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Formic Hydrogenlyase and the Photoassimilation of Formate by a Strain of *Rhodopseudomonas palustris*

S. M. HUSSAIN QADRI[1] AND D. S. HOARE
Microbiology Department, The University of Texas, Austin, Texas 78712

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A photosynthetic bacterium isolated by enrichment on media containing formate as major source of cell carbon was identified as a strain of *Rhodopseudomonas palustris*. It grew on a wide range of simple organic compounds including alcohols, fatty acids, and hydroxyacids, on a chemically defined medium with biotin and *p*-aminobenzoic acid as essential growth factors. The organism grew on formate or photoautotrophically with molecular hydrogen or thiosulfate only in the presence of yeast extract. Ability to photoassimilate formate could be shown only in organisms grown in the presence of formate. The organism contained an inducible formic hydrogenlyase consisting of a soluble formic dehydrogenase, a particulate hydrogenase, and one or more intermediate, but as yet unidentified, electron carriers. The formic hydrogenlyase could be reconstituted from a particulate hydrogenase and a partially purified soluble formic dehydrogenase. Some properties of the formic dehydrogenase and hydrogenase have been compared with that of the formic hydrogenlyase system.

The purple nonsulfur photosynthetic bacteria or *Athiorhodaceae* grow anaerobically in the light on diverse simple organic compounds (42). However, their ability to grow autotrophically or on organic "one-carbon compounds" such as methanol or formate is not well established. Van Niel (41, 42) showed that members of the *Athiorhodaceae* assimilated formate in media containing yeast extract, and cell suspensions of *Rhodospirillum rubrum* have been shown to decompose formate to carbon dioxide and molecular hydrogen (2, 3, 23). Autotrophic growth with thiosulfate as reductant, in media containing yeast extract, was found to be characteristic of *Rhodopseudomonas palustris*. Autotrophic growth on a chemically defined medium with molecular hydrogen has been established with a strain of *R. rubrum*, but growth was exceedingly slow (33). Similarly, a strain of *Rhodopseudomonas gelatinosa* was recently reported to grow photoautotrophically with molecular hydrogen or photosynthetically on methane, although growth under these conditions was also exceedingly slow (45). It is not clear why members of the *Athiorhodaceae* grow so slowly under photoautotrophic conditions, and no detailed studies have been carried out to determine the mechanisms of assimilation of organic one-carbon compounds by photosynthetic bacteria.

The major objectives of our investigation were to isolate pure cultures and to study the metabolism of *Athiorhodaceae* which would grow photosynthetically on one-carbon compounds. The present paper describes the isolation, characterization, and physiological properties of one isolate, identified as a strain of *R. palustris*, with particular reference to its ability to grow photoautotrophically and to metabolize formate. The mechanism of formate assimilation by this strain is discussed in relation to its photoautotrophic growth, and some studies on an inducible formic hydrogenlyase are described. A preliminary report on the formic hydrogenlyase of *R. palustris* has been published (S. M. H. Qadri and D. S. Hoare, Bacteriol. Proc., p. 121, 1967).

MATERIALS AND METHODS

Enrichment and isolation of a formate-utilizing photosynthetic bacterium. Larsen's modification of Winogradsky's procedure (25) was used to produce crude enrichment cultures which were subsequently used to inoculate an "enrichment medium" (42) of the following composition (percentage, w/v): $NaHCO_3$, 0.5; NaCl, 0.2; $(NH_4)_2SO_4$, 0.1; K_2HPO_4, 0.05;

[1] Present address: Department of Microbiology, University of Karachi, Karachi, Pakistan.

$MgSO_4 \cdot 7H_2O$, 0.01; $Na_2S \cdot 9H_2O$, 0.01; Difco yeast extract, 0.05; and sodium formate, 0.2. Completely filled glass-stoppered bottles were incubated in the light at 28 to 30 C. Pure cultures were isolated by standard procedures using shake dilutions on the enrichment medium. Cultures were assumed to be pure when the morphology of the organisms in the pigmented colonies of two consecutive series of shake dilutions appeared to be the same. Stocks were stored at +2 C as stab cultures on a chemically defined medium (24) with 20 mM sodium acetate as organic carbon source. Cultures for growth experiments were maintained in the chemically defined medium in completely filled (20 ml) screw-capped tubes at 28 to 30 C in the light and were transferred (0.1 ml of inoculum per 20 ml) every 4 to 5 days.

Growth experiments. Screw-capped tubes completely filled (20 ml) with a chemically defined medium (24) containing organic carbon sources at 20 mM (final concentration) were inoculated with 0.1 ml of a stock culture adjusted to a concentration at 0.7 mg (dry weight) per ml. Tubes, in triplicate for each organic compound tested, were incubated in a water bath at 28 to 30 C and illuminated at a light intensity of 140 ft-c. Growth was followed by periodic determinations of the absorbance at 600 mμ in a Bausch and Lomb Spectronic-colorimeter 20. For photoautotrophic growth, the enrichment medium containing yeast extract (20 ml) was dispensed in 50-ml Pyrex tubes fitted with rubber stoppers and gassing tubes. Cultures were gassed, with hydrogen-carbon dioxide (95:5, v/v) or nitrogen-carbon dioxide (95:5, v/v) as controls, for 15 min every 12 hr.

Electron microscopy. Specimens for thin-sectioning were fixed by a modification of the method of Kellenberger et al. (21), and standard procedures were used for shadow-cast preparations.

Batch cultures of organisms for metabolic experiments. For most large-scale experiments, the enrichment medium with a mixture of 10 mM sodium formate and 10 mM sodium acetate was used as organic carbon source. Media (4- or 20-liter batches) were inoculated with 2.5 ml of cell suspension (0.7 mg, dry weight, per ml) per liter, gassed with carbon dioxide-hydrogen (95:5, v/v) for 10 min, and incubated for 4 days at 28 to 30 C in the light. Cultures grown on media containing formate as sole organic carbon source were incubated for 6 to 7 days.

Dry weight and protein estimations. Absorbance of suspensions at 680 mμ, measured in a Beckman DU-2 spectrophotometer, was related to dry weight from a prepared calibration curve. Protein was determined from the absorbance at 260 and 280 mμ (44).

Preparation of cell-free extracts. Organisms were centrifuged at 27,000 × *g* at 2 C and were washed twice in 50 mM potassium phosphate buffer, *p*H 7.0. Washed organisms (6 g, wet weight) were suspended in 30 ml of 20 mM potassium phosphate buffer, of appropriate *p*H for the assay to be studied, with 15 g of washed, fine glass beads (Superbrite Type 110, 3M Company, St. Paul, Minn.). The suspension was treated with a model L sonic disintegrator (Measuring and Scientific Equipment Ltd., London) fitted with a 1-cm diameter probe for two periods of 5 min at 1 C. The distintegrated suspension was centrifuged for 30 min at 34,800 × *g*, and the residue (R1) was usually discarded. The supernatant fluid (S1) was centrifuged for 1 hr at 100,000 × *g* in a Spinco model L ultracentrifuge, yielding a pigmented residue (R2) and a clear supernatant fluid (S2). In some cases, the supernatant S2 was centrifuged for an additional 4 hr at 100,000 × *g*, yielding a supernatant fluid (S3) and a residue (R3).

Manometric experiments. A Gilson photosynthetic Warburg bath and conventional double side-bulb reaction flasks were used. For photoassimilation experiments, illumination was provided by a bank of 25-w incandescent bulbs which were cooled by a current of air generated by an electric fan. All manometric assays were performed at 30 C with a reaction mixture of 3.0 ml total volume.

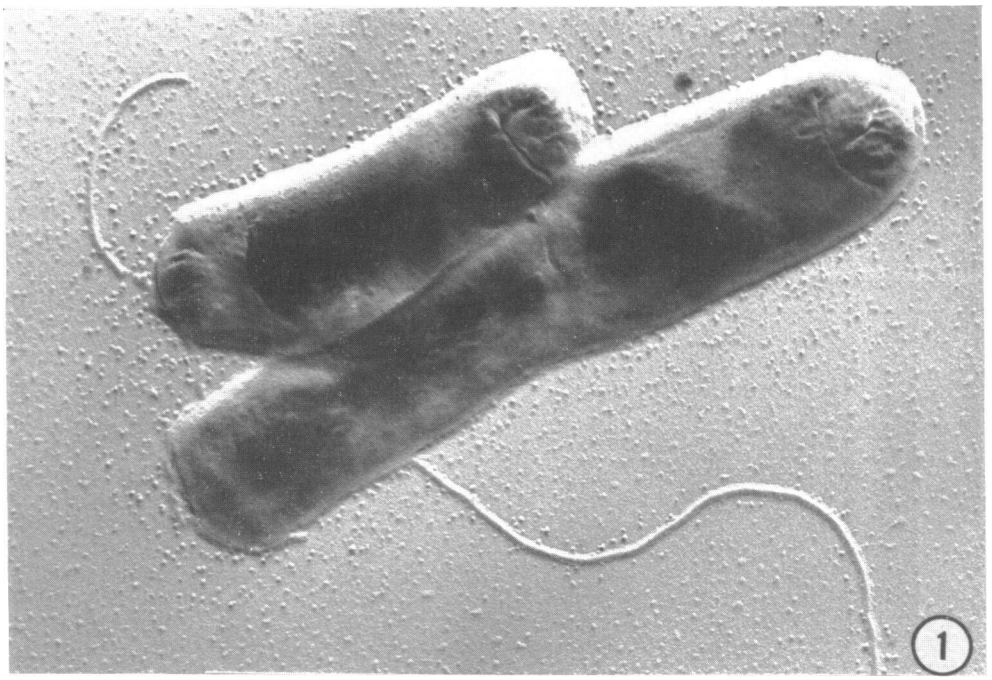
The following standard manometric assays were used. Photoassimilation of organic substrates: the main flask contained cell suspension (20 mg, dry weight) in sodium bicarbonate buffer (75 μmoles), and the side bulb contained the organic substrate (10 to 20 μmoles of Na acetate or Na formate in bicarbonate buffer). The flasks were gassed with a mixture (95:5, v/v) of nitrogen and carbon dioxide. Controls were included without organic substrate, and dark controls were run in flasks covered with aluminum foil. The manometric basis for the observed pressure changes when the sodium salt of an organic acid is photometabolized by cell suspensions of photosynthetic bacteria has been described by Elsden and Ormerod (8). Photoassimilation of molecular hydrogen and carbon dioxide was measured under similar conditions, without any organic substrate; the flasks were gassed with a mixture (95:5, v/v) of hydrogen and carbon dioxide. Rates were expressed as nanomoles of total gas (i.e., $CO_2 + H_2$) uptake per minute. Hydrogenase was assayed by gas consumption in the presence of potassium ferricyanide as electron acceptor. Reaction mixture (3 ml) contained (in μmoles): potassium phosphate buffer (*p*H 6.5), 50; $K_3Fe(CN)_6$, 50; and enzyme preparation. Center wells contained 0.2 ml of 20% (w/v) KOH and the flasks were gassed with hydrogen.

Formic dehydrogenase assays (3 ml total volume) contained (in μmoles): potassium phosphate buffer (*p*H 6.3), 50; $K_3Fe(CN)_6$, 50; sodium formate, 50; and enzyme preparation. Flasks were gassed with argon or nitrogen; substrate and $K_3Fe(CN)_6$ were added from separate side-bulbs. One unit of enzyme is defined as that amount of enzyme which catalyzed the utilization of 1 nanomole of substrate per min. Specific activity is defined as enzyme units per milligram of protein.

Formic hydrogenlyase assays (3 ml total volume) contained (in μmoles): potassium phosphate buffer (*p*H 6.5), 50; sodium formate, 50; and enzyme preparation. The reaction was followed as total gas (H_2 plus CO_2) production, or as hydrogen production when the center wells contained 0.2 ml of 20% (w/v) KOH. The flasks were gassed with hydrogen.

RESULTS

General morphological and cultural characteristics. The pure culture isolate was a gram-



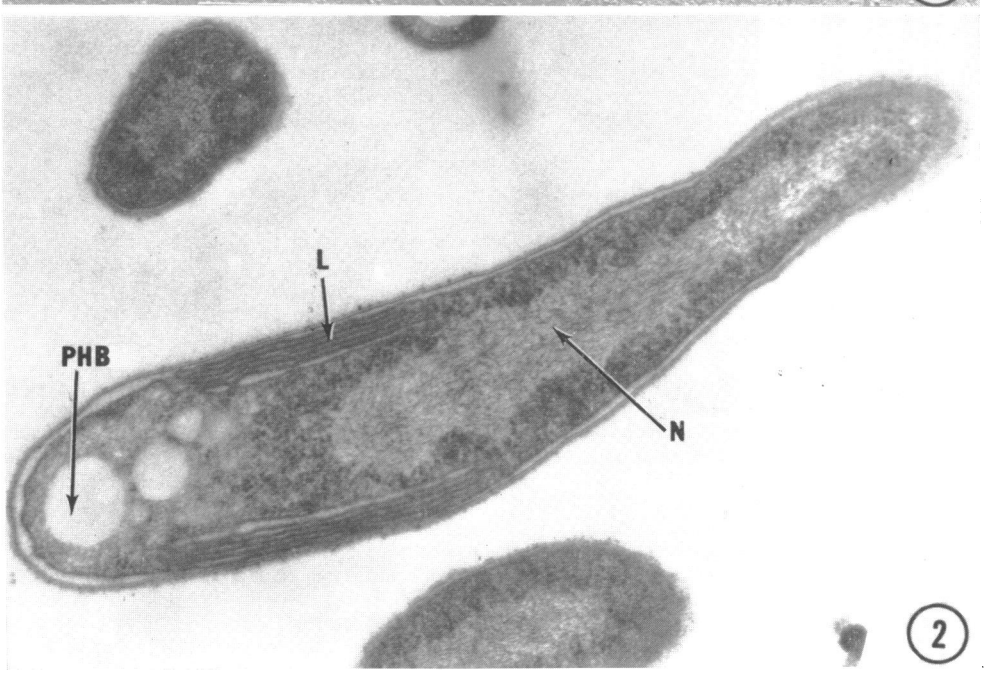


FIG. 1. *General morphological features of Rhodopseudomonas palustris, showing a single flagellum. A shadow-cast preparation photographed at a magnification of 60,000 ×.*

FIG. 2. *Ultrastructure of Rhodopseudomonas palustris photographed at a magnification of 66,000 ×. The section shows the characteristic lamellar membrane intrusions (L), fibrillar nuclear material (N), and poly-β-hydroxybutyrate granules (PHB). Organism grown on chemically defined medium with acetate as organic carbon source and harvested in the late logarithmic phase.*

negative motile rod with terminal swellings. Even under constant, controlled growth conditions, the cell population showed a range of sizes and not all cells were motile. Some nonmotile long or bent rods were present in most cultures, and there was a tendency to form rosette-like clusters. Examination of wet mounts under phase contrast at a magnification of 900 × showed organisms with characteristic opacities at the terminal swellings; dumbbell-shaped organisms were prominent in most preparations. Similar observations were reported recently by Whittenbury and McLee (46) for strains of *R. palustris* and *Rhodopseudomonas viridis*. The staining procedure of Leifson (26) showed the cells to have a single polar flagellum. Polar flagellation was confirmed by electron microscopy of platinum-shadowed preparations (Fig. 1). The organism also has a characteristic ultrastructure displaying a system of parallel lamellae underlying, and possibly continuous with, the cytoplasmic membrane (Fig. 2). This is in accord with recent studies on the ultrastructure of *R. palustris* by Tauschel and Drews (40). Photosynthetic cultures were pink or brownish pink, the pigmentation depending to some extent on the cultural conditions. The organism was a facultative photoheterotroph growing either anaerobically in the light or aerobically in the dark on a wide range of simple organic compounds. Cultures grown aerobically in the dark were a very pale grey-pink. Although the organism was isolated by enrichment on a medium containing yeast extract with formate as major source of organic carbon, it grew very slowly on this medium, and many other organic carbon sources supported more rapid growth. Optimal growth conditions and nutritional requirements were established with acetate as organic carbon source on the synthetic medium of Kornberg and Lascelles (24). The organism grew over the *p*H range 6.0 to 8.0, with an optimum *p*H 7.0, and had an absolute requirement for biotin and *p*-aminobenzoic acid; growth rate and cell yield were increased if thiamine or nicotinic acid were also added. Growth rates and cell yields on the minimal synthetic medium with single organic carbon sources are summarized in Table 1. The following compounds (tested at a final concentration of 20 mM) failed to support growth of the organism on the chemically defined medium: citrate, oxalate, methanol, isopropanol, *p*-aminobenzoate, *m*-hydroxybenzoate, salicylate, phthalate, mandelate, phenylacetamide, alanine, glycine, lysine, threonine, valine, phenylalanine, isoleucine, histidine, methionine, tryptophan, tyrosine, proline, cysteine, glucose, mannose, ribose, xylose, arabinose, glucosamine, *N*-acetylglucosamine, maltose, sucrose, and lactose. There was good growth of the organism on 2 mM octanoate, but there was no growth on 20 mM octanoate. Higher fatty acids have been found to inhibit growth of photosynthetic bacteria (42). Growth on certain organic compounds, such as the higher fatty acids and alcohols, was

TABLE 1. *Photosynthetic growth of Rhodopseudomonas palustris on organic compounds in a minimal chemically defined medium*[a]

Substrate	Generation time (hr)		Max OD (600 mμ)[b]	
	+HCO_3	−HCO_3	+HCO_3	−HCO_3
Sodium acetate	12	12	2.0	2.0
Sodium pyruvate	13	18	1.8	1.1
Sodium lactate	15	15	2.0	2.0
Sodium malate	17	17	1.8	1.8
Sodium fumarate	20	22	1.1	0.88
Sodium succinate	14	16	1.2	1.1
Sodium glutarate	16	16	1.2	1.2
Sodium malonate	15	15	1.6	1.6
Sodium β-hydroxybutyrate	8	10	1.8	1.2
Sodium crotonate	15	17	1.7	1.6
Sodium aspartate	12	12	1.2	1.2
Sodium glutamate	18	18	1.0	1.0
Asparagine	18	18	1.5	1.5
DL-Serine	11	12	2.2	2.0
D-Serine	10	NT[c]	2.0	NT
L-Serine	9	NT	2.2	NT
L-Leucine	22	>40	0.72	0.1
Benzoyl-glycine	12	12	2.0	1.9
Sodium-*p*-hydroxybenzoate	14	15	2.1	1.9
Sodium benzoate	12	14	2.8	2.8
Sodium hydrocinnamate	10	10	2.1	2.1
Sodium propionate	14	>40	2.0	0.41
Sodium butyrate	12	>40	1.9	0.39
Sodium isobutyrate	13	>40	1.9	0.17
Sodium valerate	10	>40	2.0	0.27
Sodium isovalerate	13	>40	2.0	0.25
Sodium caproate	13	>40	1.8	0.34
Sodium octanoate[d]	12	>40	2.0	0.17
Glycerol	16	>40	1.4	0.4
Ethyl alcohol	13	>40	0.95	0.27
Propyl alcohol	18	>40	0.75	0.19
Butyl alcohol	20	>40	0.7	0.16

[a] Organisms grown on 20 mM organic substrates as described in Materials and Methods.

[b] An OD (600 mμ) of 1.0 corresponds to 0.42 mg (dry weight) per ml.

[c] Not tested.

[d] Final concentration of octanoate, 2 mM.

TABLE 2. *Growth of Rhodopseudomonas palustris on formate, thiosulfate, and hydrogen*

Substrate	Yeast extract (%, w/v)	Generation time (hr)	Max OD (600 mμ)
Nil	0.05	35	0.27
Sodium formate (20 mM)	0.0	>40	0
Sodium formate (20 mM)	0.05	18	1.4
Sodium thiosulfate (10 mM)	0.0	>40	0
Sodium thiosulfate (10 mM)	0.05	33	0.97
$CO_2 + H_2$	0.05	24	0.90
$CO_2 + N_2$	0.05	35	0.35

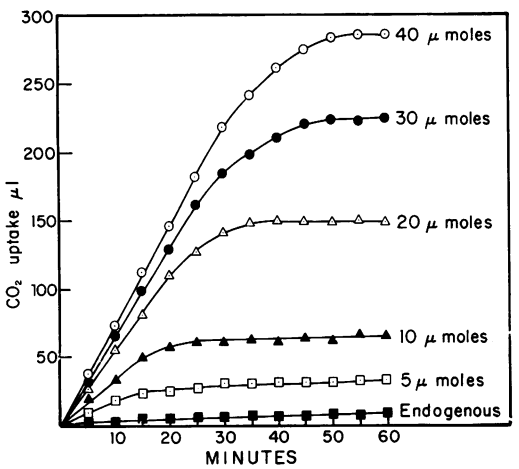


FIG. 3. *Photoassimilation of formate. Cell suspensions (20 mg, dry weight) of formate-grown organisms incubated under standard conditions with different amounts of formate as indicated.*

greatly reduced in the absence of bicarbonate. Although the organism was isolated by enrichment on media containing formate, the chemically defined medium did not support growth on formate in the presence or absence of bicarbonate. Yeast extract was essential for growth on formate. Attempts to grow the organism on a chemically defined medium with formate and a complete mixture of B vitamins, including vitamin B_{12}, were unsuccessful. A similar situation was found with respect to growth on bicarbonate with thiosulfate or molecular hydrogen. In all three cases, the growth rate was slow, but the cell yield and growth rate were greater than on the basal yeast extract-supplemented media without formate, thiosulfate, or hydrogen (Table 2). The culture was maintained through eight transfers under "autotrophic" conditions with molecular hydrogen. Identification of the isolate as a strain of *R. palustris* is based on the above morphological, cultural, and physiological studies (16, 17, 38, 42).

TABLE 3. *Stoichiometry of formic dehydrogenase and formic hydrogenlyase reactions*[a]

Formate oxidized (μmoles)	Gas produced (μmoles)	
	Formic dehydrogenase CO_2	Hydrogenase H_2
5	5.0	4.9
10	9.7	9.6
15	14.9	14.0
20	21.0	17.5[b]

[a] Reaction mixtures (3 ml) incubated in the dark under nitrogen at 30 C contained: 52 μmoles of potassium phosphate buffer (*p*H 7.0), sodium formate as indicated, and suspension of formate-grown organisms (20 mg, dry wt, for formic dehydrogenase; 93 mg, dry weight, for formic hydrogenlyase). Reaction mixtures for formic dehydrogenase contained, in addition, 50 μmoles of $K_3Fe(CN)_6$; central wells contained 0.2 ml of 20% KOH in assays for formic hydrogenlyase.
[b] Reaction incomplete after 210 min of incubation.

Experiments with washed cell suspensions. Cell suspensions of *R. palustris* grown on media containing formate were shown to photoassimilate formate (Fig. 3). This was demonstrated by the manometric procedure of Gaffron (9). However, suspensions of *R. palustris* grown on acetate media were unable to photoassimilate formate. Acetate was readily photoassimilated by suspensions of organisms grown on acetate or formate media. It therefore appeared that the ability to photoassimilate formate was induced by growth on media containing formate. A comparison was therefore made of the biochemical activities of suspensions of formate and of acetate-grown organisms, with particular reference to biochemical activities which might be associated with the assimilation of formate. Cell suspensions of formate-grown organisms produced carbon dioxide and molecular hydrogen from formate. The reaction did not proceed at a rapid rate, but was shown to be stoichiometric with different amounts of formate (Table 3). This reaction could not be demonstrated in suspensions of acetate-grown organisms. The overall reaction is formally analogous to the formic hydrogenlyase reaction. Cell suspensions also displayed formic dehydrogenase activity. The stoichiometric production of carbon dioxide from formate by suspensions of formate-grown organisms in the presence of potassium ferricyanide as electron acceptor is also illustrated in Table 3. Formic dehydrogenase activity was demonstrable in suspensions of both formate- and acetate-grown

TABLE 4. *Intracellular distribution of formic dehydrogenase and hydrogenase*

Treatment	Fraction	Formic dehydrogenase			Hydrogenase		
		Specific activity[a]	Dry wt	Total enzyme recovered	Specific activity	Dry wt	Total enzyme recovered
			mg	%		*mg*	%
None	Cell suspension	5.2	ND[b]	ND	30	ND	ND
Sonic treatment	Crude extract	5.9	2,400	100	30	892	100
Centrifugation, 30 min at 18,000 × *g*	Supernatant S1	7.6	744	42	34	244	30
	Residue R1	4.4	1,160	36	36	412	54
Centrifugation, S1, 60 min at 100,000 × *g*	Supernatant S2	10.4	486	35	32	120	14
	Residue R2	3.7	543	6	42	112	17
Centrifugation, S2, 240 min at 100,000 × *g*	Supernatant S3	17.1	258	31	4	65	1
	Residue R3	3.7	192	5	60	62	14

[a] Specific activity expressed as nanomoles × min^{-1} × (mg of dry weight)$^{-1}$.
[b] Not determined.

organisms. The rate of carbon dioxide production in the formic dehydrogenase reaction was always greater than the rate of gas production (carbon dioxide and hydrogen) in the formic hydrogenlyase reaction. Thus, formic dehydrogenase activity of cell suspensions was 7.4 to 8.9, whereas the formic hydrogenlyase activity was 3 to 4.5

Suspensions of formate-grown organisms also catalyzed a very rapid photoassimilation of molecular hydrogen in the presence of carbon dioxide. This reaction was exceedingly slow or absent in suspensions of acetate-grown organisms. Thus, cell suspensions of formate-grown organisms had a specific activity of 48 to 59, whereas the specific activity of acetate-grown organisms was 0 to 4.

The activities which are found only in formate-grown organisms, and which may therefore be associated with the ability of formate-grown organisms to assimilate formate, are: (i) the formic hydrogenlyase reaction and (ii) the ability to photoassimilate molecular hydrogen with carbon dioxide. Since the ability to photoassimilate molecular hydrogen implies the presence of a hydrogenase, i.e., an enzyme system activating molecular hydrogen, the above results further suggest that the photoassimilation of formate by formate-grown organisms may proceed via a formic hydrogenlyase system consisting of a formic dehydrogenase and a hydrogenase. Further support for this proposition was therefore sought by studying the enzyme activities of cell-free extracts.

Experiments with cell-free extracts. Crude extracts of formate-grown organisms, prepared by sonic disintegration and centrifugation for 30 min at 35,000 × *g*, showed both formic dehydrogenase and formic hydrogenlyase activities. Such preparations also showed a hydrogenase activity demonstrated by a light-independent uptake of molecular hydrogen in the presence of potassium ferricyanide as electron acceptor.

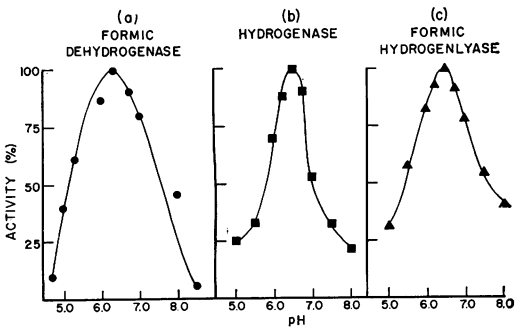


FIG. 4. *pH curves for: (a) formic dehydrogenase, (b) hydrogenase, and (c) formic hydrogenlyase. Crude extracts (S1) incubated under standard conditions at the following protein concentrations (mg per 3-ml assay): a, 19.8; b, 12.2; c, 31.6. The maximal specific activities [nanomoles × min^{-1} × (mg of protein)$^{-1}$] were: a, 8.4; b, 42.6; c, 2.9.*

Differential centrifugation of crude cell-free extracts demonstrated that the formic dehydrogenase of *R. palustris* was a soluble enzyme (Table 4). After 5 hr of centrifugation at 100,000 × *g*, extracts were completely free of chromatophore fragments, and the specific activity of the extract was more than double that of the crude extract.

Optimal assay conditions and some general

TABLE 5. *Comparison of the electron acceptor specificities of formic dehydrogenase and hydrogenase*

Electron acceptor	Activity[a]	
	Formic dehydrogenase	Hydrogenase
	%	%
Phenazine methosulfate	100	100
2,6-dichlorophenol indophenol	56	50
Methylene blue	50	43
Potassium ferricyanide	47	43
Benzyl viologen	31	32
Methyl viologen	17	23
Triphenyl tetrazolium	13	22
FMN	0	0
FAD	25	18
NAD	0	0
NADP	0	0
Oxygen	0	NT[b]

[a] Maximal specific activities [nanomoles × min^{-1} × (mg of protein)$^{-1}$] were: formic dehydrogenase, 44; hydrogenase, 132.

[b] Not tested.

properties of the formic dehydrogenase reaction were determined in extracts obtained after 1 hr of centrifugation at 100,000 × *g*. Potassium ferricyanide was used routinely as the electron acceptor, and the reaction was followed manometrically under an atmosphere of nitrogen as described above. Formic dehydrogenase was active over the *p*H range 5.5 to 8.0 and had an optimum *p*H of 6.3 in 20 mM potassium phosphate buffer (Fig. 4a). Under optimal conditions, the rate of carbon dioxide production was directly proportional to the concentration of enzyme over the range of 0 to 25 mg (dry weight) per assay (3 ml). The apparent K_m for formate was 1.45×10^{-3}, and for potassium ferricyanide the K_m was 1.16×10^{-2} M.

Formic dehydrogenase activity was slowly lost when extracts were stored at −10 C. Extracts prepared from organisms which were suspended in phosphate buffer containing formate (3.3 mM) were consistently more active in the formic dehydrogenase reaction. The presence of formate and storage under anaerobic conditions improved the stability of formic dehydrogenase. Formic dehydrogenase activity was retained in acetone-dried cells but could not be extracted into 20 mM potassium phosphate buffer unless the suspension of acetone-dried cells was sonically treated. Attempts to purify formic dehydrogenase by conventional methods met with limited success. Two- to threefold purification was achieved

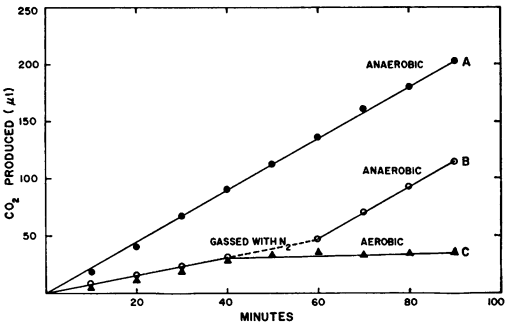


FIG. 5. *Effect of oxygen on formic dehydrogenase. A partially purified preparation (2.4 mg of protein per 3-ml assay) incubated under nitrogen (A) and under air (B and C). After 40 min of incubation with the complete reaction mixture, flask (B) was flushed with nitrogen.*

by fractional precipitation with ammonium sulfate, but the recovery of enzyme was poor and rarely exceeded 50%. For example, 86 mg of protein with a specific activity of 51 was recovered by precipitation with ammonium sulfate to 40% saturation from an extract containing 500 mg of protein and a specific activity of 18. Extracts or ammonium sulfate-precipitated fractions could be dialyzed against 20 mM potassium phosphate buffer (*p*H 6.3) containing 3 mM sodium formate for 24 hr at +2 C without significant loss of enzyme activity. The activity of dialyzed extracts was not increased by the addition of nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP). It thus seemed unlikely that the soluble formic dehydrogenase of *R. palustris* was a pyridine nucleotide-dependent dehydrogenase like the NAD-dependent enzyme in *Pseudomonas* AM1 (20) or the NADP-dependent enzyme in *Clostridium thermoaceticum* (27). This was further confirmed by studies on the electron acceptor specificity of the formic dehydrogenase (Table 5). A number of artificial electron acceptors could be substituted for potassium ferricyanide in the formic dehydrogenase assay, but common biological electron acceptors, including the pyridine nucleotides, NAD or NADP, and flavin nucleotides, flavin mononucleotide (FMN) and flavin adenine nucleotide (FAD), were ineffective. Oxygen did not serve as an electron acceptor for the formic dehydrogenase. Thus, preparations did not respire under standard manometric procedures, i.e., there was no gas uptake aerobically when extracts, or fractions precipitated with ammonium sulfate, were incubated with formate without an artificial electron acceptor and when the center wells of the reaction vessels contained 0.2 ml of 20% (w/v)

TABLE 6. *Sulphydryl sensitivities of formic dehydrogenase, hydrogenase, and formic hydrogenlyase*

PCMB concn (M × 10^4)	Activity (% of control)					
	Formic dehydrogenase		Hydrogenase		Formic hydrogenlyase	
	No cysteine	+3.3 mM Cysteine	No cysteine	+3.3 mM Cysteine	No cysteine	+3.3 mM Cysteine
0 (control)	100	100	100	100	100	100
0.5	90	99	89	100	83	92
0.75	73	92	64	90	67	78
1.0	68	86	46	80	45	75
3.0	23	79	31	64	25	64
4.0	8	72	21	68	16	66

[a] Extracts (S1) of organisms grown on acetate plus formate were used at the following concentrations (mg of protein per assay): formic dehydrogenase, 16.8; hydrogenase, 9.3; formic hydrogenlyase, 20.4. The maximal specific activities [nanomoles × min^{-1} × (mg of protein)$^{-1}$] were: formic dehydrogenase, 10.8; hydrogenase, 39; formic hydrogenylyase, 2.7.

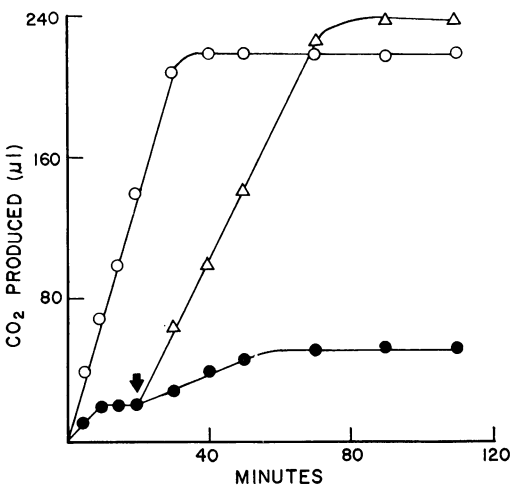


FIG. 6. *Coupled reaction between formic dehydrogenase and fumarate reductase. Crude extract (S1, 19.8 mg of protein) was incubated at 30C (pH 7.0) with 10 µmoles of sodium formate, (○) 10 µmoles of dichlorophenol-indophenol (DCPIP), (●) 1 µmole of DCPIP, and 10 µmoles of sodium fumarate in the dark. After 20 min, (as indicated by the arrow) one flask was illuminated* (△).

KOH. However, oxygen inhibited the formic dehydrogenase reaction with ferricyanide or dichloroindophenol as electron acceptor. Inhibition was not prevented by the addition of catalase, but activity was restored when the system was subsequently made anaerobic (Fig. 5). The mechanism of oxygen inhibition of formic dehydrogenase activity is not clear.

TABLE 7. *Stoichiometry of the coupled systems with fumarate reductase*[a]

Expt no.	Hydrogen donor system	Rate-limiting reactant (µmoles)	Measurement (µmoles)
1	Hydrogenase	Fumarate	Hydrogen consumption
		5	4.8
		10	9.7
		15	14.6
		20	19.7
2	Formic dehydrogenase	Fumarate	CO_2 production
		5	4.5
		10	10.4
		15	15.0
		20	9.9
3	Formic dehydrogenase	Formate	CO_2 production
		5	4.8
		10	9.8
		15	14.7
		20	19.6

[a] Reactions were carried out manometrically at 30 C in a volume of 3 ml. Experiment 1 contained: supernatant S1 (20 mg of protein) in potassium phosphate buffer (*p*H 6.3), 52 µmoles; dichlorophenol-indophenol, 1 µmole; sodium fumarate, as indicated; and gas-phase hydrogen. Experiment 2 contained: crude extract (20 mg of protein) in potassium phosphate buffer (*p*H 6.3), 52 µmoles; sodium formate, 40 µmoles; benzyl viologen, 2 µmoles; sodium fumarate, as indicated; and gas-phase nitrogen. Experiment 3 as in experiment 2, but with 30 µmoles of sodium fumarate and sodium formate as indicated. All measurements are corrected for endogenous reaction with dye in the absence of sodium fumarate.

Formic dehydrogenase was a sensitive sulphydryl enzyme, inhibited by very low concentrations of *p*-chloromercuribenzoate (PCMB). Inhibition could be completely reversed by the addition of an excess of cysteine (Table 6).

Characteristics of hydrogenase. Hydrogenase activity, unlike formic dehydrogenase, was almost exclusively confined to the "particulate" fraction of cell-free extracts (Table 4). With potassium ferricyanide as electron acceptor, hydrogenase activity was demonstrated over the range of *p*H 5.0 to 8.0 in 20 mM potassium phosphate buffer and showed maximal activity at

TABLE 8. *Resolution and reconstitution of the coupled systems with fumarate reductase*

Treatment	Specific activity [nanomoles × min^{-1} × (mg of protein)$^{-1}$] of coupled systems with			
	Fraction	Total protein (mg)	Formic dehydrogenase	Hydrogenase
None	Crude extract	137	7.6	11.4
Centrifugation, 4 hr at 100,000 × *g*	Supernatant fluid	55	1.5	1.5
	Particles	72	1.1	20.0
Reconstituted	Supernatant fluid + particles	127	7.3	10.7

*p*H 6.5 (Fig. 4b). The *p*H curve was very similar to that for formic dehydrogenase (Fig. 4a). At *p*H 6.5 in 20 mM potassium phosphate, the rate of the hydrogenase reaction was proportional to the amount of enzyme up to a concentration of 16 mg (dry weight) per assay (3-ml volume). The particulate hydrogenase showed the same electron acceptor specificity as was shown with the soluble formic dehydrogenase; the relative rates for the two enzymes are compared in Table 5. Stoichiometry of the hydrogenase reaction was established by measuring total hydrogen uptake with a range (10 to 50 μmoles) of limiting concentrations of ferricyanide. Two moles of ferricyanide were reduced per mole of hydrogen oxidized. The hydrogenase was more stable to storage at −10 C than was formic dehydrogenase. The particulate hydrogenase also resembled formic dehydrogenase in its sensitivity to inhibition by *p*-chloromercuribenzoate and reversal of the inhibition by the addition of cysteine (Table 6).

Coupling of formic dehydrogenase and hydrogenase with fumarate reductase. Both the formic dehydrogenase and hydrogenase could be coupled to a fumarate reductase, but only in the presence of catalytic amounts of an artificial electron acceptor. Benzyl viologen catalyzed a slow, light-independent, coupled reaction.Dichlorophenol-indophenol coupled a slightly faster reaction, but light was essential (Fig. 6). Analogous results have been reported for the coupled hydrogenase and fumarate reductase reactions in *R. rubrum* (5). The optimal *p*H for these coupled reactions was 7.0. The stoichiometry of the coupled reactions is illustrated in Table 7. Resolution of the crude extracts into "soluble" and "particulate" fractions established that the coupled reaction between the formic dehydrogenase and fumarate reductase required both the soluble and particulate fractions, whereas the coupled reaction between the hydrogenase and fumarate reductase was confined to the particulate fraction. These results thus confirm that the formic dehydrogenase is a soluble enzyme, whereas both the hydrogenase and fumarate reductase are particulate enzymes (Table 8).

Characteristics of the formic hydrogenlyase system. Results of experiments outlined above were consistent with the proposition that the formic hydrogenlyase system of *R. palustris* consists of a soluble formic dehydrogenase and a particulate hydrogenase, with one or more unidentified intermediate electron carriers. Gas production resulting from the anaerobic incubation of formate with crude cell-free extracts of formate-grown *R. palustris* in the absence of an electron acceptor was shown to be a mixture of hydrogen and carbon dioxide. The reaction was followed to completion with a limiting concentration of formate, after which an excess of potassium ferricyanide was added (Fig. 7). Addition of ferricyanide resulted in a rapid gas uptake equivalent to half of the gas production from formate. Since the ferricyanide was reduced, and such preparations have been shown to catalyze a rapid uptake of molecular hydrogen in the presence of ferricyanide, the experiment confirmed the production of molecular hydrogen from formate.

Formic hydrogenlyase activity was reconstituted from soluble fractions containing formic

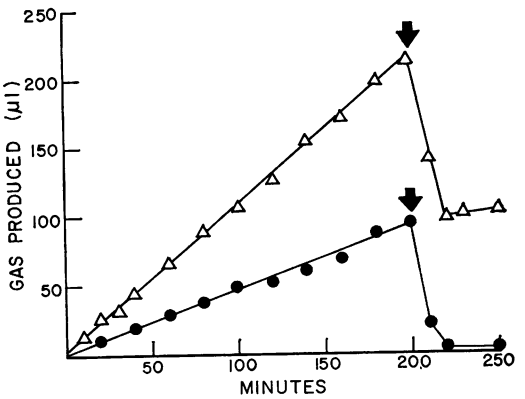


FIG. 7. *Evidence for hydrogen production in the formic hydrogenlyase reaction. Reaction mixture containing 19 mg of protein was incubated at 30 C (pH 6.5) under anaerobic conditions with 50 μmoles of sodium formate, (●) with 0.2 ml of 20% KOH in center well, (△) without KOH; 50 μmoles of $K_3Fe(CN)_6$ added as indicated by arrows.*

TABLE 9. *Resolution and reconstitution of formic hydrogenlyase*

Treatment	Fraction	Total protein (mg)	Specific activity [nanomoles × min^{-1} × (mg of protein)$^{-1}$][a]	Total enzyme units
None	Crude extract (S1)	252	2.2	554
Centrifugation, 4 hr at 100,000 × *g*	Supernatant	53	0.8	42
	Particles	175	0.4	70
Reconstituted	Supernatant fluid + particles	228	2.5	570
Centrifugation, 1 hr at 100,000 × *g*	Supernatant	280	4.2	1,180
40% Saturation with $(NH_4)_2SO_4$	Precipitate (P1)	77	10.9	840
Centrifugation of redissolved fraction	Supernatant (S2)	42	2.1	88
P1, 1 hr at 100,000 × *g*	Precipitate i/(P2)	29	2.7	78
Reconstituted	Supernatant S2 + precipitate P2	71	8.6	610

[a] As nanomoles of total gas: $H_2 + CO_2$ produced. One enzyme unit is the amount of enzyme catalyzing the formation of 1 nmole of gas ($H_2 + CO_2$) per min.

TABLE 10. *Reconstitution of formic hydrogenlyase from formic dehydrogenase of acetate-grown organisms and hydrogenase of formate-grown organisms*

Organism grown on	Fraction	Protein concn (mg/assay)	Formic dehydrogenase	Specific activity[a]	
				Hydrogenase	Formic hydrogenlyase
Acetate	Partially purified[b]	5.2	16.6	1.1	0
Acetate + formate	Particulate (chromatophores)	10.1	2.2	53.0	0.6
	Reconstituted 1 + 2	15.3	6.7	35.0	4.6

[a] Expressed as nanomoles × min^{-1} × (mg of protein)$^{-1}$.

[b] Supernatant S2 precipitated by 40% saturation with ammonium sulfate, redissolved in buffer, and centrifuged for 1 hr at 100,000 × *g* to remove pigmented particles.

dehydrogenase and a particulate fraction containing hydrogenase (Table 9). The optimal *p*H for the overall formic hydrogenlyase reaction in crude extracts was the same as that for formic dehydrogenase and hydrogenase: namely *p*H 6.5 (Fig. 4c). The K_m for formate was 2.3×10^{-3} M. Formic hydrogenlyase was also reconstituted from an ammonium sulfate (0 to 40% saturation) fraction freed of chromatophore fragments by centrifugation for 1 hr at 100,000 × *g*, together with the particulate hydrogenase (Table 9). It was also reconstituted from the particulate hydrogenase of formate-grown cells, and a partially purified formic dehydrogenase from acetate-grown cells which do not themselves produce formic hydrogenlyase (Table 10).

Attempts to gain information on the possible nature of intermediate electron carriers in the formic hydrogenlyase reaction by the use of a variety of inhibitors met with limited success. Thus, compounds which were inhibitory in low concentrations, such as cyanide and azide, inhibited formic dehydrogenase and hydrogenase as well as formic hydrogenlyase (Table 11). Other inhibitors of electron transport had no significant effect on the activity of these enzymes.

DISCUSSION

"Autotrophic" growth of R. palustris. The photosynthetic organism was in part identified as a strain of *R. palustris* on the basis of its ability to grow autotrophically with thiosulfate on a medium containing yeast extract; this property was found to be characteristic of all strains of *R. palustris* studied by van Niel (42). Thiosulfate utilization by *R. palustris* has been reexamined recently by Rolls and Lindstrom (36, 37). Our strain of *R. palustris* could not be grown strictly autotrophically on chemically defined media which supported heterotrophic growth on a wide variety of organic compounds. An exactly comparable situation was found with respect to

TABLE 11. *Inhibitors of formic dehydrogenase, hydrogenase, and formic hydrogenlyase*[a]

Inhibitor	Concn ($M \times 10^4$)	Inhibition (%)		
		Formic dehydrogenase	Hydrogenase	Formic hydrogenlyase
Control		0	0	0
Sodium azide	0.01	18	10	12
	0.05	37	22	27
	0.1	56	28	41
	0.5	62	33	59
Potassium cyanide	0.1	25	22	22
	0.5	58	28	53
	1.0	70	41	77
	5.0	81	61	87
Antimycin A	0.01	13	5	5
	0.5	19	13	23
2-*n*-Heptyl-4-hydroxyquinoline-*N*-oxide	0.5	11	13	14
	1.0	16	18	16
Rotenone	1.0	0	0	0
Chloropromazine	1.0	0	0	0
Atebrine	10.0	0	NT	NT
α,α-Dipyridyl	5.0	4	NT	NT
8-Hydroxyquinoline	0.5	8	13	15
	1.0	41	48	42
O-Phenanthroline	15.0	18	12	NT
	30.0	43	37	NT
	60.0	66	51	NT

[a] Reaction mixtures (3 ml), incubated at 30°C under standard assay conditions, contained cell-free extract S1 (16 to 20 mg of protein). Specific activities [nanomoles $\times$ min^{-1} $\times$ (mg of protein)$^{-1}$] of the uninhibited enzymes were: formic dehydrogenase, 10.4 to 14.8; hydrogenase, 37 to 45; formic hydrogenlyase, 2.2 to 4.4.

growth of *R. palustris* autotrophically with molecular hydrogen or with formate. Indeed, the response to formate could be taken to indicate that our strain of *R. palustris* grows autotrophically on formate as it does with molecular hydrogen. It is still not clear why yeast extract is required for growth under the above conditions. Analogous situations are known. *Vibrio oxaliticus* was found to grow on formate only in the presence of yeast extract, although it grew on a chemically defined medium with oxalate, pyruvate, or acetate (4). Likewise, *Micrococcus denitrificans* grows autotrophically with hydrogen by nitrate respiration only in the presence of yeast extract, although it grows heterotrophically by nitrate respiration in a chemically defined medium (43). A possible explanation for these effects is that energy or reducing power may be derived from the inorganic hydrogen donor (molecular hydrogen or thiosulfate) or from formate, but that organic substances present in the yeast extract are indispensable for growth, i.e., contribute to cellular carbon. Lwoff (28) first applied the term "chemo-metatrophy" to this phenomenon, and similar proposals have been made to explain earlier claims for the autotrophic growth of sulfate-reducing bacteria in media which contained yeast extract (29). It is evident that our isolate of *R. palustris* is unable to grow strictly autotrophically, as can members of the purple sulfur bacteria (*Thiorhodaceae*).

Formate metabolism by photosynthetic bacteria. Although utilization of formate by unspecified strains of *Athiorhodaceae* has been reported by van Niel (41), and formate degradation has been demonstrated in *R. rubrum* (3, 10, 23), this is the first report of attempts to establish the biochemical mechanism of formate assimilation by a photosynthetic bacterium. During the course of these investigations, a preliminary account of formate metabolism by another strain of *R. palustris* was published (48). Formate did not support growth of purple sulfur bacteria (31). Present evidence with our strain of *R. palustris* indicates that formate is assimilated via an inducible formic hydrogenlyase system, i.e., formate is assimilated autotrophically as carbon dioxide and molecular hydrogen. Formate is assimilated in this way by *Pseudomonas oxaliticus* (35). However, further evidence that this is the sole or major mechanism of formate assimilation by *R. palustris* remains to be established by isotope techniques with ^{14}C-formate.

Formic hydrogenlyase in photosynthetic bacteria. Decomposition of formate to carbon dioxide and hydrogen in photosynthetic bacteria was first reported by Nakamura (32) in "*Rhodobacillus palustris.*" It was later shown by Kohlmiller and Gest (23) in *R. rubrum*; this was confirmed by Bennett, Rigopoulos, and Fuller (3). However, in contrast to *R. palustris*, formate decomposition to carbon dioxide and hydrogen was demonstrated in *R. rubrum* grown on a medium containing malate and glutamate. In *R. palustris*, formic hydrogenlyase appears to be an inducible enzyme system comprising formic dehydrogenase, hydrogenase, and one or more as yet unidentified intermediate electron carriers. Ability to photoassimilate formate, presence of formic hydrogenlyase, and hydrogenase all

appear to be dependent upon growth of the organism on media containing formate. The formic hydrogenlyase system of *R. palustris* has many properties in common with that of coli-aerogenes organisms (11, 34). Thus, the system from *Escherichia coli* is inducible (12, 14), and appears to consist, in part at least, of a soluble formic dehydrogenase and a particulate hydrogenase. In coli-aerogenes organisms, an active hydrogenlyase system has been reconstituted from a partially purified soluble formic dehydrogenase (which uses viologens as electron acceptors) and a particulate hydrogenase from another variant which is not gas-producing. However, a second formic dehydrogenase, using methylene blue as electron acceptor, could not be coupled with the particulate hydrogenase (34). A particulate hydrogenase has been reported in a strain of *R. palustris* grown on peptone plus lactate (18). The formic hydrogenlyase system from *R. palustris* is readily resolved into, and reconstituted from, a soluble formic dehydrogenase and a particulate hydrogenase. Even a partially purified formic dehydrogenase from acetate-grown cells incapable of forming formic hydrogenlyase can reconstitute formic hydrogenlyase when combined with the particulate hydrogenase.

The soluble formic dehydrogenase of *R. palustris* does not function with known biological electron acceptors. Thus, it is distinguished from the soluble formic dehydrogenase of *Pseudomonas* AM1 which uses NAD (20), the soluble NADP-linked formic dehydrogenase of *C. thermoaceticum* (27), and the particulate membrane-bound formic dehydrogenase II (cytochrome b_1 oxido-reductase) of aerobically grown *E. coli* (14). A ferredoxin-dependent oxidation of formate has been reported in the strictly anaerobic methane bacterium *Methanobacillus omelianskii* (6). Ferredoxin has not been unequivocally excluded as the natural electron acceptor for the formic dehydrogenase of *R. palustris*. However, this seems unlikely, since cell-free extracts treated with diethylaminoethyl cellulose under conditions which remove ferredoxin still retain formic dehydrogenase activity. Furthermore, the very slow reaction rate with the viologen dyes, relative to that with dyes of more electropositive redox potential, is not in accord with the properties of many ferredoxin-linked dehydrogenases. Ferredoxin has been isolated from *R. palustris* by Yamanaka and Kamen (47).

The natural electron acceptor for the formic dehydrogenase remains to be identified. It may be significant that a nonphotosynthetic *Pseudomonas* species capable of growing on one-carbon compounds has been found to contain an alcohol dehydrogenase, with an unidentified prosthetic group thought to be a pteridine derivative; the enzyme dehydrogenates methanol (1). Attempts to study spectral changes in the presence and absence of formate with preparations of high specific activity gave no clear indication of any chromophoric prosthetic group. Presumably, even the best preparations available were still too crude for such studies, and further purification steps would be essential to obtain meaningful results. The extreme sensitivity to oxygen may be a function of the prosthetic group, although the dehydrogenase is a sensitive sulfhydryl enzyme. However, addition of thiols did not prevent or reverse the inhibition of activity by oxygen. It was also somewhat surprising that the formic dehydrogenase could not be linked directly to any other biological electron acceptor system except the particulate hydrogenase. Coupling to the particulate fumarate reductase could be affected only via artificial electron acceptors. However, Jacobs and Wolin (19) were able to couple formate oxidation directly to fumarate reduction in the strict anaerobe *V. succinogenes*.

The survey of inhibitors showed no inhibitor which was specific for either the formic dehydrogenase, the particulate hydrogenase, or the formic hydrogenlyase reaction. Indeed, the electron acceptor specificities of the soluble formic dehydrogenase and the particulate hydrogenase were remarkably similar (Table 5). This suggests that the two reactions may be linked directly in the formic hydrogenlyase system via a common electron carrier as shown in the upper part of Fig. 8. An alternative scheme which must be considered is one involving at least two intermediate electron carriers (Fig. 8, bottom). This scheme would involve three enzyme steps: I, the soluble formic dehydrogenase; II, an intermediate oxido-reductase; and III, the particulate hydrogenase. Such a scheme could explain the observations that the rate of hydrogen production in the formic hydrogenlyase reaction is very slow, whereas the rates of the formic dehydrogenase and hydrogenase reactions are relatively rapid. The intermediate oxido-reductase II in the above scheme would then be envisaged as the rate-limiting step in the formic hydrogenlyase reaction.

A similar scheme, in which one or the other of the intermediate electron carriers X_1 and X_2 is postulated to be a "*c*-type" cytochrome of low redox potential, has been proposed by Gray and Gest (12). This is supported by direct evidence for the participation of cytochrome c_3 as an electron carrier in the formic hydrogenlyase of *Desulfovibrio desulfuricans* (J. P. Williams, J. T.

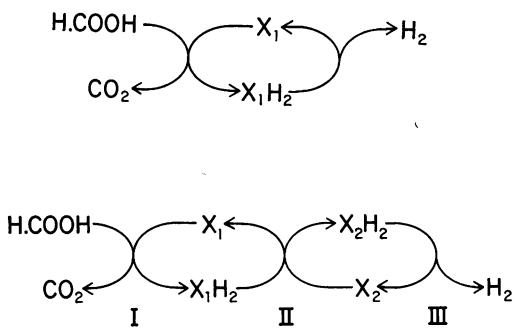


FIG. 8. *Schemes showing how soluble formic dehydrogenase and particulate hydrogenase may be linked in the formic hydrogenlyase system either directly via a common electron carrier (top) or in a three-step enzyme system involving at least two intermediate electron carriers (bottom). X_1 = unidentified electron carrier.*

Davidson, and H. D. Peck, Bacteriol. Proc., p. 110, 1964), and by the demonstration of a low potential cytochrome C_{552} in *E. coli* grown under anaerobic conditions (7, 13). A cytochrome C_{552} has been isolated from *R. palustris* (15, 22, 30).

Spectral studies with the formic dehydrogenase of our strain of *R. palustris* gave no evidence in support of a cytochrome involvement in the reaction. Traces of a cytochrome *c* were present in the preparation, and, although this was oxidized by the addition of a very low concentration of potassium ferricyanide, the subsequent addition of an excess of formate did not reduce the cytochrome but did completely reduce the ferricyanide. Further support for the second scheme, involving a rate-limiting intermediate oxido-reductase, may be derived from the findings that acetate-grown cells, although virtually devoid of formic hydrogenlyase activity, do have a hydrogenase (i.e., as assayed by ferricyanide reduction). Failure to detect formic hydrogenlyase in acetate-grown organisms might therefore be attributed to the failure to form the intermediate oxido-reductase under such growth conditions. The extreme sensitivity of the formic hydrogenlyase system of *R. palustris* to cyanide, confirming much earlier results with the *E. coli* system (39), may be attributable to a cyanide-sensitive haem protein electron carrier, but further clarification of this effect must depend upon the preparation of a formic dehydrogenase of much higher purity (specific activity) than has been obtained hitherto.

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LITERATURE CITED

1. Anthony, C., and L. J. Zatman. 1967. The microbial oxidation of methanol. The prosthetic group of the alcohol dehydrogenase of *Pseudomonas* sp. M27. Biochem. J. **104**:960–969.
2. Bennett, R., and R. C. Fuller. 1964. The pyruvate phosphoroclastic reaction in *Chromatium*. A probable role for ferredoxin in a photosynthetic bacterium. Biochem. Biophys. Res. Commun. **16**:300–307.
3. Bennett, R., N. Rigopoulos, and R. C. Fuller. 1964. The pyruvate phosphoroclastic reaction and light-dependent nitrogen fixation in bacterial photosynthesis. Proc. Natl. Acad. Sci. U.S. **52**:762–768.
4. Bhat, J. V., and H. A. Barker. 1948. Studies on a new oxalate-decomposing bacterium, Vibrio oxaliticus. J. Bacteriol. **55**:359–368.
5. Bose, S. K., and H. Gest. 1962. Electron transport systems in purple bacteria. Hydrogenase and light stimulated electron transfer reactions in photosynthetic bacteria. Nature **195**:1168–1172.
6. Brill, W. J., E. A. Wolin, and R. S. Wolfe. 1964. Anaerobic formate oxidation: a ferredoxin-dependent reaction. Science **144**:297–298.
7. Cole, J. A., and J. W. T. Wimpenny. 1966. The interrelationship of low redox potential cytochrome C_{552} and hydrogenase in facultative anaerobes. Biochem. Biophys. Acta **128**:419–425.
8. Elsden, S. R., and J. G. Ormerod. 1956. The effect of monofluoroacetate on the metabolism of *Rhodospirillum rubrum*. Biochem. J. **63**:691–701.
9. Gaffron, H. 1933. Über den Stoffwechsel der schwefelfreien Purpurbakterien. Biochem. Z. **260**:1–17.
10. Gest, H. 1951. Metabolic patterns in photosynthetic bacteria. Bacteriol. Rev. **15**:183–210.
11. Gest, H., and H. D. Peck, Jr. 1955. A study of the hydrogenlyase reaction with systems derived from normal and anaerogenic coli-aerogenes bacteria. J. Bacteriol. **703**:26–334.
12. Gray, C. H., and H. Gest. 1965. Biological formation of molecular hydrogen. Science **148**:186–192.
13. Gray, C. H., D. E. Hughes, and M. Ranlett. 1963. A soluble c-type cytochrome from anaerobically grown *Escherichia coli* and various Enterobacteriaceae. Biochim. Biophys. Acta **67**:157–160.
14. Gray, C. T., J. W. T. Wimpenny, D. E. Hughes, and M. R. Mossman. 1966. Regulation of metabolism in facultative bacteria. I. Structural and functional changes in *Escherichia coli* associated with shifts between the aerobic and anaerobic states. Biochim. Biophys. Acta **117**:22–32.
15. Henderson, R. W., and D. D. Nankiville. 1966. Electrophoretic and other studies on haem pigments from *Rhodopseudomonas palustris*: cytochtome 552 and cytochromoid C. Biochem. J. **98**:587–593.

16. Hutner, S. H. 1946. Organic growth essentials of the aerobic nonsulfur bacteria. J. Bacteriol. **52**:213–221.
17. Hutner, S. H. 1950. Anaerobic and aerobic growth of purple bacteria (Athiorhodaceae) in chemically defined media. J. Gen. Microbiol. **4**:286–293.
18. Izawa, S. 1962. Hydrogenase reactions in *Rhodopseudomonas palustris*. Plant Cell Physiol. **3**:23–42.
19. Jacobs, N., and M. J. Wolin. 1963. Electron-transport system of *Vibrio succinogenes*. I. Enzymes and cytochromes of the electron-transport system. Biochim. Biophys. Acta **69**:18–28.
20. Johnson, P., and J. R. Quayle. 1964. Microbial growth on C-1 compounds. Oxidation of methanol, formaldehyde and formate by methanol grown Pseudomonas AM1. Biochem. J. **93**:281–289.
21. Kellenberger, E., A. R. Ryter, and J. Sechaud. 1958. Electron microscope study of DNA-containing plasms. II. Vegetative and mature phage DNA as compared with normal bacterial nucleoids in different physiological states. J. Biophys. Biochem. Cytol. **4**:671–678.
22. Klerk, H. de, R. G. Bartsch, and M. D. Kamen. 1965. Atypical soluble haem proteins from a strain of *Rhodopseudomonas palustris*. sp. Biochim. Biophys. Acta **97**:275–280.
23. Kohlmiller, E. F., Jr., and H. Gest. 1951. Comparative study of the light and dark fermentations of organic acids by Rhodospirillum rubrum. J. Bacteriol. **61**:269–282.
24. Kornberg, H. L., and J. Lascelles. 1960. The formation of iso-citratase by the Athiorhodaceae. J. Gen. Microbiol. **23**:511–517.
25. Larsen, H. 1953. On the microbiology and biochemistry of the photosynthetic green sulfur bacteria. Kgl. Norske Videnskab. Selskabs Skrifter, p. 11.
26. Leifson, E. 1951. Staining, shape and arrangement of bacterial flagella. J. Bacteriol. **62**:377–389.
27. Li, L.-F., L. Ljungdahl and H. G. Wood. 1966. Properties of nicotinamide adenine dinucleotide phosphate-dependent formate dehydrogenase from *Clostridium thermoaceticum*. J. Bacteriol. **92**:405–412.
28. Lwoff, A. 1943. L'evolution physiologique. Etude des pertes de fonctions chez les microorganisms, Paris.
29. Mechalas, B. J., and S. C. Rittenberg. 1960. Energy coupling in *Desulfovibrio desulfuricans*. J. Bacteriol. **80**:501–507.
30. Morita, S. 1960. Crystallization of *Rhodopseudomonas palustris* cytochrome 552. J. Biochem. (Tokyo) **48**:870–873.
31. Muller, F. M. 1933. On the metabolism of the purple sulphur bacteria in organic media. Arch. Mikrobiol. **4**:131–166.
32. Nakamura, H. 1937. Über das Vorkommen der Hydrogenlyase in *Rhodobacillus palustris* und über ihre Rolle in Mechanismus der bakteriellen Photosynthese. Acta Phytochim. (Japan) **10**:211–218.
33. Ormerod, J. G., and H. Gest. 1962. Symposium on metabolism of inorganic compounds. IV. Hydrogen photosynthesis and alternative metabolic pathways in photosynthetic bacteria. Bacteriol. Rev. **26**:51–66.
34. Peck, H. D., Jr., and H. Gest. 1957. Formic dehydrogenase and the hydrogenlyase enzyme complex in coli-aerogenes bacteria. J. Bacteriol. **73**:706–721.
35. Quayle, J. R., and D. B. Keech. 1959. Carbon assimilation by *Pseudomonas oxaliticus* (OX1). I. Formate and carbon dioxide utilization during growth on formate. Biochem. J. **72**:623–630.
36. Rolls, J. P., and E. S. Lindstrom. 1967. Induction of a thiosulfate-oxidizing enzyme in *Rhodopseudomonas palustris*. J. Bacteriol. **94**:784–785.
37. Rolls, J. P., and E. S. Lindstrom. 1967. Effect of thiosulfate on the photosynthetic growth of *Rhodopseudomonas palustris*. J. Bacteriol. **94**: 860–866.
38. Scher, S., B. Scher, and S. H. Hutner. 1963. Notes on the natural history of *Rhodopseudomonas palustris*, p. 580–587. *In* C. H. Oppenheimer [ed.], Symposium of marine microbiology. Charles C Thomas, Springfield, Ill.
39. Stephenson, M., and L. H. Stickland. 1932. Hydrogenlyases. Bacterial enzymes liberating molecular hydrogen. Biochem. J. **26**:712–724.
40. Tauschel, H. D., and G. Drews. 1967. Thylakoidmorphogenese bei *Rhodopseudomonas palustris*. Biochem. Z. **63**:691–701.
41. Van Niel, C. B. 1941. Bacterial photosyntheses and their importance for the general problems of photosynthesis. Advan. Enzymol. **1**:263–328.
42. Van Niel, C. B. 1944. The culture, general physiology, morphology, and classification of the non-sulfur purple and brown bacteria. Bacteriol. Rev. **8**:1–118.
43. Verhoeven, W. A., A. L. Koster and M. C. A. van Nievelt. 1954. Studies on true dissimilatory nitrate reduction. 3. *Micrococcus denitrificans* Beijerinck, a bacterium capable of using molecular hydrogen. Antonie van Leeuwenhoek J. Microbiol. Serol. **20**:273–284.
44. Warburg, O., and W. Christian. 1942. Isolierung und Kristallization des Gärungsferment Enolase. Biochem. Z. **310**:384–421.
45. Wertlieb, D., and W. Vishniac. 1967. Methane utilization by a strain of *Rhodopseudomonas gelatinosa*. J. Bacteriol. **93**:1722–1724.
46. Whittenbury, R., and G. A. McLee. 1967. *Rhodopseudomonas palustris* and *Rh. viridis*: photosynthetic budding bacteria. Arch. Microbiol. **59**:324–334.
47. Yamanaka, T., and M. D. Kamen. 1965. Purification of an NADP reductase and of ferredoxin derived from the facultative heterotroph *Rhodopseudomonas palustris*. Biochim. Biophys. Res. Commun. **18**:611–616.
48. Yoch, D. C., and E. S. Lindstrom. 1967. Photosynthetic conversions of formate and CO_2 to glutamate by *Rhodopseudomonas palustris*. Biochim. Biophys. Res. Commun. **28**:65–69.