ROLE OF FERREDOXIN IN THE METABOLISM OF MOLECULAR HYDROGEN

R. C. VALENTINE AND R. S. WOLFE

Department of Microbiology, University of Illinois, Urbana, Illinois

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ABSTRACT

VALENTINE, R. C. (University of Illinois, Urbana) AND R. S. WOLFE. Role of ferredoxin in the metabolism of molecular hydrogen. J. Bacteriol. **85**:1114–1120. 1963.—The metabolism of molecular hydrogen by *Clostridium pasteurianum*, *Micrococcus lactilyticus* (*Veillonella alcalescens*), and several other anaerobic bacteria was studied. Oxidation of hydrogen, using several electron-accepting substrates including triphosphopyridine nucleotide, uric acid, xanthine, nitrite, and hydroxylamine, required ferredoxin in conjunction with hydrogenase. Evolution of hydrogen from pyruvate, α-ketoglutarate, hypoxanthine, and dithionite was mediated by ferredoxin. On the basis of these findings, a unitary hypothesis for biological hydrogen evolution is proposed in which ferredoxin plays a key role.

In recent studies concerned with the mechanism of H_2 evolution by *Clostridium pasteurianum*, it has been shown that ferredoxin functions as an electron coupler between pyruvic dehydrogenase and hydrogenase (Mortenson, Valentine, and Carnahan, 1962; Valentine, Jackson, and Wolfe, 1962*a*). In *Micrococcus lactilyticus* (*Veillonella alcalescens*), H_2 evolution from hypoxanthine, as well as the reduction of uric acid by H_2, is ferredoxin-dependent (Valentine et al., 1962*a*). On the basis of these studies, a general scheme for H_2 formation has been formulated.

$$\text{Substrate} + \text{ferredoxin (ox)} \xrightarrow{\text{dehydrogenase}} \text{ferredoxin (red)} + \text{oxidized substrate}$$

$$\text{ferredoxin (red)} \xrightarrow{\text{hydrogenase}} \text{ferredoxin (ox)} + H_2$$

To elucidate further the role of ferredoxin in the metabolism of H_2, we investigated a number of bacterial systems which evolve or oxidize molecular hydrogen; the results are presented in this communication.

MATERIALS AND METHODS

Organisms. Growth conditions for *C. pasteurianum* W-5 and preparation of crude extracts were as described by Carnahan et al. (1960). *Desulfovibrio desulfuricans* 8303 was kindly supplied by J. M. Akagi and L. Leon Campbell, Jr., and was grown on Postgate's modification of medium C of Butlin, Adams, and Thomas (1949) as described by Baker, Papiska, and Campbell (1962). Cells of the thermophilic sulfate-reducing bacterium, *C. nigrificans*, were supplied by J. M. Akagi and were grown on PYL medium as described by Campbell, Frank, and Hall (1957). *M. lactilyticus* 221 was grown on lactate as described by Whiteley and Douglas (1951), and extracts were prepared as described by Valentine et al. (1962*a*). *Peptostreptococcus elsdenii* (*LC*) was grown on the lactate medium of Elsden and Lewis (1953). *Clostridium acidiurici*, kindly supplied by R. D. Sagers, was grown on the uric acid medium described by Barker and Beck (1941). *Streptococcus allantoicus* was grown on allantoin as described by Barker (1943), and *C. tyrobutyricum* (*C. lactoacetophilum*) was grown on glucose according to the procedure of Bhat and Barker (1947). *Rhodospirillum rubrum* was grown photosynthetically on the glutamate medium of Gest and Kamen (1949). *Butyribacterium rettgeri* was grown on lactate according to the method of Barker and Haas (1944).

Extract preparation. Extracts of the above organisms were prepared by crushing the freshly harvested cells in a Hughes press. The crushed cells were suspended in water (at 0 C) which contained deoxyribonuclease (75 μg per ml). The cell debris was removed by centrifugation at 15,000 × *g* for 15 min; extracts containing 25 to 30 mg of protein per ml were prepared by this procedure.

Removal of ferredoxin. Ferredoxin was removed by passage of the crude extracts through a column of diethylaminoethylcellulose (DEAE cellulose) as described previously (Mortenson et al., 1962; Valentine et al., 1962*a*).

Purification of ferredoxin. Ferredoxin from the DEAE cellulose column noted above was purified, and purification units were employed according to Mortenson et al. (1962) and Valentine et al. (1962*a*). For most of the experiments described here, a dried preparation of ferredoxin (60 units per mg) from *C. pasteurianum* was used. For certain experiments, ferredoxin was purified further by a second chromatography on DEAE cellulose (phosphate). A linear gradient from 0 to 0.5 M phosphate buffer at pH 6.5 was used for elution. This procedure was found to remove small amounts of hydrogenase which were present in the dried ferredoxin preparation.

Hydrogenase assay. Hydrogenase activity was assayed by a manometric procedure similar to that described by Peck and Gest (1956). For certain experiments, ferredoxin (0.0 to 0.15 mg) was used in place of methyl viologen in this assay.

Assay of hydroxylamine and nitrite reductase. The standard reaction mixture in each Warburg vessel contained: 50 μmoles of potassium phosphate buffer (pH 6.5), 20 μmoles of hydroxylamine, or 10 μmoles of sodium nitrite, extract, and ferredoxin (usually 0.5 mg). Methyl viologen (1 μmole) was substituted for ferredoxin when using extracts of *C. nigrificans*, and, similarly, 0.08 mg of cytochrome c_3 were substituted when using extracts of *D. desulfuricans*. H_2 was the gas phase; incubation was at 30 C, except for extracts of *C. nigrificans* which were incubated at 50 C. Utilization of H_2 was measured manometrically.

Reduction of diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN). Oxidation of H_2 with reduction of DPN or TPN was followed manometrically; each Warburg vessel contained, in 3 ml, 0 to 8 μmoles of DPN or TPN, enzyme, 100 μmoles of potassium phosphate buffer (pH 6.5), and ferredoxin (usually 0.5 mg). Incubation was at 30 C. Hydrogen utilization was followed manometrically and reduced diphosphopyridine nucleotide (DPNH) or reduced triphosphopyridine nucleotide (TPNH) production was determined by measuring the increase in optical density at 340 mμ in a Cary spectrophotometer.

Formic dehydrogenase. Formic dehydrogenase from *C. acidiurici* was measured by following H_2 evolution from formate in a coupled reaction with hydrogenase from *C. pasteurianum*. Each Warburg vessel contained, in 3 ml, 7.2 mg of extract from *C. acidiurici* containing formic dehydrogenase, 3.5 mg of *C. pasteurianum* extract containing hydrogenase, 50 μmoles of sodium formate, 50 μmoles of potassium phosphate buffer (pH 6.5), and 1 μmole of methyl viologen. Incubation was at 30 C; N_2 was the gas phase. KOH (0.2 ml of a 20% solution) was placed in the center well.

H_2 evolution from α-ketoglutarate. H_2 evolution from α-ketoglutarate by *M. lactilyticus* (McCormick, Ordal, and Whiteley, 1962) was measured in a Warburg vessel containing 30 μmoles of sodium α-ketoglutarate, 150 μmoles of potassium phosphate buffer (pH 6.5), 30 units of coenzyme A (CoA), 14 mg of *M. lactilyticus* extract, ferredoxin (usually 0.2 mg), and water to 3 ml. Incubation was at 30 C. N_2 was the gas phase. KOH (0.2 ml of a 20% solution) was in the center well. Other products of α-ketoglutarate degradation were not determined.

Sulfate and sulfite reduction. The adenosine triphosphate (ATP)-dependent reduction of sulfate by *D. desulfuricans* was measured manometrically by following H_2 utilization according to the procedure of Peck (1959). Sulfite reduction using H_2 also was determined manometrically; each Warburg vessel contained, in a volume of 3 ml, 100 μmoles of potassium phosphate buffer (pH 6.5), 10 μmoles of sodium sulfite, 0.5 μmole of methyl viologen, and 14 mg of crude extract of *D. desulfuricans*.

Colorimetric assays. Protein was determined by the Lowry modification of the Folin procedure (Lowry et al., 1951). Ammonia was determined by the method of Conway (1950). Nitrite was determined by using the sulphanilamide-α-naphthylene diamine reagents as described by Nicholas, Medina, and Jones (1960). Acetyl phosphate was determined by the method of Lipmann and Tuttle (1945).

Chromatography. DEAE cellulose was generated in the phosphate form by soaking DEAE cellulose (Brown Co., Berlin, N.H.) with 1 M potassium phosphate buffer (pH 7.5) for at least 24 hr. Columns were prepared by adding a slurry of DEAE cellulose to the top of chromatographic tubes which contained a small amount of glass

TABLE 1. *Role of ferredoxin in pyruvate oxidation by different bacteria**

Source of extract	Crude extract	DEAE cellulose-treated extract		
	No carrier added	No carrier added	Ferredoxin added	Methyl viologen added
Clostridium pasteurianum	4.1†	0.2	8.1	7.4
Micrococcus lactilyticus	2.1	0.1	4.3	3.9
Peptostreptococcus elsdenii	1.8	0.2	4.5	5.2
C. lactoacetophilum	2.2	0.1	5.0	5.2
C. nigrificans‡	0.1	0.1	0.1	24.0
Desulfovibrio desulfuricans	0.4	0.4	1.6	3.4
Butyribacterium rettgeri	0.1	0.0	2.7	2.9
C. acidi-urici	0.1	0.1	0.1	0.3
Streptococcus allantoicus	0.0	0.0	0.0	0.0
Rhodospirillum rubrum	0.0	0.0	0.0	0.0

* A complete reaction mixture in 1 ml contained a suitable amount of extract (pyruvate clastic enzymes), 100 μmoles of sodium pyruvate, 30 units of coenzyme A, 50 μmoles of potassium phosphate buffer (pH 6.5), and 0.2 mg of ferredoxin or 0.5 μmole of methyl viologen as indicated. After 15 min at 30 C, the reaction was terminated by addition of 1 ml of 2 M neutral hydroxylamine.

† Results expressed as μmoles of acetyl phosphate formed per mg of protein per hr.

‡ Incubation temperature was 51 C.

wool firmly packed at the bottom. Residual phosphate was removed by passing 10 resin-bed volumes of water through the column prior to use. The column was then packed to constant flow rate by gently tamping the resin with a glass rod.

Chemicals. Crystalline cytochrome *c* was obtained from Nutritional Biochemical Corp., Cleveland, Ohio, and cytochrome c_3 (95% pure) was kindly supplied by L. Leon Campbell, Jr.; methyl and benzyl viologen were purchased from British Drug House, Ltd.

RESULTS

Role of ferredoxin in pyruvate oxidation. Table 1 represents a survey of pyruvate clastic reactions in several anaerobic bacteria. The specific activities (measured in terms of acetyl phosphate formed per milligram of extract protein per hour) of the crude bacterial extracts are compared with the specific activities of extracts treated with DEAE cellulose to remove ferredoxin. In the case of *C. pasteurianum*, 4.1 μmoles of acetyl phosphate were formed per hr per mg of extract protein; after passage of the crude extract through DEAE cellulose to remove ferredoxin, the eluate (containing 95% of the protein) no longer carried out the pyruvate clastic reaction. The reconstituted system catalyzed the production of 8.1 μmoles of acetyl phosphate per mg of protein per hr. The specific activity of our enzyme preparations varied considerably, but all preparations showed a requirement for ferredoxin. Pyruvate oxidation by crude extracts of *C. pasteurianum* may be stimulated fivefold by addition of 0.2 mg of ferredoxin. Using the resolved extract, 0.5 μmole of methyl viologen also was effective in restoring activity. Benzyl viologen was found to be about one-fourth as effective as methyl viologen. These results indicate that the limiting reaction in pyruvate oxidation by *C. pasteurianum* was concerned with electron transport. H_2 formation from pyruvate showed an identical stimulation by ferredoxin. Addition of ferredoxin to extracts of *M. lactilyticus*, *P. elsdenii*, *C. lactoacetophilum*, *D. desulfuricans*, and *B. rettgeri* showed marked stimulation of pyruvate oxidation (Table 1). Crude extracts of *B. rettgeri* and *D. desulfuricans*, before removal of ferredoxin, showed only weak oxidation of pyruvate. It was interesting to note that cytochrome c_3 was readily reduced by pyruvic dehydrogenase from *C. pasteurianum*, but did not promote H_2 evolution from pyruvate. *C. acidiurici* does not evolve H_2 from pyruvate (hydrogenase is absent); pyruvate was oxidized only in the presence of electron acceptors such as DPN, TPN, or benzyl viologen. In the normal fermentation of uric acid by *C. acidiurici*, electrons from pyruvate (or formate) are presumably transferred to uric acid to form xanthine; the electron-transport steps involved in this process have not been elucidated but, by analogy to uric acid reduction by *M. lactilyticus*, they may involve ferredoxin. Methyl viologen served as electron mediator for pyruvate oxidation by *C. nigrificans*, resulting in the formation of 24 μmoles of acetyl phosphate per hr per mg of protein, but ferredoxin was not effective.

Lack of coupling of ferredoxin with formic dehydrogenase. A model formic hydrogenlyase system similar to that described by Gest and Peck (1955) was composed of formic dehydro-

genase from *C. acidiurici* (Hug and Sagers, 1957), hydrogenase from *C. pasteurianum*, and methyl viologen as electron carrier. The complete system catalyzed the linear evolution of 224 μliters of H_2 in 23 min. When ferredoxin replaced methyl viologen, no H_2 evolution was observed. Under the conditions used, ferredoxin did not couple with formic dehydrogenase; similar findings were observed with crude formic dehydrogenase preparations from *D. desulfuricans*.

Evolution of H_2 from dithionite. Extracts of *M. lactilyticus* have been found to catalyze the evolution of H_2 from a solution of dithionite (Whiteley and Ordal, 1957; Valentine et al., 1962*a*). Ferredoxin is required for H_2 evolution (Table 2). Methyl and benzyl viologen replaced ferredoxin. In the complete reaction mixture (flask 4), 6.2 μmoles of H_2 were evolved in 15 min when 2 mg of *M. lactilyticus* protein, free of ferredoxin, were incubated with 20 μmoles of dithionite and 0.2 mg of ferredoxin. Hydrogen evolution from dithionite by *C. pasteurianum* extracts also requires ferredoxin as catalyst (Mortenson et al., 1962).

Reduction of electron-accepting dyes. Extracts of *C. pasteurianum* or *M. lactilyticus* which were free of ferredoxin readily reduced benzyl viologen with hydrogen or pyruvate as reductant. Extracts of *C. pasteurianum* which were free of ferredoxin readily reduced methylene blue with H_2 and carried out the reduction of cytochrome c_3, methylene blue, methyl viologen, flavins, and neotetrazolium chloride with pyruvate. These results indicate that either hydrogenase or pyruvic dehydrogenase may reduce certain electron acceptors without the intervention of ferredoxin.

α-Ketoglutarate oxidation. As shown in Fig. 1, ferredoxin is required for H_2 evolution from α-ketoglutarate. In the complete reaction mixture containing 14 mg of ferredoxin-resolved extract from *M. lactilyticus* and 0.2 mg of ferredoxin, 170 μliters of H_2 were formed in 40 min. Omission of ferredoxin resulted in a marked decrease in H_2 production (to 18 μliters). Other products of α-ketoglutarate oxidation were not determined.

Nitrite reduction. Extracts of *C. pasteurianum*, prepared from cells grown on $(NH_4)_2SO_4$ or N_2 as nitrogen source, readily reduce nitrite to NH_3 with H_2. The stoichiometry of nitrite reduction with H_2 is presented in Table 3; in this experiment, disappearance of nitrite was compared with ammonia synthesis and hydrogen utilization. Flasks 1 and 2 are controls without nitrite and enzyme, respectively. Flasks 3 and 4 represent complete reaction mixtures containing 10.2 mg of *C. pasteurianum* extract. All of the nitrite in flasks 3 and 4 was used for ammonia synthesis. These data are consistent with a reaction in which 1 mole of nitrite is reduced by 3 moles of H_2 to yield 1 mole of ammonia.

Figure 2 shows that ferredoxin is required for nitrite reduction. In this experiment, 50 ml of crude extract of *C. pasteurianum* containing 1.25 g of protein were added to a DEAE cellulose

TABLE 2. *H_2 evolution from dithionite by Micrococcus lactilyticus**

Flask no.	Component omitted	H_2 evolved per 15 min
		μmoles
1	Dithionite	0.0
2	Ferredoxin	0.2
3	Enzyme	0.2
4	None	6.2

* Each Warburg vessel contained (in 3 ml) 2 mg of extract protein, 100 μmoles of potassium phosphate buffer (pH 6.5), 20 μmoles of sodium dithionite, and 0.2 mg of ferredoxin as indicated. KOH (0.2 ml of 20%) was in the center well; incubation was at 30 C for 15 min. Nitrogen was the gas phase. H_2 evolution was followed manometrically.

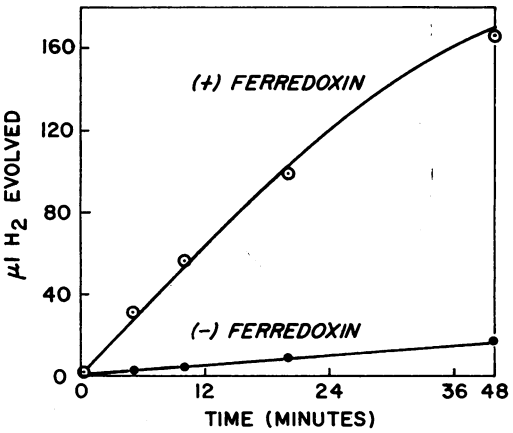

FIG. 1. *H_2 evolution from α-ketoglutarate. Each Warburg flask contained (in 3 ml) 30 μmoles of sodium α-ketoglutarate, 150 μmoles of potassium phosphate (pH 6.5), 30 units of CoA, 14 mg of Micrococcus lactilyticus extract free of ferredoxin, and 0.2 mg of ferredoxin from M. lactilyticus. Incubation was at 30 C; KOH (0.2 ml of 20%) was in the center well. Control flasks without enzyme and α-ketoglutarate showed no H_2 evolution.*

TABLE 3. *Nitrite reduction by Clostridium pasteurianum**

Flask no.	Nitrite added	Nitrite used	NH_3 formed	H_2 used
1	0.0	0.0	0.27	0.10
2	9.8	0.0	0.0	0.00
3	9.8	9.8	8.58	29.10
4	9.8	9.8	10.26	28.50

* Complete reaction mixture contained sodium nitrite as indicated, 50 μmoles of potassium phosphate buffer (pH 6.5), 10.2 mg of extract, and water to a final volume of 3 ml; KOH (0.2 ml of a 20% solution) was in the center well; hydrogen was the gaseous phase. Incubation was at 30 C for 30 min. Enzyme was omitted from flask 2. Results expressed in μmoles.

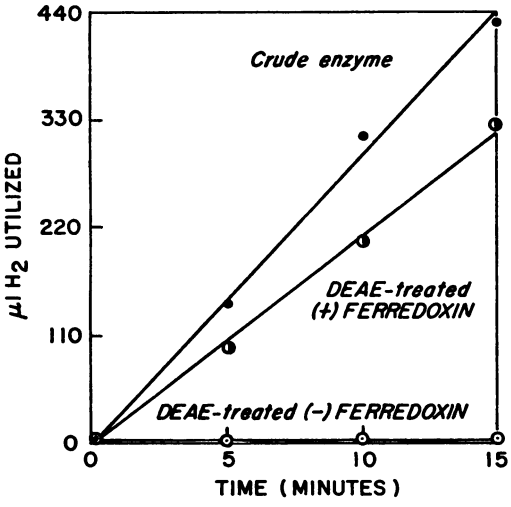

FIG. 2. *Ferredoxin requirement for nitrite reduction. The complete reaction mixture contained 10 μmoles of sodium nitrite, 50 μmoles of potassium phosphate buffer (pH 6.5), 8.8 mg of crude or DEAE-cellulose-treated C. pasteurianum enzyme, 0.5 mg of ferredoxin, where indicated, and water to a final volume of 3 ml. H_2 was the gas phase. KOH (0.2 ml of 20% solution) was in the center well. Incubation temperature was 30 C.*

column (1 by 4 cm). As shown, the DEAE-treated extract supplemented with ferredoxin utilized 320 μliters of H_2 compared with 430 μliters for the crude extract. No H_2 utilization occurred in the absence of ferredoxin (lower curve).

Hydroxylamine reduction to ammonia. The hydroxylamine reductase system of *C. pasteurianum* also required ferredoxin. Table 4 shows the effect of benzyl viologen, ferredoxin, and cytochrome c_3 on hydroxylamine reduction to ammonia by extracts of *C. pasteurianum*, *D. desulfuricans*, and *C. nigrificans*. The electron carriers required for hydroxylamine reduction in these organisms are readily apparent from Table 4. The natural electron carrier from *C. pasteurianum* is ferredoxin and that from *D. desulfuricans* is cytochrome c_3; neither of these carriers would replace benzyl viologen for hydroxylamine reduction by *C. nigrificans*.

Sulfate and sulfite reduction. In our hands, ferredoxin did not mediate electrons for the reduction of sulfite or sulfate. In one experiment, 215 μliters of H_2 were utilized in 25 min when 10 mg of crude extract of *D. desulfuricans* were incubated with 12 μmoles of sodium sulfite and 1 μmole of methyl viologen. When methyl viologen was omitted, 17 μliters of H_2 were utilized for sulfite reduction. When ferredoxin (0.2 mg) was substituted for methyl viologen, 25 μliters of H_2 were utilized, indicating that ferredoxin did not replace methyl viologen for sulfite reduction. In similar experiments with the ATP-dependent reduction of sulfate with H_2 (Peck, 1959), ferredoxin did not substitute for methyl viologen as an efficient electron carrier between hydrogenase and adenosine-5′-phosphosulfate (APS) reductase.

TABLE 4. *Electron carriers for NH_2OH reduction**

Source of extract	Electron carrier added			
	None	BV	Ferredoxin	c_3
Clostridium pasteurianum	0.0†	1.1	12.0	0.0
Desulfovibrio desulfuricans	0.0	3.5	0.0	4.1
Clostridium nigrificans	0.0	4.1	0.0	0.0

* Each Warburg flask (in 3 ml) contained: 7 to 10 mg of extract protein, 100 μmoles of potassium phosphate buffer (pH 6.5), 15 μmoles of hydroxylamine, and electron carrier as indicated [0.5 μmole of benzyl viologen (BV)], 0.4 mg of ferredoxin, 0.08 mg of cytochrome c_3. Incubation was at 30 C, except for *C. nigrificans* which was at 50 C. *D. desulfuricans* extract was freed from cytochrome c_3 by passage through Amberlite resin GC 50, and *C. pasteurianum* was freed of ferredoxin as described. Hydroxylamine reduction was measured by following H_2 utilization.

† Values represent μmoles of H_2 utilized per mg of extract protein per hr.

Pyridine nucleotide reduction. Freshly prepared extracts of *C. pasteurianum* recently have been found to catalyze the rapid reduction of TPN with H_2 or pyruvate (Valentine, Brill, and Wolfe, 1962*b*); the viologen dyes (methyl and benzyl) substituted for ferredoxin. Extracts prepared from cells grown with N_2 or with $(NH_4)_2SO_4$ as a source of cellular nitrogen were equally active with respect to TPN reduction. H_2 was not evolved from TPNH, and ferredoxin did not mediate the direct reduction of nitrite or hydroxylamine from TPNH. Using the system described by Valentine et al. (1962*b*), the oxidation of H_2 and reduction of DPN occurred only when the crude extract was supplemented with catalytic amounts (0.3 μmole) of benzyl viologen, the stoichiometry of DPN reduction being 5.0 μmoles of H_2 utilized and 4.35 μmoles of DPNH formed. Ferredoxin did not stimulate DPN reduction. Addition of small amounts of TPN to the extract did not mediate the reduction of DPN from H_2, indicating that an active pyridine nucleotide transhydrogenase was not present in the extract.

Discussion

Koepsell and Johnson (1942) found that extracts of *C. butylicum* readily cleaved pyruvate, yielding CO_2, H_2, and acetyl phosphate; this reaction was believed responsible for the rapid evolution of hydrogen gas by these organisms. Wolfe and O'Kane (1953) later studied this reaction in more detail and elucidated the role of CoA and thiamine pyrophosphate as cofactors for pyruvate cleavage. The marked stimulation of pyruvate oxidation by certain electron-accepting dyes indicated that the limiting reaction in pyruvate breakdown was concerned with electron transport. The present concept of pyruvate oxidation is that electrons of the pyruvic dehydrogenase complex are first transferred to ferredoxin, which functions as an electron coupler between pyruvic dehydrogenase and hydrogenase. Ferredoxin also serves as an electron mediator for hydrogen evolution from hypoxanthine and α-ketoglutarate as well as for the oxidation of hydrogen coupled with reduction of nitrite, hydroxylamine, uric acid, and xanthine. On the basis of these studies, a general pathway for the biological formation of hydrogen has been formulated in which ferredoxin plays a key role. In this scheme, specific dehydrogenases reduce ferredoxin, which is oxidized by hydrogenase to yield hydrogen. That the dehydrogenase-ferredoxin reaction involves a degree of specificity is best illustrated by formic dehydrogenase which did not couple with ferredoxin. This result is in agreement with earlier findings by Gest and Peck (1955), who found that crude extracts of *M. lactilyticus*, now known to contain ferredoxin, did not mediate hydrogen evolution from formate using formic dehydrogenase from a variant strain of *E. coli*. Specificity also exists between ferredoxin and hydrogenase, certain hydrogenases being unable to couple effectively with ferredoxin (e.g., the hydrogenase of *C. nigrificans*).

Extracts of *C. pasteurianum* recently have been found to catalyze the rapid reduction of TPN but not DPN with molecular hydrogen (Valentine et al., 1962*b*). TPN also served as electron acceptor for pyruvate oxidation, reduction from pyruvate being dependent on ferredoxin. Reduction of DPN from hydrogen was not mediated by ferredoxin but required benzyl viologen. Ferredoxin did not replace methyl viologen for reduction of sulfate and sulfite with hydrogen using extracts of *D. desulfuricans* which contained cytochrome c_3, indicating the requirement for an additional carrier(s) in these reactions.

In addition to the functions of ferredoxins described above, it is interesting to speculate on other reactions in which ferredoxin might participate. The low redox potential of ferredoxin makes it an ideal carrier or reductant for conversion of N_2 to ammonia in the nitrogen-fixing organisms; its requirement in pyruvate oxidation by extracts of *C. pasteurianum* and its close association with hydrogenase support this view.

The role of ferredoxins as electron carriers in photosynthesis has recently been described by Tagawa and Arnon (1962). These authors confirmed the previous findings with ferredoxin from *C. pasteurianum* and found that crystalline clostridial ferredoxin replaced photosynthetic pyridine nucleotide reductase in the light-dependent generation of TPNH by spinach chloroplasts as studied by San Pietro (1961) and Fry and San Pietro (1962). Tagawa and Arnon (1962) also reported the isolation of ferredoxin from the photosynthetic bacteria *R. rubrum* and *Chromatium* indicating its general occurrence in photosynthetic tissues.

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