REGULATION OF PHOSPHOENOLPYRUVATE CARBOXYLASE FROM *ACINETOBACTER CALCOACETICUS* BY NUCLEOTIDES

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1. Introduction

PEP carboxylase (orthophosphate:oxaloacetate carboxylase (phosphorylating) EC 4.1.1.31) of bacteria is a characteristic allosteric enzyme whose activity is controlled by a multiplicity of effectors. For instance, acetyl-CoA and fructose-1,6-bisphosphate have been found to activate the enzyme, and aspartate and certain other dicarboxylic acids to inhibit it [1]. These effects are generally interpreted, metabolically, in terms of the enzyme's amphibolic properties [2–5].

The influence of various nucleoside mono-, di- and triphosphates on the enzyme from bacteria is quite different. This communication shows that nucleoside mono- and diphosphates are the most potent activators of PEP carboxylase from *Acinetobacter calcoaceticus*.

2. Materials and methods

PEP carboxylase from *Ac. calcoaceticus*, partially purified by fractionation with ammonium sulphate (30–40% saturated) and gel filtration through Sephadex G-200 to give a product of spec. act. 24.8 mkat/kg [7], was used throughout this work.

Enzyme activity was measured at 20°C in a coupled spectrophotometric assay by following NADH oxidation at 340 nm. Unless otherwise stated, assayed mixtures contained 0.1 M Tris–HCl (pH 7.4), 2 mM PEP, 0.075 mM NADH, 10 mM $MgCl_2$, 10 mM $NaHCO_3$, 10 μg malate dehydrogenase and an adequate amount of enzyme in 3 ml. The reaction was initiated by adding PEP. The coupled reaction was shown experimentally not to be rate limiting.

Protein was determined by the method in [8]. All chemicals were analytical grade reagents of the highest grades available.

Abbreviations: AcCoA, acetyl CoA; FBP, fructose-1,6-bisphosphate; PEP, phosphoenolpyruvate

3. Results and discussion

As shown in table 1, the PEP carboxylase is activated by nucleotides. In all cases the activation is much stronger without addition of AcCoA. Among the nucleotides tested, nucleoside monophosphates were the most effective activators. Adenine and adenosine showed no effect on the rate of catalysis.

Table 1
Activation of PEP carboxylase by nucleotides

Nucleotide (5 mM)	Relative activity (%)	
	Without AcCoA	With AcCoA (87 μM)
–	100	100
c-AMP	275	127
AMP	295	139
ADP	270	126
ATP	112	100
GMP	321	143
GDP	271	132
GTP	183	101
CMP	315	144
CDP	265	131
CTP	160	94
UMP	261	121
UDP	219	118
UTP	107	103

Assay conditions were as in section 2

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In contrast to our results the PEP carboxylase from *E. coli* is inhibited by GMP and ATP [6]. In *Pseudomonas* ADP is an inhibitor of the enzyme [9], while for *Salmonella* CMP, CDP and GTP are reported to be the most effective activators [10].

AMP served as a model activator in the following experiments. As described for AcCoA [11], the enzyme reaction velocity curve with PEP as the variable substrate becomes much less sigmoidal with increasing concentrations of AMP [7]. Being a positive allosteric effector of the *v–K*-type [12], AMP increases the maximal velocity as well as the affinity of the enzyme for PEP. The K_m value decreases from 1.68 mM without activator to 0.92 mM at infinite AMP concentration, while V_{max} is increased 2.66-fold. Values for infinite AMP concentration were obtained from plots of 1/[AMP] versus $1/V_{max}$ and $1/K_m$. In contrast to the other activators (AcCoA and FBP) of PEP carboxylase from *Ac. calcoaceticus* [11], AMP appears to have a greater effect on V_{max} than on K_m for PEP. In this connection it should be mentioned that Hill coefficients are significantly lowered with increasing concentrations of AMP [7].

Plots of $1/V$ versus 1/[AMP] at different concentrations of PEP show a dependence of the K_s values for AMP on the substrate concentration, suggesting an allosteric effect of the substrate on the activator AMP. Values are given in table 2. A second calculation from plots of the slopes of the velocity plots [7] provided the same result for infinite PEP concentration.

Since PEP influences the K_s for AMP, it could be expected that the interaction coefficient for AMP could also be affected by PEP. The interaction coefficients obtained from a double logarithmic plot are given in table 2. Such a decrease of interaction coefficients for the activator caused by the substrate is quite uncommon even for an allosteric enzyme.

Since the effectiveness of nucleotides as activators decreased in the order NMP > NDP > NTP it could be suspected that the activation of PEP carboxylase depends on the energy charge [13]. Therefore AMP and ATP were combined up to an energy charge of 0.8 at total concentrations of nucleotides of 0.5–10 mM. In all cases we obtained the same activation as when only AMP was used, suggesting that energy charge is not the determining factor.

When AMP and AcCoA are combined, it is remarkable that the highest activity is not found with the highest concentrations used of both activators but at only 28.7 μM AcCoA and 1 mM AMP. Both activators show a significant effect on the K_s value of the other, which is decreased with increasing activator concentrations [7].

Exact calculations of the synergistic effect of AMP and AcCoA show that it cannot be explained as a single additive one (table 3). Low concentrations of both activators result in higher activation rates than are expected by simple addition, while higher concentrations give lower activation rates than the additive values.

Finally, it should be noted that AMP seems only to activate the tetrameric form of the enzyme from *Ac. calcoaceticus*. It was shown by polyacrylamide gel electrophoresis that the enzyme dissociates into its dimeric form on keeping for some weeks [7]. The degree of activation by AMP decreases in the same way as the enzyme dissociates, while the degree of activation of the enzyme in the presence of AcCoA remains nearly constant.

Table 2
K_s values and Hill coefficients (n) for AMP at different substrate (PEP) concentrations

PEP (mM)	K_s AMP (mM)	n AMP
0.5	1.94	1.11
1.0	0.98	1.02
2.0	0.70	0.57
∞	0.48	

Table 3
Influence of a combination of AMP and AcCoA on PEP carboxylase activity

AcCoA (μM)	AMP (mM)	Relative activity		Q
		Expected (%)	Found (%)	
5.8	0.25	286	370	1.29
5.8	0.5	369	506	1.37
5.8	1.0	551	726	1.33
28.7	0.25	438	624	1.40
28.7	0.5	521	790	1.52
28.7	1.0	703	887	1.25
87.0	0.25	740	720	0.98
87.0	0.5	823	800	0.97
87.0	1.0	1005	880	0.87

Expected (additive) and found relative activities and the quotient (Q) (found/expected) are given

The activation of PEP carboxylase from *Ac. calcoaceticus* by nucleoside mono- and diphosphates and the results in [11] concerning the effect of amino and organic acids on the enzyme lead to the assumption that the regulation of PEP carboxylase is mostly connected with the citric acid cycle and to a far lower extent with amino acid metabolism. The activation depends upon the absolute concentrations of mononucleotides rather than the energy charge and cannot be interpreted as compensatory for regulatory effects on aspartate transcarbamylase as has been suggested for other bacteria [3,10]. The activation of PEP carboxylase by a high AMP content may be physiologically advantageous because under such conditions there may be a high demand for oxaloacetate by the energy-generating pathway of the citric acid cycle. This argument finds support from earlier observations in other microorganisms that one form of pyruvate kinase [14,15] and pyruvate dehydrogenase [16] are both activated by AMP, a compound whose level is expected to be quite low when the ATP:ADP concentration is high [17]. Our results on inhibition of NADP-linked malic enzyme by ATP in *Ac. calcoaceticus* [18] also fit into this concept.

Furthermore the different kinetic behavior of the activators, their synergistic effect and the inhibition behavior (AMP effects are not inhibitable by fumarate or L-aspartate, whereas AcCoA effects are [11]) lead to the conclusion that there are different binding sites for nucleotides and the other activators, as has been found for the enzyme from other bacteria [19–21]. A model, based on a combination of the multiconformational concept [22] and the Monod model [12] for allosteric enzymes, that takes into consideration all these results is provided and discussed elsewhere (in preparation).

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