INHIBITION OF THE CARBON DIOXIDE FIXATION IN E. COLI BY THE COMPOUNDS RELATED TO TCA CYCLE

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It is well known that the CO_2 fixation-reaction to produce C_4-dicarboxylic acid in TCA cycle is the key reaction which enables this cycle to play a biosynthetic role (Wiame, 1957). With the growing cells of various microorganisms, the assimilation of labelled CO_2 has been reported to be reduced by the addition of the compounds related to TCA cycle to the medium (Lardy et al., 1949; Wiame and Bourgeois, 1955; Abelson et al., 1955). Although this phenomenon was attributed to the isotopic dilution caused by the addition of the fixation products (Roberts et al., 1955), the authors obtained with E. coli cells the results which suggest that the CO_2 fixation is controlled by these compounds (unpublished work). The present communication describes the inhibition of the CO_2 fixation by aspartate (Asp), glutamate(Glu), or some members in TCA cycle in cell-free extracts of E. coli.

Reaction system for the CO_2 fixation : Table I shows the dependence of the reaction on pyruvate(PA), CoA and acetyl phosphate(AP). The requirement for ATP was slight, and that for NAD^+ was not noticed in the crude extracts as well as in the extracts treated with active charcoal or

TABLE I. Component Requirements for the CO_2 Fixation System

Reaction system	Total c.p.m. fixed (x 10^{-3})
Complete	90.0
-PA	17.2
-CoA	14.2
-AP	12.6
-AP, -CoA	4.6
-ATP	66.6
-NAD^+	97.8

The complete reaction system contained the following constituents (μmoles in 0.7 ml); $NaH^{14}CO_3$, 15 (1.3 x 10^6c. p.m.); sodium pyruvate, 20; lithium acetyl phosphate, 8.2; CoA, 0.25; ATP, 6.8; NAD^+, 2.9; $MgSO_4 \cdot 7H_2O$, 5; $MnSO_4 \cdot 5H_2O$, 0.5; Tris-HCl buffer (pH 7.4), 70; malic dehydrogenase, 3,000 units; and 0.1 ml of the crude sonic extracts of *E. coli* W grown in a glucose-salts medium (centrifuged at 10^5 x G for 2.5 hrs. 17 mg protein/ml). The mixture was incubated for 90 min. at 30°C under H_2. After the reaction was stopped, the total radioactivity fixed was measured by the usual method.

Sephadex G-25. The non-requirement for pyridine nucleotide suggests that malic enzyme is not concerned in this reaction system. The radioactive reaction products were separated by paper chromatography and about 90 percent of the radioactivity was found in citrate. Furthermore, it was shown in a separate experiment that ^{14}C of bicarbonate was incorporated into oxaloacetate(OAA) which was added for trapping. In view of these facts, OAA seems to be the primary product and to condense yielding citrate with acetyl-CoA which is presumably generated from CoA and AP by an endogenous phosphotransacetylase.

As the presence of phosphoenolpyruvate(PEP) carboxylase in *E. coli* was reported by Cánovas and Kornberg (1965), PEP was tested as the substrate instead of PA. As shown in Table II, PEP was more effective than PA. Upon further incu-

TABLE II. Inhibition of the CO_2 Fixation by Asp

Additions (μmoles)	Incubation time (min.)	Total c.p.m. fixed (x 10^{-3})	Inhibition (%)
Control	30	44.8	
+PEP (7)	30	820	
+PEP, +Asp (3.5)	30	266	68
+PA (20)	30	65.5	
+PA, +Asp	30	17.7	82
Control	90	71.8	
+PA	90	195	

The reaction mixture of the control was the same as in Table I except the omissions of PA, NAD^+ and malic dehydrogenase, and the addition of glutathione (2 μmoles). The reaction was carried out under air.

bation, the effect of PA became more remarkable. Therefore, it is conceivable that the true substrate is rather PEP than PA and that PA is a precursor of PEP, though the existence of PA carboxylase could not be excluded. Presumably PEP may be generated from PA by the reverse reaction by a PA kinase (Krimsky, 1959), owing to the high ratio of ATP/ADP which may be brought by the generation of ATP from AP and ADP by an endogenous acetokinase. Whether PEP is derived by this mechanism or by any other way remains to be elucidated.

Inhibition of the CO_2 fixation by the compounds related to TCA cycle : The effect of various compounds on the CO_2 fixation in the system containing PA as the substrate is shown in Table III. Asp, Glu, their corresponding amides, or some carboxylic acids in TCA cycle showed inhibitory effects, but the other compounds virtually did not. Asp, one of the most powerful inhibitors, showed 86 percent inhibition at the concentration of 5 x 10^{-3}M as is seen in

TABLE III. Effects of Various Compounds on the CO_2 Fixation

Additions	Exp. 1		Exp. 2		Exp. 3	
(5×10^{-3}M)	A*	B**	A	B	A	B
None	110	-	109	-	111	-
L-Aspartate	39.1	65	39.6	64	6.4	94
L-Glutamate	69.0	37	58.2	47	55.4	50
L-Arginine	104	5				
DL-Threonine	110	0				
L-Proline	118	-7				
L-Lysine	93.6	15				
L-Asparagine			53.0	51		
L-Glutamine			59.2	46		
L-Alanine					103	7
Citrate			76.2	30	70	37
DL-Isocitrate					77	31
α-Keto-glutarate			73.6	32	89	20
Succinate			28.6	74	84	24
Fumarate					21	81
DL-Malate			39.0	64	54	51
DL-Lactate					94	15
Butyrate					110	1
DL-Citramalate					116	-4
DL-Tartarate					117	-5
Malonate					111	0
Glutarate					110	1

* A ; Total c.p.m. fixed ($\times 10^{-3}$)
** B ; Inhibition (%)

The reaction mixture in Exp. 1 was the same as that in Table I, except the omissions of NAD^+ and malic dehydrogenase, the addition of glutathione (2 μmoles), and the use of $KH^{14}CO_3$ (10 μmoles, 2.2×10^6 c.p.m.) in place of its sodium salt. The reaction mixture in Exp. 2 was the same as that in Exp. 1, except the concentration of CoA (0.082 μmoles). The reaction mixture in Exp. 3 was the same as that in Exp. 1 except the concentration of $KH^{14}CO_3$ (20 μmoles, 10^6 c.p.m.) and of CoA (0.32 μmoles). The reaction was carried out under H_2 for 90 min. in Exp. 1 and 2, and under air for 30 min. in Exp. 3.

Fig. 1. The inhibition of the CO_2 fixation by Asp was also observed in the system containing PEP instead of PA. The results are shown in Table II.

Mode of the inhibition : Recently Cánovas and Kornberg

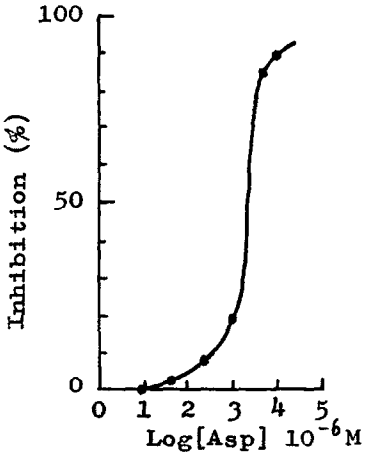

Fig. 1

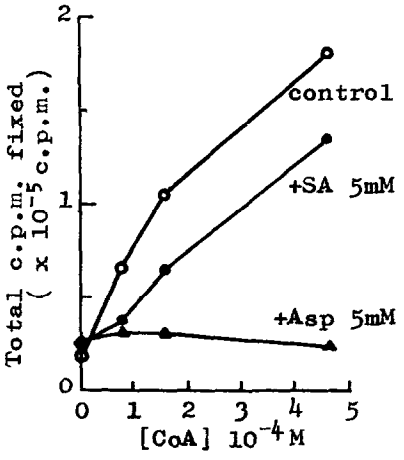

Fig. 2

Fig. 1. Inhibition of the CO_2 fixation by Asp. The reaction mixture was the same as that in Exp. 1, Table III, except the concentration of $KH^{14}CO_3$ (20 μmoles, 10^6 c.p.m.) and of CoA (0.32 μmoles). The reaction was carried out under air for 30 min.

Fig. 2. Inhibition of the CO_2 fixation by Asp or SA with the various concentrations of CoA. The reaction conditions were the same as in Fig. 1.

(1965) found that the CO_2 fixation on PEP was stimulated by acetyl-CoA. Similar results were also obtained by the authors. As to the mode of the inhibition by these compounds described in the previous section, a possibility is raised that they might inhibit the fixation reaction by producing OAA which depletes acetyl-CoA, the stimulator of the fixation reaction, to form citrate by a condensation reaction. If so, the inhibition should be relieved by increasing the concentration of acetyl-CoA. In Fig. 2 is shown the relationship between the inhibition by Asp or succinate(SA) and the increasing concentrations of CoA*. The inhibition by

* The supplemental experiments had shown that the generation of acetyl-CoA from CoA and AP by a phosphotransacetylase was proportional to the concentration of CoA and was not affected by Asp or SA.

Asp overcame the stimulation by CoA, whereas the inhibition by SA was progressively relieved along with the increasing amount of CoA. The mode of the inhibition by Asp seems to be different from that of the inhibition by SA.

Since malonate is known to block the conversion of Glu to OAA by inhibiting succinic dehydrogenase, the effect of this compound on the inhibition by Glu was studied in order to examine the possibility discussed above. However, the inhibition by Glu was not affected at all by malonate even at the concentrations enough to inhibit succinic dehydrogenase. Therefore, the mode of the inhibition by Asp or Glu seems to be not compatible with that possibility. As another possibility, the direct effect of these compounds on PEP carboxylase remains to be examined. Further work along this line is now in progress.

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