ROLE OF FERREDOXIN IN HYDROGEN METABOLISM OF *MICROCOCCUS LACTILYTICUS*[1,2]

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One of the simplest fermentations yielding molecular hydrogen as an end product is the oxidation of hypoxanthine to hydrogen by *Micrococcus lactilyticus*:

$$\text{Hypoxanthine} = \text{Xanthine} + H_2$$

Xanthine oxidase and hydrogenase were found to be constituents of this system, and an unidentified electron carrier mediating electrons between xanthine oxidase and hydrogenase was postulated (Whiteley 1951,1956). In this connection it was interesting to find that extracts of *M. lactilyticus* contained high concentrations of ferredoxin, an electron carrier recently isolated from *Clostridium pasteurianum* (Mortenson et al., 1962). The present communication is concerned with the role of ferredoxin in hydrogen formation from hypoxanthine.

M. lactilyticus strain 221, kindly supplied by H. R. Whiteley, was grown on the lactate medium as described by Whiteley (1951). Demineralized water was used in place of tap water. Extracts were prepared by crushing the freshly harvested cells in a Hughes press. The crushed cells were suspended in 50 ml of water at 0° which contained 75 µg of DNAse. The cell debris was removed by centrifugation at 15,000 x G for 15 minutes. Approximately 50 ml of the amber colored extract containing 25 to 30 mg of protein per ml were next passed through a DEAE-cellulose (phosphate)

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column (1 x 4 cm) to remove ferredoxin. The column quickly became saturated with protein so that most of the protein passed through the column. The eluate prepared in this manner no longer carried out the production of hydrogen from hypoxanthine. Ferredoxin formed a distinct brown band on top of the column and was eluted with 0.5 M potassium phosphate buffer at pH 7.2. A partially purified ferredoxin fraction (prepared as above by selective adsorption on DEAE-cellulose) was dialyzed for 48 hours against 8 liters of water to remove phosphate ions. Following dialysis ferredoxin was again adsorbed on a DEAE-cellulose column. As shown in Fig. 1, a linear phosphate gradient from 0 to 0.5 M potassium phosphate at pH 7.2 was used, ferredoxin being eluted in a sharp peak at approximately 0.25 M phosphate buffer.

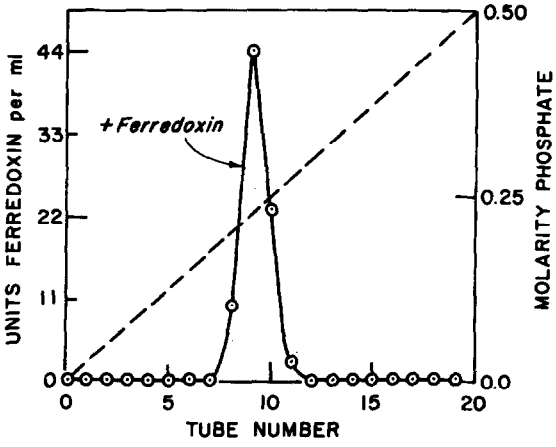


Figure 1. Chromatography of Ferredoxin from M. lactilyticus on DEAE-cellulose (phosphate). Partially purified ferredoxin (1240 units) was chromatographed on a 0.5 x 10 cm column at a flow rate of 80 ml per hour. Fractions of 10 ml were collected. Potassium phosphate buffer at pH 7.2 was used.

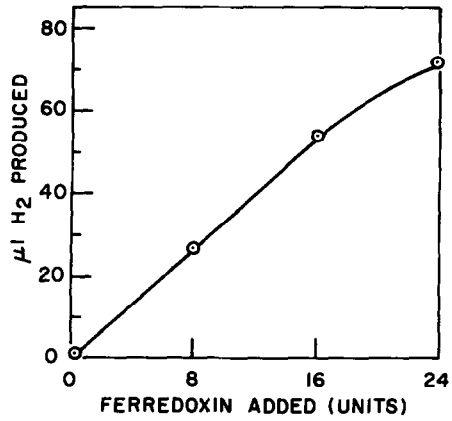


Figure 2. Effect of Ferredoxin concentration on hydrogen production from hypoxanthine. Each Warburg vessel contained crude extract, 11 mg protein; potassium phosphate buffer, 100 μmoles at pH 6.5; hypoxanthine, 20 μmoles; ferredoxine as indicated; water to a final volume of 3 ml; 0.2 ml 20 per cent KOH, center well. Reaction time, 40 min. at 30°. Gas phase, nitrogen.

The activity of ferredoxin is expressed in units as determined in the enzymatic procedure described by Mortenson, et al. (1962). Ferredoxin prepared in this manner was found to contain no detectable acetate kinase, pyruvate oxidizing system, transacetylase, hydrogenase, or xanthine oxidase. Partially purified ferredoxin was found to reactivate the DEAE-cellulose-treated enzyme fraction with respect to hydrogen formation from hypoxanthine. Figure 2 shows that hydrogen production from hypoxanthine was proportional to ferredoxin concentration. A blank value of 11 µl of hydrogen was subtracted from the points in Fig. 2. Other cofactors including iron and molybdenum would not substitute for ferredoxin in hypoxanthine oxidation. Whiteley (1951,1956) observed that the formation of xanthine from hydrogen and uric acid was stimulated by methyl and benzyl viologen. Figure 3 illustrates the effect of ferredoxin on uric acid reduction.

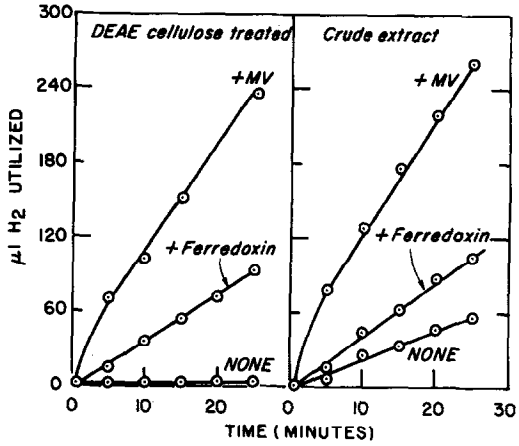
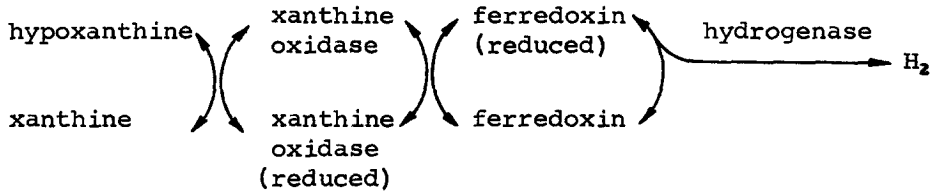


Figure 3. Reduction of uric acid using hydrogen.

Each Warburg vessel contained 11 mg protein as crude extract (right) or as DEAE-cellulose treated extract (left); potassium phosphate buffer at pH 6.5, 100 µmoles; uric acid, 20 µmoles; methyl viologen where indicated, 1 µmole; ferredoxin where indicated, 16 units; water to a final volume of 3 ml; 0.2 ml of 20 per cent KOH was in the center well. Gas phase hydrogen.

DEAE-cellulose-treated extract does not catalyze the reduction of uric acid; activity is restored by the addition of methyl viologen or ferredoxin.

A proposed scheme for production of molecular hydrogen from hypoxanthine is given below:



No differences in the properties of ferredoxin isolated from ammonia or nitrogen grown C. pasteurianum or that isolated from M. lactilyticus have been detected; in all systems tested purified ferredoxin is completely interchangeable. In C. pasteurianum reduction of hydroxylamine has been found to require ferredoxin; in M. lactilyticus ferredoxin has been found to serve as the electron carrier for the oxidation of pyruvate and α-ketoglutarate as well as for the evolution of hydrogen from dithionite. Pyruvate oxidation by extracts of Peptostreptococcus elsdenii, Butyribacterium rettgeri, Clostridium tetanomorphum, and Clostridium butyricum is stimulated by ferredoxin. However, ferredoxin does not substitute for methyl viologen in the hydrogen reduction of fumarate (Peck et al 1957) or in the formic dehydrogenase system described by Hug and Sagers (1957).

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