THE ROLE OF C_2-DERIVATIVES IN THE DECOMPOSITION OF FORMATE

by

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Formate is converted to equimolar quantities of H_2 and CO_2 by *Escherichia coli* and related organisms through the "formic hydrogenlyase" enzyme system. The hydrogenlyase activity of concentrated cell-free extracts from anaerobically-grown *E. coli* is lost upon moderate dilution or dialysis[1]. However, if *Clostridium butylicum* extract, which does not metabolize formate[2], is added to the dilute *E. coli* enzyme preparation together with low levels of pyruvate or diacetyl, formate decomposition is observed[3]. Pyruvate and diacetyl similarly activate the hydrogenlyase reaction in dialyzed *E. coli* preparations[1]. These observations suggest that a common metabolic product derived from pyruvate and diacetyl is required for formate conversion to H_2 and CO_2. Further study has disclosed that catalytic amounts of acetyl phosphate "spark" the reaction in the dilute and dialyzed preparations.

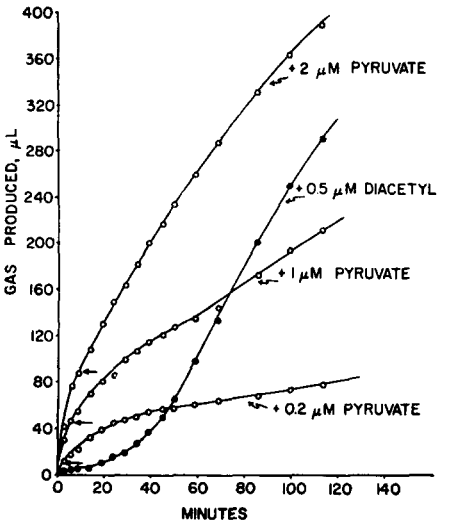


Fig. 1. Activation of formic hydrogenlyase in the dilute *E. coli* enzyme preparation by *Cl. butylicum* extract and pyruvate or diacetyl. The vessels contained 0.05 ml of *E. coli* extract[1] and 0.1 ml of *Cl. butylicum* extract[2] in 0.1 *M* potassium phosphate buffer pH 6.2 (final fluid volume, 1.2 ml). At zero time, 15 μM of sodium formate and sodium pyruvate (0.20 μM, 1 μM, or 2 μM) or diacetyl (0.5 μM) were added from the side arms. Gas phase, helium. Temperature, 30° C. The arrows indicate the total amount of $CO_2 + H_2$ expected from complete decomposition of the added pyruvate through the phosphoroclastic reaction. In the absence of pyruvate or diacetyl there was no gas production from formate by either enzyme preparation alone or by the combined extracts.

Activation of formate decomposition in the dilute preparation by addition of *Cl. butylicum* extract and pyruvate or diacetyl is illustrated in Fig. 1. It is evident that, within limits, the activation showed a dependence on the quantity of pyruvate metabolized by *Cl. butylicum* extract. In contrast to the results with pyruvate, "sparking" of the reaction by diacetyl was characterized by a short induction period. Acetyl phosphate in catalytic concentrations has also been observed to activate in a similar manner (short induction period).

Considerable variation has been noted in the response of dilute *E. coli* extracts to the "sparking" substances; this variation appears to be due to a number of factors, most important of which are extent of dilution of the extract and incubation time in the absence of formate. In experiments where the empirically-determined conditions required for demonstration of "sparking" have not been satisfactorily met, formate decomposition frequently begins after an induction period of variable length and proceeds with kinetics which resemble those of an autocatalytic reaction. Under the latter circumstances, *Cl. butylicum* extract alone may significantly reduce the length of the induction phase. In view of the exacting experimental requirements, it is of importance to stress that the results described were obtained by close adherence to the conditions noted in the legends of Fig. 1 and 2. The nature and significance of these experimental factors will be further detailed elsewhere.

Using the dilute system, negative results were observed with several other potential "sparking" substances, viz., vinylacetate, acetoacetate and butyrate, in experiments in which pyruvate or diacetyl were effective. Conversely, there was also no activation when *Cl. butylicum* extract was replaced by certain other crude preparations which metabolize pyruvate such as yeast carboxylase and *A. aerogenes* extracts which produce acetoin. The inability of reducing agents to reactivate either the dilute or dialyzed extracts indicates that the "sparking" substances do not act by simply maintaining an essential coenzyme or other constituent of the system in an active reduced state.

For technical reasons (noted above), dialyzed preparations have proved to be more suitable than dilute extracts for closer study of the "sparking" effect. After anaerobic dialysis against water, free of dissolved oxygen, the extract is incapable of producing gas from formate or does so only after an extended induction phase[1]. Formate is immediately decomposed by the dialyzed extract, however, in the presence of boiled preparations from *E. coli*, *Cl. butylicum*, *S. cerevisiae* and pigeon liver. Diacetyl, pyruvate and acetyl phosphate are also individually capable of activating the reaction (induction period). Unlike the dilute preparation, the concentrated dialyzed extract can apparently convert these compounds to the actual "sparking" intermediate in the absence of additional enzyme preparations. Typical results showing the activation by pyruvate and acetyl phosphate are summarized in Fig. 2. As indicated, pyruvate is generally more effective than acetyl phosphate but in some trials equivalent "sparking" was observed. None of the common coenzymes tested thus far have "sparking" activity for the dialyzed system[1]. Acetyl coenzyme A (kindly supplied by Drs. G. D. NOVELLI and I. C. GUNSALUS) also was incapable of activating the dialyzed extract and in fact caused inhibition of the "sparking" observed with acetyl phosphate. In addition, it has not been possible to demonstrate a coenzyme A requirement for hydrogenlyase activity.

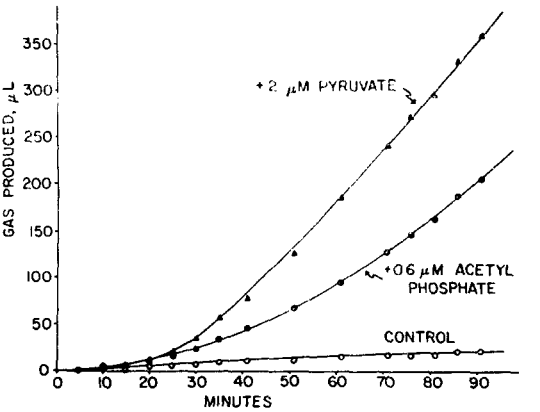
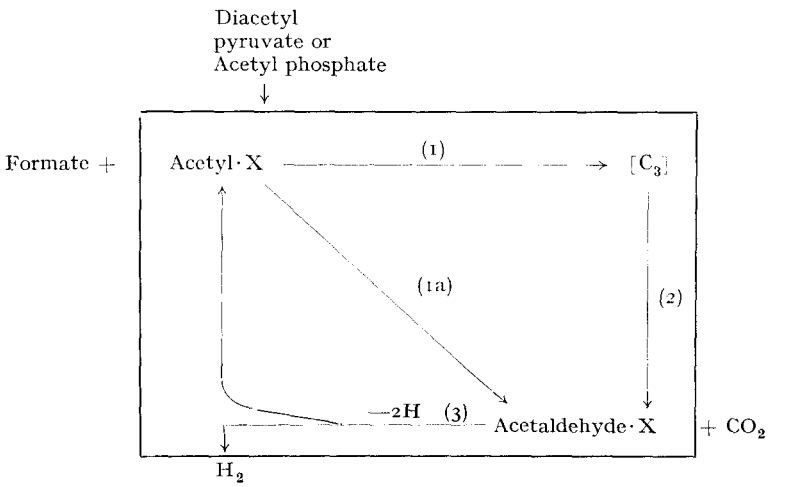


Fig. 2. Activation of formic hydrogenlyase in the dialyzed *E. coli* enzyme preparation by pyruvate or acetyl phosphate. The vessels contained 0.2 ml of *E. coli* extract (dialyzed for 17 hours) in 0.0625 *M* sodium potassium phosphate buffer pH 6.1 (final fluid volume, 1.2 ml). At zero time, 15 μM of sodium formate and sodium pyruvate (2 μM) or dilithium acetyl phosphate (0.6 μM) were added from the side arms. Gas phase, helium. Temperature, 30° C.

The foregoing observations suggest the participation of C_2 derivatives in the conversion of formate to H_2 and CO_2. Two alternative mechanisms which are compatible with the present results are indicated by schemes A and B below.



Scheme A = Reactions 1, 2, 3
Scheme B = Reactions 1a, 3

According to scheme A (1, 2, 3) an intermediate C_3 compound is formed which undergoes decarboxylation yielding CO_2 and an acetaldehyde complex. The latter is then oxidized, thus regenerating acetyl·X; the electrons are transferred to hydrogenase with the ultimate formation of H_2. In the alternative scheme B (1a, 3) formate is dehydrogenated (decarboxylated) directly with

acetyl·X serving as hydrogen acceptor. Molecular hydrogen is subsequently formed and acetyl·X regenerated as in scheme A. It is suggested that acceptors other than hydrogenase may act as oxidants for the acetaldehyde derivative in which case molecular hydrogen would not be produced. The rate of CO_2 evolution in the presence of such oxidants could be construed as a measure of the "formic dehydrogenase" activity which is always observed in active hydrogenlyase preparations. Both sequences are represented in terms of formate decomposition; for purposes of clarity, the reverse reactions, which could account for the synthesis of formate from CO_2 and H_2 are not indicated.

Reaction (3) is common to both schemes which emphasizes the suggested role of C_2 derivatives as intermediate hydrogen carriers. In this connection, it is of interest that the inference may be drawn from a recent report by PEEL AND BARKER[4] that the reduction of vinylacetate by H_2, catalyzed by dried cell preparations of *Cl. kluyveri*, involves acyl derivatives in a similar role.

Acceptance of Scheme A implies that formation of H_2 and CO_2 from pyruvate by *E. coli* and *Cl. butylicum* occurs through a common pathway. Thus both organisms would activate pyruvate to $[C_3]$ and thence through reactions (2) and (3) to acetate, H_2 and CO_2. The inability of *Cl. butylicum* to convert formate to H_2 and CO_2 could be explained as due to absence of reaction (1). Reversal of reaction (1) would be visualized as one of the initial stages in the classical phosphoroclastic split of pyruvate (to acetate and formate), which can be readily demonstrated in *E. coli* when the organism is grown so as not to produce H_2 and CO_2 from formate or pyruvate.

The nature of the C_2 derivatives in the above schemes is not specified. Conceivably, any of the currently postulated C_2-coenzyme compounds (C_2 lipoic, C_2 lipothiamide, or C_2 cocarboxylase)[5,6,7], may be involved. At the present time there appears to be no definitive evidence which excludes the possibility that these C_2 derivatives can be generated without intermediation of acetyl coenzyme A[8].

The work described here can account for the observation that traces of fermentable carbohydrates stimulate the hydrogenlyase activity of intact cell preparations of *E. coli*[9]. Our results suggest that these activations may have been mediated through formation of a C_2 derivative.

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REFERENCES

1 H. GEST, *Phosphorus Metabolism*, Vol. II, 522 (1952).
2 H. J. KOEPSELL AND M. J. JOHNSON, *J. Biol. Chem.*, 145 (1942) 379.
3 H. GEST, H. E. SWIM AND S. S. BARKULIS, *Fed. Proc.*, 12 (1953) 207.
4 J. L. PEEL AND H. A. BARKER, *Biochem. J.*, 53 (1953) xxix.
5 H. D. BARNER AND D. J. O'KANE, *J. Bact.*, 64 (1952) 381.
6 M. I. DOLIN, L. P. HAGER AND I. C. GUNSALUS, *Am. Chem. Soc., Abstracts 123rd Meeting* (1953) 27C.
7 L. J. REED AND B. G. DE BUSK, *J. Am. Chem. Soc.*, 74 (1952) 3964.
8 H. CHANTRENNE AND F. LIPMANN, *J. Biol. Chem.*, 187 (1950) 757.
9 J. LASCELLES, *Proc. Linn. Soc., N.S.W.*, 73 (1948) 430.

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ON THE DISSIMILATION OF DL-ALANINE-1-^{14}C BY RAT BRAIN HOMOGENATES

by

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In a recent article, we reported that brain tissue dissimilates DL-alanine-1-^{14}C at a rate about one third of that exhibited by the kidney[1]. This interpretation was based on measurements of $^{14}CO_2$ produced from this amino acid by homogenates. It was of interest to learn whether there is a preferential utilization of one optical isomer since KREBS[2] has shown that members of the D-series are

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