CARBON DIOXIDE REGULATION OF FORMATE HYDROGENLYASE IN ESCHERICHIA COLI

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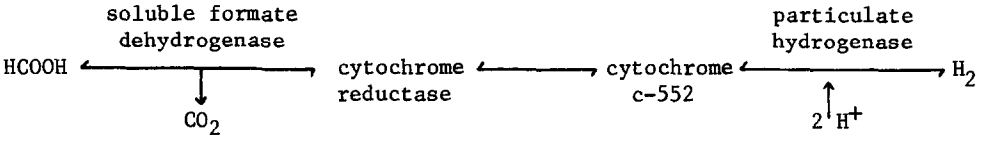
It was suggested by Wimpenny (1969) that carbon dioxide might be involved at the molecular level as a regulator molecule. Evidence has been obtained which does implicate carbon dioxide as a regulator. The enzyme analyzed and found to be affected by carbon dioxide was formate dehydrogenase which is a component of formate hydrogenlyase, an inducible anaerobic enzyme system.

The hydrogenlyase system in Escherichia coli, according to the model of Gray and Gest (1965), contains two enzymes, hydrogenase and formate dehydrogenase. End products of the reaction are carbon dioxide and molecular hydrogen as illustrated in the following diagram:



Our results indicate that carbon dioxide, as an anaerobic regulator molecule, functions as both a metabolic stimulator and inhibitor.

METHODS

Organisms: Two E. coli strains, 82/r and P6 were used in this study. E. coli 82/r is an adenine-requiring mutant of B/r that was isolated by Anderson (1951). E. coli P6, a stable large-cell strain, was isolated by Ogg and Zelle (1957) after exposing 82/r to camphor vapors. The major difference between formate hydrogenlyase activity in the two strains was a latent induction time of this enzyme in P6 as compared with 82/r (Olsen and Ogg, 1963).

Media and Cultivation: E. coli 82/r and P6 were grown in a glucose-basal salts medium containing per liter: K_2HPO_4, 7.0 g; KH_2PO_4, 2.0 g; $MgSO_4$, 0.1 g; $(NH_4)_2SO_4$, 1.0 g; glucose, 1.5 g; and adenine, 10 µg/ml. The cells were incubated at 37 C on a water bath shaker.

The two cell strains were harvested by centrifugation in the early stationary phase of the growth cycle, washed with 0.067 M phosphate buffer (pH 6.0), resuspended in the phosphate buffer and used for enzyme assays.

Enzyme Assays: Formate hydrogenlyase was measured manometrically according to the procedure of Olsen and Ogg (1963). Two separate experimental Warburg flasks were used since both carbon dioxide and hydrogen were simultaneously evolved from the cell. One flask contained 0.2 ml 20% (w/v) KOH in the center well for CO_2 absorption. The center well of the second experimental flask remained empty. Gas accumulation in the second flask was considered a combination of carbon dioxide and hydrogen. The difference, in µliters, between the two experimental flasks allowed an estimation of formate dehydrogenase activity. The determinations were performed with cells grown under aerobic conditions prior to anaerobic induction. When formate hydrogenlyase assays of pre-induced enzyme were conducted, the two E. coli strains were harvested by centrifugation after 17 hr growth under anaerobic conditions in a glucose-basal salts medium supplemented with amino acids (Olsen and Ogg, 1963).

Induction Atmosphere: To produce an "anaerobic" environment, the Warburg flasks were flushed with a commercially prepared nitrogen (95% N_2:5% CO_2), 100% nitrogen, or a commercially prepared carbon dioxide (100% CO_2). A 100% nitrogen atmosphere was obtained by flushing the commercially prepared nitrogen through a 20% (w/v) KOH solution.

Protein Determinations: Protein was determined using the Folin reagent according to the procedure of Lowry et al. (1951).

RESULTS

Formate hydrogenlyase induction, under an atmosphere of commercially

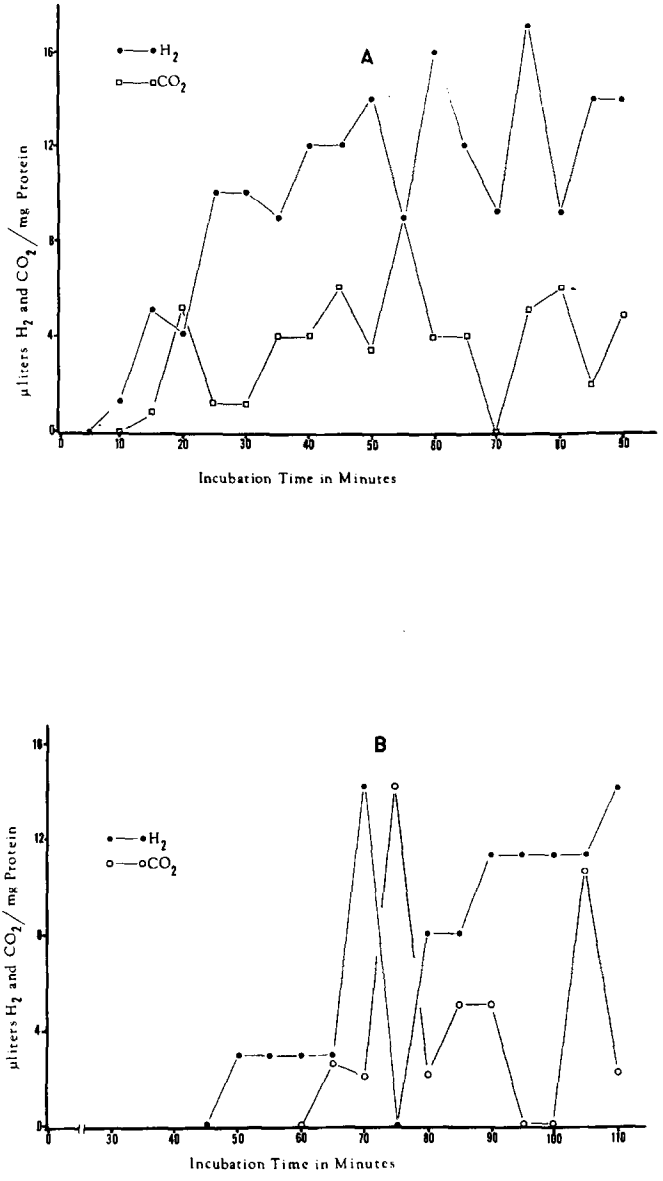


Figure 1: Formate hydrogenlyase induction and activity in Escherichia coli 82/r(A) and P6(B) under an atmosphere of 95% nitrogen:5% carbon dioxide.

prepared nitrogen, is illustrated in Fig. 1. Each point of the curve in Figures 1 and 2 represents the amount of gas evolved per five minute period and is independent of any previous measurement. A decrease in the slope of the curve did not necessarily mean that gas was consumed by the

organisms, but there occurred a decrease in enzyme activity for the five minute experimental period. An unusual feature of the formate hydrogenlyase reaction was the latent release of carbon dioxide from the cell. Hydrogen was observed 5 to 15 min prior to the first indication of carbon dioxide in the flask. Also, the quantities of both gases should theoreti-

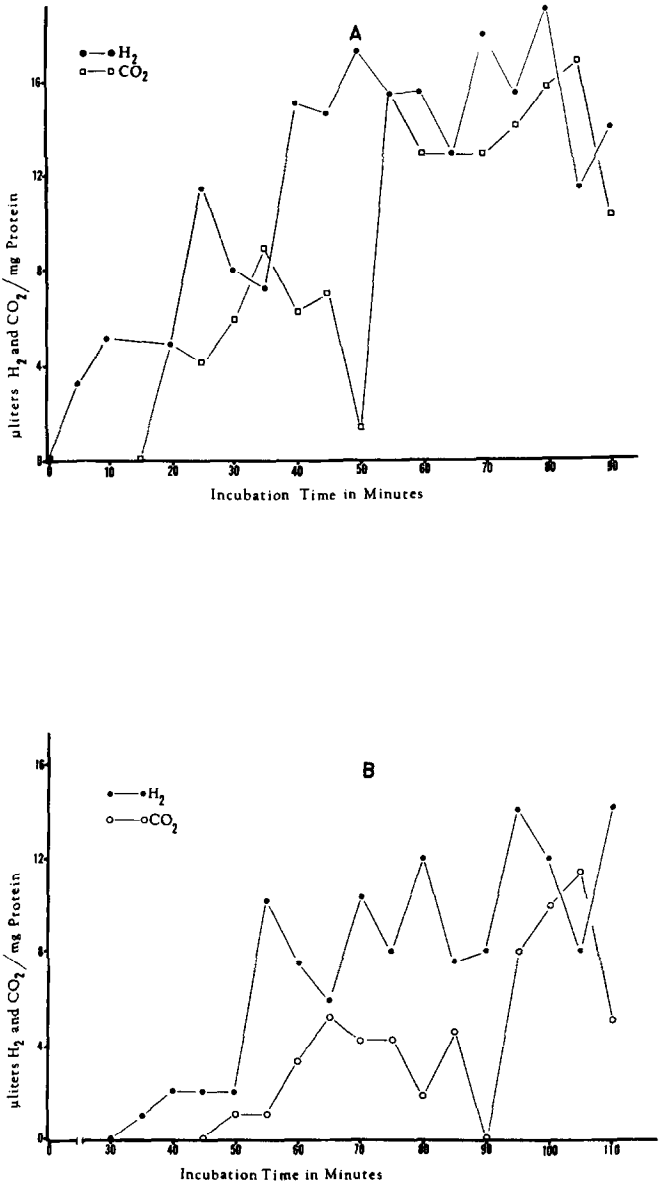


Figure 2: Formate hydrogenlyase induction and activity in Escherichia coli 82/r(A) and P6(B) under an atmosphere of 100% nitrogen.

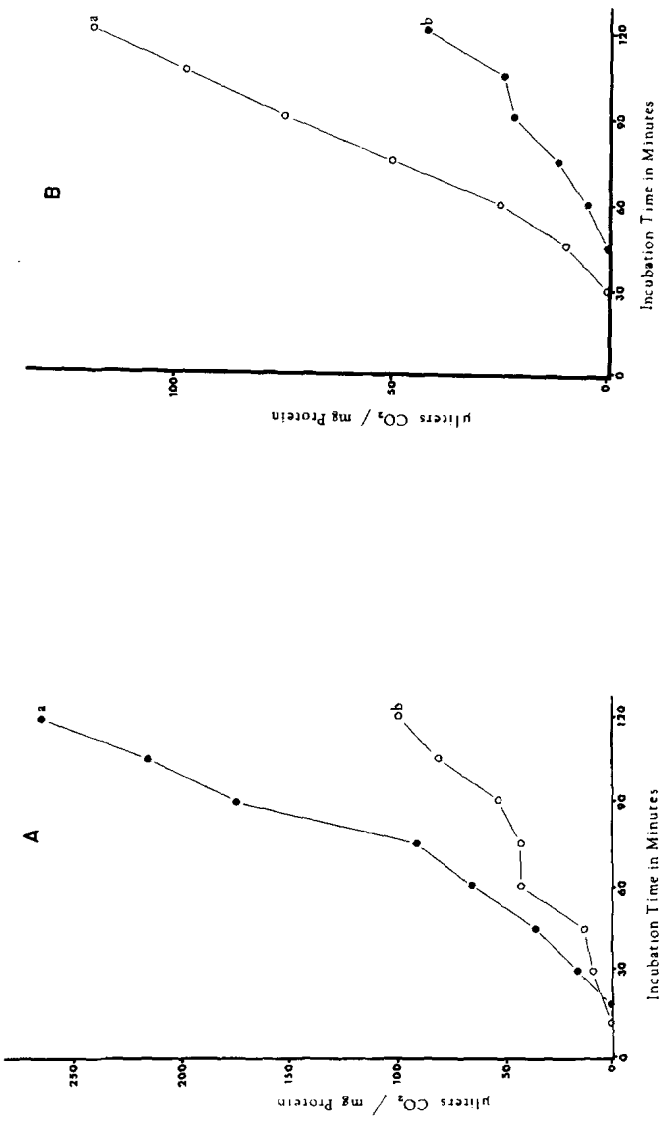


Figure 3: Formate dehydrogenase activity in Escherichia coli 82/r(A) and P6(B) under an atmosphere of 100% nitrogen or 95% N_2:5% CO_2. a = 100% nitrogen; b = 95% N_2:5% CO_2.

cally have been in equimolar proportion (Hopton, 1958). At the initial stages of induction, however, gas levels as high a 4 µliters of hydrogen per µliter of carbon dioxide were observed. Only when cells containing pre-induced formate hydrogenlyase were assayed did the two gases attain equimolar proportion (Fig. 4).

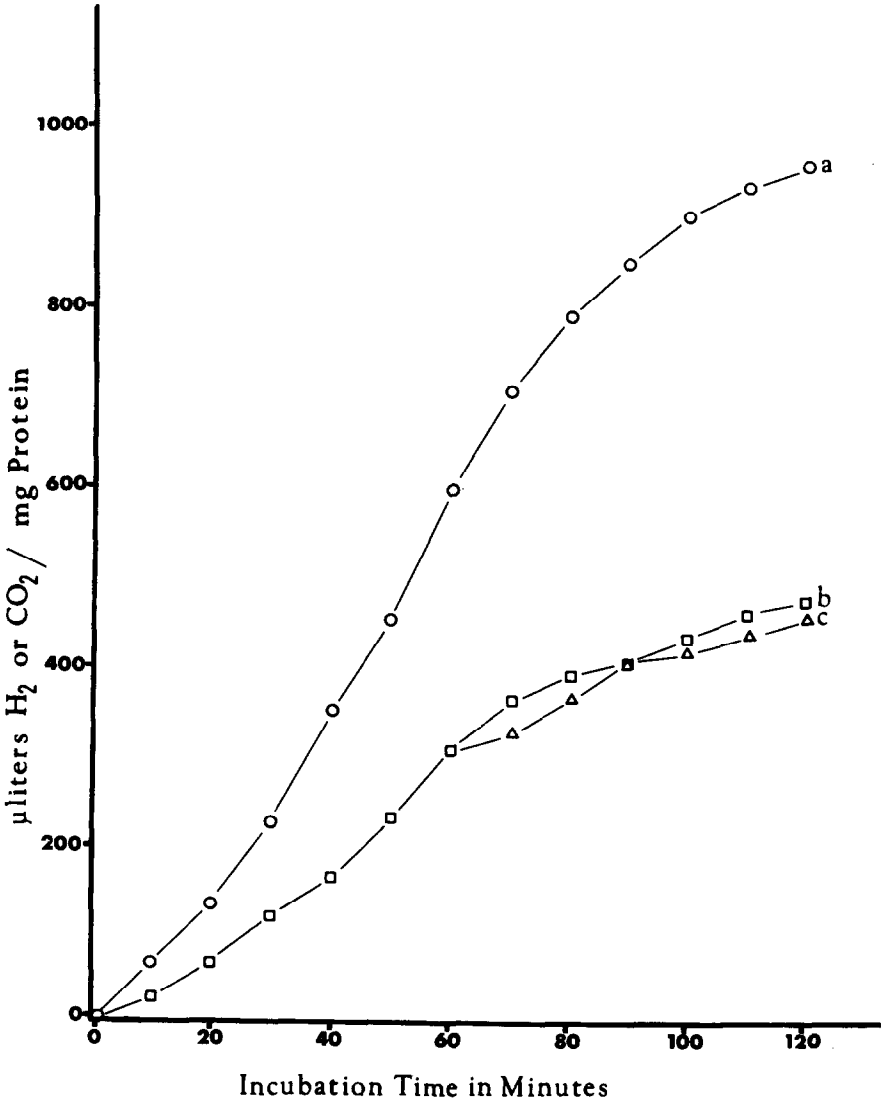


Figure 4: Hydrogen and carbon dioxide evolution from Escherichia coli pre-induced for formate hydrogenlyase and assayed under an atmosphere of 100% nitrogen or 100% carbon dioxide. a = 100% nitrogen, no KOH in flask center well; b = 100% nitrogen, 0.2 ml KOH in flask center well; c = 100% carbon dioxide, no KOH in flask center well.

Formate hydrogenlyase induction and activity under a 100% nitrogen atmosphere is shown in Fig. 2. Hydrogenase activity was measured in flasks which contained KOH in the center well. It was, therefore, not possible to observe a carbon dioxide effect on hydrogenase. Formate dehydrogenase, on the other hand, was measured with and without KOH. Removal of CO_2 from commercially prepared nitrogen resulted in a decreased induction time and increased formate dehydrogenase activity in P6 and 82/r by 60% and 68%, respectively (Fig. 3).

Incubation of cells, pre-induced for formate hydrogenlyase, under an atmosphere of commercially prepared carbon dioxide (100% CO_2) resulted in total inhibition of formate dehydrogenase activity, but no apparent effect on hydrogenase expression (Fig. 4). Incubation under similar conditions, but with non-induced cells, resulted in a complete inhibition of formate dehydrogenase activity, and hydrogenase activity was reduced by 65% and 100% in 82/r and P6, respectively.

At the termination of each enzyme assay, protein in the experimental and endogenous flasks was measured. It was found that carbon dioxide in the induction atmosphere affected protein synthesis. An atmosphere of 100% CO_2 resulted in no measurable increase in total protein during the experimental period (Table 1).

Table 1: Protein synthesis in Escherichia coli 82/r and P6 under various atmospheres.

Increase in Total Protein (%)

Atmosphere	82/r		P6	
	KOH*	No KOH**	KOH	No KOH
95% N_2:5% CO_2	26.2	13.1	13.3	1.2
100% N_2	26.3	18.0	13.1	2.7
100% CO_2	----	0.0	----	0.0

* KOH present in flask center well.
** KOH absent from flask center well.

DISCUSSION

Previous investigators (Wildiers, 1901; Walker, 1932; Gladstone, Fildes, and Richardson, 1935) reported that microorganisms being incubated under aerobic conditions require carbon dioxide for growth. Mechanical removal of carbon dioxide from the aerobic atmosphere resulted in a cessation of metabolic activities. According to Ajl, White and Werkman (1947), carbon dioxide is required by heterotrophic bacteria (e.g., E. coli) to initiate optimal growth and reproduction under normal environmental conditions. From the lag in production and reduced quantity of carbon dioxide released from the E. coli 82/r and P6 cells, it appears that this gas is similarly essential under anaerobic conditions.

The production of formate via glycolysis, phosphoroclastic reaction, is a reversible process (Lipmann and Tuttle, 1944). Werkman and Wood (1942) observed that in E. coli cells there occurs a fixation of carbon dioxide and its combination with a compound presumed to be pyruvate. Novelli (1955), using extracts of Micrococcus lactilyticus, was able to show the incorporation of $H^{14}COOH$ or $^{14}CO_2$ into pyruvate. Reversibility of the phosphoroclastic reaction appears to be more than a fortuitous process but one essential for viability of the bacterial cell. If atmospheric carbon dioxide were solely sufficient for fixation and utilization by E. coli, then its presence as 5% of the environment should have eliminated the reversible clastic reaction. This, however, was not the case. Atmospheric carbon dioxide was actually a metabolic inhibitor under anaerobic conditions which in the appropriate concentration could completely stop protein synthesis. It appears, then, that carbon dioxide can function as both a metabolic stimulator and inhibitor.

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