NOTE

THE FORMIC HYDROGENLYASE SYSTEM OF AEROBACTER AEROGENES[1]

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The formic hydrogenlyase system, so far recognized only in a limited number of bacterial species, has been a subject of some controversy. The major question involved is whether the formic hydrogenlyase system is a distinct enzyme system, or whether its action is a result of the coupled effects of the formic dehydrogenase and hydrogenase. This problem and others have been reviewed recently (Umbreit, *in* Sumner and Myrbäck, *The enzymes*, Vol. 2, 329, 1951). The present paper presents data which support the existence of a separate formic hydrogenlyase system in a strain of *Aerobacter aerogenes* (D-1). The composition of the medium employed and the techniques used for the estimation of formic hydrogenlyase, formic dehydrogenase, and hydrogenase activity of the resting cellular suspensions are given in a previous communication (Lichstein and Boyd, J. Bact., **62**, 415, 1951). Two types of incubation were employed, namely, stationary cultivation in an incubator, and aerobic conditions in which case the flasks were placed on a shaking machine in order to ensure adequate aeration. The cells were harvested by centrifugation after 15 to 18 hours' incubation at 30 C, washed once with distilled water, and resuspended in distilled water to give the desired cell concentration.

TABLE 1

Formic hydrogenlyase, formic dehydrogenase, and hydrogenase activity of aerobic and stationary grown Aerobacter aerogenes

CONDITIONS OF CULTIVATION	ADDITIONS	RATE*							
		Formic hydrogenlya			Formic dehydrogenase		Hydrogenase		
		30 min	60 min	90 min	30 min	60 min	30 min	60 min	90 min
Stationary	none	410	340		800	770	2,000	2,900	
	oleate, 0.1 mg	490	450		1,250	1,200	2,600	3,600	
	HYE,† 1 mg	750	700		940	900	2,100	2,900	
Aerobic	none	0	0	0	400	330	0	0	0
	oleate, 0.1 mg	0	0	0	650	600	0	0	0
	HYE,† 1 mg	0	200	200	400	450	0	0	0

* $\text{FHL} = \frac{\mu\text{L } H_2 \text{ produced}}{\text{hours} \times \text{mg cell N}}$; $\text{FD} = \frac{\mu\text{L } CO_2 \text{ produced}}{\text{hours} \times \text{mg cell N}}$; $\text{H} = \frac{\mu\text{L } H_2 \text{ consumed}}{\text{hours} \times \text{mg cell N}}$.

† HYE = hydrolyzed yeast extract = Difco yeast extract autoclaved at 121 C for 3 hours in 0.9 N H_2SO_4 and neutralized with $Ba(OH)_2$.

Representative data are presented in table 1. It is seen from an examination of these data that *A. aerogenes* grown under stationary conditions exhibited formic hydrogenlyase, formic dehydrogenase and hydrogenase activity. The formic hydrogenlyase system was stimulated by oleic acid and by hydrolyzed yeast extract. The formic dehydrogenase system was stimulated greatly

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by oleate, whereas hydrogenase activity was stimulated only slightly by the fatty acid in this particular experiment. It is manifest also that growth of this organism under aerobic conditions resulted in an adverse influence on all three systems. These aerobic cells showed no formic hydrogenlyase activity unless cofactors were supplied, the formic dehydrogenase system was present but significantly reduced in activity, whereas the hydrogenase enzyme appeared to be absent completely under the most rigid scrutiny.

It is of interest that, in agreement with others, it can be established that aerobic cultivation results in the growth of cells exhibiting no formic hydrogenlyase activity. However, the results given show that activity is engendered after an incubation period of 30 to 40 minutes after the addition of hydrolyzed yeast extract. It appears reasonable to conclude that aerobic cultivation results in the production of a reduced amount of apo-formic hydrogenlyase which is inactive until the necessary cofactors are available. These findings are significant in that they support the concept that formic hydrogenlyase is a distinct enzyme system (reaction 1) rather than a result of the combined activities of formic dehydrogenase and hydrogenase (reaction 2).

$$(1)\quad HCOOH \xrightleftharpoons{\text{formic hydrogenlyase}} CO_2 + H_2$$

$$(2)\quad HCOOH \xrightleftharpoons{\text{formic dehydrogenase}} CO_2 + 2H \text{ to carrier}$$

$$\text{carrier-2H} \rightleftharpoons \text{carrier} + 2e + 2H^+$$

$$H_2 \xrightleftharpoons{\text{hydrogenase}} 2H \rightleftharpoons 2e + 2H^+$$

It is clear that in order for reaction 2 to produce molecular H_2 an active hydrogenase must be present, and conversely the absence of the enzyme hydrogenase would preclude reaction sequence 2 and favor the identity of reaction 1. The results obtained in parallel studies of aerobic and stationary grown cells, tested in the presence and absence of cofactors, demonstrate the production of molecular H_2 in the absence of hydrogenase activity and therefore support the mechanism given in reaction 1.