A NOVEL REACTION OF S-ADENOSYL-L-METHIONINE CORRELATED WITH THE ACTIVATION OF PYRUVATE FORMATE-LYASE

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SUMMARY: Conversion of the inactive form of pyruvate formate-lyase to the catalytically active enzyme is accomplished by the Fe-dependent 'enzyme II'; reduced flavodoxin, S-adenosyl-L-methionine and the effector pyruvate are required. It was found that adenosylmethionine is reductively processed during activation of pyruvate formate-lyase to yield methionine, adenine and 5-deoxyribose. We suggest that transient adenosylation of enzyme II is required for its function as a converter enzyme.

Pyruvate formate-lyase (formate acetyl-transferase, EC 2.3.1.54) catalyzes the reaction pyruvate + CoA $\rightleftharpoons$ acetyl-CoA + formate, which plays a key role in the anaerobic metabolism of *Escherichia coli* and other bacteria. Its basic mechanism has only recently been delineated by the identification of an acetyl-enzyme intermediate (1). Less understood, however, is the regulation of this enzyme. It occurs in two forms of equal size (about 150,000 daltons) which are interrelated by the following activation-deactivation cycle.

The form designated PFL_I is the stable but catalytically inactive state of the enzyme; this is the form isolated from cellular extracts. PFL_I is convertible to the active form, PFL_A, by a complex enzymatic reaction which comprises a Fe-dependent protein called 'enzyme II' (molecular weight 40,000), reduced flavodoxin as electron donor and the low-molecular weight factors S-adenosyl-L-methionine and pyruvate (1,2). The latter can be replaced by oxamate thus demonstrating an effector role for pyruvate. S-Adenosyl-L-homocysteine inhibits the activation reaction (3).

Abbreviations: PFL_A and PFL_I, active and inactive forms of pyruvate formate-lyase; Fld, flavodoxin.

PFL_A is momentarily and irreversibly inactivated upon contact with air. Under anaerobic conditions, however, PFL_A decays to PFL_I (4). This occurs apparently spontaneously with a half-time of 50 to 140 min at 30 °C and pH-values of 7.5 to 9 (T. Schmitt, unpublished).

Reduction-oxidation (2) as well as alkylation-dealkylation (4) processes were proposed as possible mechanisms of the enzyme interconversion. In testing for them, it has recently been observed that adenosylmethionine is converted to methionine during the activation reaction; however, the PFL_A form produced neither contained the expected adenosyl group nor any other C-containing residue from adenosylmethionine (K. Jungermann, R. Thauer, T. Schmitt, H. Blaschkowski and J. Knappe, unpublished). In the present communication we present evidence that adenosylmethionine is reductively cleaved into L-methionine and 5'-deoxyadenosine. The disclosure of the role of adenosylmethionine as well as of reduced flavodoxin throws new light on the nature of the regulatory modification of pyruvate formate-lyase.

METHODS AND MATERIALS

Pyruvate formate-lyase (inactive form; specific activity 48 U/mg), enzyme II (DEAE-cellulose fraction), flavodoxin, and chloroplast fragments from spinach (for photoreduction of flavodoxin) were prepared as described previously (5).

Adenosylmethionine-[*adenosine*-U-^{14}C] (180,000 dpm/nmole) and adenosylmethionine-[*methionine*-U-^{14}C] (56,000 dpm/nmole) were prepared enzymatically from uniformly ^{14}C labeled ATP and methionine respectively essentially as described by Tabor and Tabor (6). Adenosylmethionine-[*methyl*-^{14}C] (36,000 dpm/nmole) was obtained from Amersham Buchler GmbH. Final purification of these compounds was by chromatography on amberlite IRC 50 (H^+-form) using a gradient to 0.08 N HCl.

5-Deoxy-D-ribose (7) was synthesized by reduction of 15 g 1-methyl-2,3-isopropylidene-5-tosyl-D-ribose with 7 g $LiAlH_4$ (cf. (8); 17 hours refluxing in ether) to methyl-2,3-isopropylidene-5-deoxyribose and hydrolysis of the latter in 0.4 N H_2SO_4 for 90 min at 90 °C; yield, 5.4 g. - ^{13}C-NMR in D_2O (22.63 MHz; TMS = 0): 96.80 ppm (C-1), 17.99 (C-5) for the α-anomer; 101.87 (C-1), 20.13 (C-5) for the β-anomer. - The phenylhydrazone, recrystallized from toluene, had the m.p. 126 °C (Found: C, 58.85; H, 7.36; N, 12.47. Calcd. for $C_{11}H_{16}O_3N_2$: C, 58.91; H, 7.19; N, 12.49).

Paper chromatography was performed on Whatman No. 3 mainly using the following solvent systems. A) Butanol-acetic acid-water (60:15:25; descending): Rf methionine, 0.6; cyanhydrins of 5-deoxy-D-ribose, 0.39-0.42; other compounds, see Fig. 1. B) Phenol-water (100:39): Rf 5-deoxyribose, 0.84;

2-deoxyribose, 0.79; ribose, 0.66; L-lyxose, 0.54. C) 0.1 N NH_3: Rf adenine, 0.46; adenosine, 0.55; xanthine, 0.60; hypoxanthine, 0.67; inosine, 0.83; ribose and deoxyriboses, 0.93.

RESULTS

Products of Adenosylmethionine. Adenosylmethionine labeled with ^{14}C in various parts of the molecule was employed in the system for PFL activation; with flavodoxin being continously photoreduced by added chloroplasts. A series of products was obtained which could be separated by paper chromatography (Fig. 1).

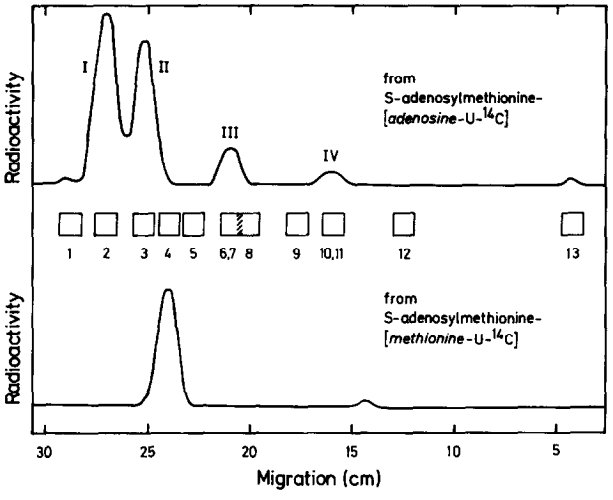

Figure 1. Radiopaperchromatography of S-adenosylmethionine products. PFL activation reactions were carried out at 30 °C in closed tubes under argon as described previously (1,5). They contained in a volume of 0.5 ml: 0.1 M 2-(N-morpholino)propanesulfonate pH 7.7, 9 mM dithiothreitol, 0.4 mM $Fe(NH_4)_2(SO_4)_2$, 20 µM dichlorphenolindophenol, 10 µM 3(3,4-dichlorophenyl)-1,1-dimethyl urea, chloroplast fragments (10 µg chlorophyll), 20 µg flavodoxin, 2.8 mg PFL (inactive form), 0.9 mg enzyme II-fraction, 4 mM oxamate, and 6.4 µM [^{14}C]adenosylmethionine. The reactions were stopped with perchloric acid after 3 hours illumination from a daylight lamp; the (neutralized) supernatants were applied on Whatman paper No. 3 at a width of 15 cm and chromatographed in solvent A. Radioactivity scanning was by a windowless counter. Radioactive zones were eluted with 1 % acetic acid. - Squares denote the migration of the following compounds: 1, 5'-Methylthioadenosine; 2, 5-deoxyribose; 3, adenine; 4, methionine; 5, 2-deoxyribose; 6, hypoxanthine; 7, adenosine; 8, α-amino-γ-butyrolactone; 9, ribose; 10, inosine; 11, homoserine; 12, adenosylhomocysteine; 13, adenosylmethionine.

A single radioactive compound appeared when adenosylmethionine labeled in the methionine moiety was used. It was identified as methionine by co-chromatography (in several solvents) with the amino acid. Following treatment with H_2O_2 the labeled material behaved as expected for methionine sulfone.

Compound II (Fig. 1) from an experiment with adenosylmethionine uniformly labeled in the adenosine moiety was disclosed as adenine by its chromatographic properties. From this it became evident that the other major product, compound I, must be related to ribose. First, the possible identity with D-*erythro*-2,3-dihydroxy-Δ4-pentenal was examined. This compound is known to arise from adenosylcobalamin (9,10) and likewise could have been formed in the reaction studied as it obviously involved an adenosyl transfer. However, treatment with OsO_4, which should have given rise to a mixture of D-ribose and L-lyxose, was ineffective. Compound I was finally identified as 5-deoxy-D-ribose by cochromatography with the authentic compound and with the cyanhydrin(s) thereof following reaction with KCN. Furthermore, when the ^{14}C labeled product was mixed with carrier 5-deoxyribose and converted to the phenylhydrazone, the crystalline derivative had the expected specific radioactivity.

Of the minor products, compound III was disclosed as hypoxanthine while compound IV, having a mobility similar to inosine, has not been identified as yet.

Methionine, 5-deoxyribose, adenine and hypoxanthine were recovered from the two corresponding experiments at molar ratios of 1 : 0.88 : 0.72 : 0.19. This result clearly demonstrated that in this system adenosylmethionine is processed by reduction. Most likely the primary product besides methionine is 5'-deoxyadenosine. Nucleosidase and deaminase activities (as measured by use of [^{14}C]adenosine) were present in the enzyme preparations employed in these experiments and can easily explain the conversion of 5'-deoxyadenosine to the products identified.

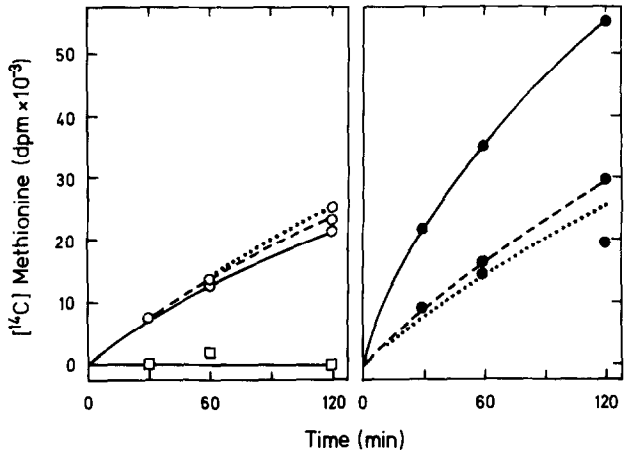

Figure 2. Time course of methionine production. Reaction conditions were as in Fig. 1, employing 4.4 nmoles of [*methyl*-^{14}C]adenosylmethionine and the following amounts of enzymes: 0.3 mg PFL (inactive form (□) or 0.27 mg enzyme II-fraction (o) or the combination of both (•). Dashed lines (---) refer to experiments without oxamate; dotted lines (...) to experiments with additionally 20 nmoles of adenosylhomocysteine. Methionine was measured as radioactivity passing a Dowex 50 (Na^+-form) column. In the series of the complete system (right part, upper curve) also the amount of active PFL formed was determined, using the coupled optical assay (1); results are given in Table 1.

Correlation between Adenosylmethionine Cleavage and Activation of Pyruvate Formate-Lyase. In subsequent experiments (see Fig. 2) the formation of methionine was used as a measure for adenosylmethionine processing. The reaction was found to be mediated by enzyme II along with reduced flavodoxin as electron donor. (When flavodoxin was omitted and the reaction run in the dark, methionine production was diminished about tenfold.)

Upon addition of PFL_I, however, the reaction proceeded at an increased rate (Fig. 2, right part). The additional release of methionine coincided with the generation of active pyruvate formate-lyase since it (largely) depended on oxamate and was abolished by adenosylhomocysteine. A transfer of the adenosyl group to pyruvate formate-lyase, as might be suggested by this observation, had previously been excluded by analysis of the PFL_A form as isolated from the activation system by anaerobic gel filtration (cf. (1) and introduction). The present experiments, along with the above product

analysis, then indicate that the transformation of the enzyme occurs concurrently with the adenosyl group of adenosylmethionine being transferred to formally a hydride ion.

The continued methionine release after maximum PFL_A activation is observed (Table 1) can readily be explained by decay of PFL_A to the PFL_I form (see introduction). The present data suggest that the generation of one enzyme-active site is related to the consumption of one or two molecules of adenosylmethionine.

Table 1. Oxamate-dependent Formation of Methionine and Appearance of Active Pyruvate Formate-Lyase

Reaction time (min)	PFL_A (units)	PFL_A (nmole)	Δ Methionine (nmole)
30	8.5	0.17	0.35
60	13	0.26	0.52
120	14	0.28	0.71

Data are from the pertinent experiments of Fig. 2 and the relationship that 1 unit PFL_A is an equivalent of 0.02 nmole catalytic site (1).

DISCUSSION

The results presented in this communication suggest that the activation of pyruvate formate-lyase occurs according to the following equation:

$$PFL_I + Fld_{red} + \text{adenosylmethionine} \xrightarrow[\text{(pyruvate)}]{\text{enzyme II (Fe)}}$$

$$PFL_A + Fld_{ox} + \text{methionine} + \text{5'-deoxyadenosine}.$$

It ascribes to adenosylmethionine the function of an adenosyl group donor which is a hitherto unknown biological property of this compound.

The system might resemble adenosylcobalamin-dependent enzyme reactions in which 5'-deoxyadenosine occurs as an intermediate of the respective substrate rearrangements (for a recent review, cf. (11)). Participation of a cobalamin coenzyme can be excluded since the pyruvate formate-lyase system is present in B_{12} auxotrophic mutants (12). However, the enzyme II-fraction comprises a Fe-protein (cf. (2,5)). This is noteworthy as a metal-adenosyl compound appears to be most qualified as a precursor of 5'-deoxy-adenosine.

It is proposed that an adenosyl-enzyme II complex is the actual mediator of PFL activation and that this is accompanied by release of 5'-deoxyadenosine (or 5-deoxyribose plus adenine, the glycosidase activity possibly exerting a driving force). Each cycle of PFL activation then requires the *de novo* formation of adenosyl-enzyme II from adenosylmethionine and concurrently consumes a reductant. (The observed PFL_I-independent reaction could possibly stem from defective species in the only partially purified enzyme II-fraction.)

The previous concept (2) of pyruvate formate-lyase being activated by reduction (of a buried disulfide bond) of the protein appears quite unlikely now since reduced flavodoxin is acting in adenosylmethionine cleavage; which presumably is its sole function. Instead we propose that rearrangements in the protein structure of pyruvate formate-lyase are involved in the interconversion of the two forms of the enzyme. In the PFL_A form the protein might be under conformational strain which would provide a rationale for the apparent spontaneity of its conversion to the PFL_I form. Redistribution of a disulfide bond and SH-group(s), for example, would be a feasible rearrangement process.

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