8.

On the basis of the extinction coefficient for coenzyme Q_8 given by Lester *et al.* (1959) the amount of coenzyme Q present in the *Thiobacillus* extracts can be estimated from the change in optical density upon reduction. Calculation of the coenzyme Q content of *T. thiooxidans* made in this manner ranged from 1.6 to 4.8 mg per g of dry cells. The average value for three separate determinations was 3.2 mg per g (or 4.5 μmoles per g).

Discussion

The present demonstration in *T. thiooxidans* of a cytochrome system that apparently is capable of opera-

tion with molecular oxygen makes inhibition data reported for whole cells seem more meaningful. Evidently Szczepkowski and Skarzynski (1952) were misled regarding cytochromes in this organism.

Furthermore, a close link exists between thiosulfate oxidation and the cytochromes in *Thiobacillus* X (Trudinger, 1961b). Extracts contained a thiosulfate oxidase which catalyzed the oxidation of thiosulfate to tetrathionate with one of the cytochromes (cytochrome 553.5) acting as the electron acceptor. When the extract containing soluble cytochromes and thiosulfate oxidase was combined with a particulate fraction (which also contained cytochrome), the oxidation of thiosulfate was accompanied by oxygen uptake. Oxygen uptake could be abolished by addition of cyanide. Thus the participation of a complete cytochrome chain in thiosulfate oxidation by *Thiobacillus* X is clearly established.

Since *T. thiooxidans* grows only on sulfur, the insolubility of which prevents it from acting in the cell-free systems so far studied, evidence for the participation of cytochromes and coenzyme Q in the electron transport system starting from sulfur is necessarily indirect. This evidence consists of inhibition of whole cell respiration on sulfur by cyanide, azide, and carbon monoxide, and the presence in cell-free extracts of a cyanide-inhibited cytochrome oxidase. Since, however, this is a unique organism growing on sulfur in an extremely acid environment and deriving all its carbon from CO_2, the presence of cytochromes and the high level of coenzyme Q found are themselves of interest. Whether the real oxidation substrate is sulfur or an adenosine phosphosulfate derivative (Peck, 1962) is a matter for further study.

The compounds of the coenzyme Q family have been found in many but not all bacteria. However, this is, to our knowledge, the first demonstration of coenzyme Q in an obligate chemosynthetic autotrophic bacterium. While the occurrence of coenzyme Q in the thiobacilli is perhaps not surprising in view of its widespread distribution among bacteria, its presence does strengthen the case for a cytochrome system in *T. thiooxidans*. Coenzyme Q has not been found in bacteria devoid of cytochromes, such as *Streptococcus* and *Clostridium*, but is universally present in cytochrome-containing organisms, including the strict anaerobe *Desulfovibrio desulfuricans* (Lester and Crane, 1959).

It is of some interest to compare the amount of coenzyme Q found in *T. thiooxidans* with that reported for other bacteria. The average value of 4.5 μmoles per gram for *T. thiooxidans* compares favorably with 4.3 μmoles per gram for *Rhodospirillum rubrum*, which is the highest value reported for bacteria (Lester and Crane, 1959). Such a high level of coenzyme Q would imply an important respiratory function.

References

Aubert, J. P., Milhaud, G., Moncel, C., and Millet, J. (1958), *Compt. rend. 246*, 1616.

Crane, F. L., Widmer, C., Lester, R. L., and Hatefi, Y. (1959), *Biochim. et Biophys. Acta 31*, 476.

Dixon, M., and Webb, E. C. (1958), Enzymes, New York, Academic Press, Inc., p. 166.

Klimek, R., Skarzynski, B., and Szczepkowski, T. W. (1956), *Acta Biochim. Polon. 3*, 261.

Lester, R. L., and Crane, F. L. (1959), *J. Biol. Chem. 234*, 2169.

Lester, R. L., Hatefi, Y., Widmer, C., and Crane, F. L. (1959), *Biochim. et Biophys. Acta 33*, 169.

Page, A. C., Gale, P., Wallick, H., Walton, R. B., McDaniel, L. E., Woodruff, H. B., and Folkers, K. (1960), *Arch. Biochem. Biophys. 89*, 318.

Peck, H. D. (1962), *Bacteriol. Rev. 26*, 67.

Postgate, J. R. (1956), *J. Gen. Microbiol. 14*, 545.

Starkey, R. L. (1935), *J. Gen. Physiol. 18*, 325.

Straus, W. (1954), *J. Biol. Chem. 207*, 733.

Szczepkowski, T. W., and Skarzynski, B. (1952), *Acta Microbiol. Polon. 1*, 93.

Trudinger, P. A. (1961a), *Biochem. J. (London) 78*, 673.

Trudinger, P. A. (1961b), *Biochem. J. (London) 78*, 680.

Vogler, K. G., LePage, G. A., and Umbreit, W. W. (1942), *J. Gen. Physiol. 26*, 89.

Vogler, K. G., and Umbreit, W. W. (1941), *Soil Sci. 51*, 331.

Wagner, H., Hörhammer, L., and Dingler, B. (1962), *J. Chromatog. 7*, 211.

Coenzyme Q. XXXVI. Isolation and Characterization of Coenzyme Q_{10} (H-10)

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Received August 2, 1962

A new naturally occurring member of the coenzyme Q group has been isolated and crystallized from cells of *Gibberella fujckuroi*. Comparison of ultraviolet, infrared, and nuclear magnetic resonance spectra of this compound with spectra of coenzyme Q_{10} and related known compounds has revealed a structure differing from coenzyme Q_{10} in that the terminal unit in the ten-unit side-chain is isopentanyl, rather than isopentenyl. This new compound is designated coenzyme Q_{10}(H-10).

Coenzyme Q_{10} was described by Crane *et al.* (1957) after observations that a lipid in beef heart mitochondria was active in a succinoxidase system. This same quinone, which was designated ubiquinone (50), was described by Morton *et al.* (1957). It was shown to have structure I (Wolf *et al.*, 1958; Morton *et al.*, 1958).

Interest in the biochemical role of this quinone has prompted investigation of the distribution of this quinone in a wide variety of potential source materials. Coenzyme Q_{10} has been found in all mammals that have been examined. There have also been reports of its presence in plants (Crane, 1959; Page *et al.*, 1959), microorganisms (Lester *et al.*, 1959; Page *et al.*, 1960; Erickson *et al.*, 1960), and an insect (Heller *et al.*, 1960).

In addition to coenzyme Q_{10}, there are four other naturally occurring coenzyme Q's (Q_6–Q_9) differing structurally from one another only in the number of isoprenoid units in the side-chain. Coenzymes Q_6–Q_9 occur most frequently in microbial species (Lester *et al.*, 1958), but Q_9 has been reported in some rodents (Linn