ACTIVATION OF GLUTAMATE DEHYDROGENASE BY 3′, 5′-CYCLIC ADENOSINE MONOPHOSPHATE

M. S. SETCHENSKA, J. G. VASSILEVA-POPOVA and E. M. RUSSANOV

Central Laboratory of Biophysics and Institute of Physiology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

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1. Introduction

Glutamate dehydrogenase (EC 1.4.1.3) is an allosteric protein with a large number of powerful catalytic modifiers of diverse nature, e.g. nucleotides (GTP, ADP, ATP) [1,2], metal ions (Zn^{2+}) [3], steroids (diethylstilbestrol) [4], amino acids (leucine, isoleucine, methionine, norvaline) [5]. The nucleotides are the most interesting allosteric effectors because of their opposite effects. ADP is an activator [1,6,7] while GTP is an inhibitor [1,2,6] of both NAD(P)H oxidation and $NAD(P)^+$ reduction. ATP inhibits the NADH oxidation, does not influence the NADPH oxidation and activates the NAD^+ and $NADP^+$ reduction [1,6]. 5′-AMP activates and 5′-GMP inhibits both the forward and backward reactions with the two cofactors [6].

In the present study we have found that $5 \cdot 10^{-4}$ M cAMP activates the reductive amination of oxoglutarate about two-fold with NADH and three-fold with NADPH and does not effect the oxidative deamination of glutamate with both NAD and NADP. Dibutyryl-cAMP inhibits glutamate dehydrogenase, which is probably due to the butyric moiety of the molecule, because the free butyric acid has an inhibitory effect. Cyclic 3′, 5′-guanosine monophosphate (cGMP) also inhibits the enzyme.

2. Materials and methods

Crystalline bovine liver glutamate dehydrogenase was obtained from Boehringer whereas mitochondrial extracts from rat liver, brain and testis were used as crude enzyme preparations. A stock solution of 200 μg/ml of Boehringer glutamate dehydrogenase was prepared in Tris–HCl buffer, pH 7.6, from which 0.010 ml aliquots were taken per 2 ml reaction mixture. Mitochondria from rat liver were prepared according to Schneider [8], from rat brain by the method of Whittaker and Barker [9] and from rat testis according to Sulimovici [10]. The particles were disrupted with 0.5% Triton X-100 and then centrifuged at 140 000 *g* for 1 h and the resulting supernatant was used for glutamate dehydrogenase activity estimation. The reaction mixture (final vol 2 ml) for determination of enzyme activity in the reductive direction consisted of 50 mM Tris–HCl buffer, pH 7.6, 5 mM oxoglutarate, 0.1 mM NAD(P)H, 50 mM NH_4Cl and the enzyme; in the oxidative direction it consisted of 50 mM Tris–HCl buffer, pH 7.6, 2.5 mM glutamate, 1 mM $NADP^+$ and the enzyme. Cyclic AMP and cyclic GMP (free acids) were dissolved in the assay mixture buffer, dibutyryl-cAMP in distilled water. These substances were added to the reaction mixture at the concentrations shown in the figs. and tables. The changes in the optical density at 340 nm were measured at 25°C in a cell with 1 cm light path at 30 sec intervals. The specific activity of the enzyme was expressed as μmoles NAD(P)H oxidized or $NAD(P)^+$ reduced per min per mg protein.

cAMP, cGMP, dibutyryl-cAMP, Na-oxoglutarate, glutamate, NAD, NADP, NADH, NADPH and Tris were from Boehringer; NH_4Cl was from Merck.

3. Results

Fig.1 shows that cAMP enhanced the activity of crystalline bovine liver glutamate dehydrogenase with

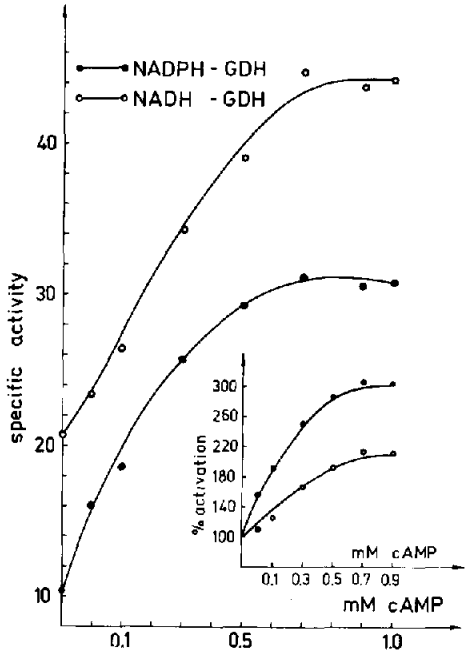

Fig.1. Cyclic AMP concentration-dependent activation of bovine liver glutamate dehydrogenase. See text for details.

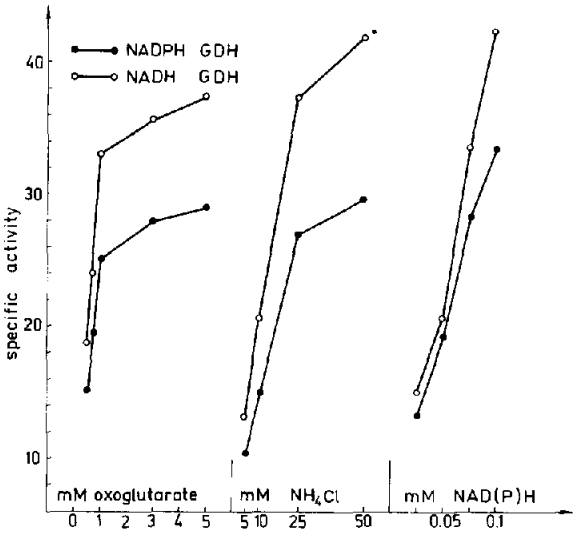

Fig.2. Activation of bovine liver glutamate dehydrogenase by cAMP in relation to the substrate concentrations. See text for details.

oxoglutarate, NH_4 and NAD(P)H as substrates. The NADPH-dependent reaction was more sensitive to cAMP than the NADH-dependent one: a 50% activation was observed at $5 \cdot 10^{-5}$ M cAMP with NADPH and at $2 \cdot 10^{-4}$ M cAMP with NADH. At $5 \cdot 10^{-4}$ M, cAMP activated the reductive amination of oxoglutarate with NADH about two-fold and with NADPH about three-fold. The saturation plateau is between $7 \cdot 10^{-4}$ and $1 \cdot 10^{-3}$ M. The activation was independent of the incubation time.

The oxidative deamination of glutamate was not influenced by cAMP even at 1 mM (5.9 μmol NADP and 8.8 μmol NAD reduced per min per mg protein in comparison with 7.0 μmol and 9.2 μmol, respectively, in the presence of cAMP).

The activation by cAMP increased with the saturation of the enzyme with the substrates: oxoglutarate, NH_4^+ and NAD(P)H (Fig.2). We did not find any pH dependence (between 7.0 and 9.0) for the activation of glutamate dehydrogenase by cAMP (results not shown here). Only at pH 9.0, when the enzyme activity was very low, did the extent of activation at 0.8 mM cAMP rise to 430 and 330% for the NADPH- and NADH-dependent reactions, respectively.

At 0.5 mM, dibutyryl-cAMP (table 1) inhibited the reaction with NADPH and NADH to the extent of 35 and 80%, respectively. At 0.8 mM, cAMP restored the NADPH-linked activity up to the control levels, but not the NADH-linked activity. This inhibition was probably due to the butyric moiety of the molecule because free butyric acid itself at equivalent concentrations had an inhibitory effect. Cyclic GMP at 0.5 mM also inhibited the enzyme by 53 and 31% for NADPH- and NADH-dependent glutamate dehydrogenase, respectively (table 1). When 0.8 mM cAMP were added to the cGMP-inhibited enzyme the activity was enhanced as if cGMP were not present in the reaction mixture.

We also tested the effect of cAMP on glutamate dehydrogenase in mitochondrial matrix from rat liver, brain and testis (table 2). In all mitochondrial preparations cAMP activated glutamate dehydrogenase, 2.5- to 3-fold with NADH and 6- to 7-fold with NADPH. This confirmed the results obtained with the crystallyne enzyme that the NADPH-linked reaction was more sensitive than the NADH one. The oxidative deamination of glutamate with mitochondrial enzyme was also unaffected by cAMP.

Table 1
Influence of cAMP, dibutyryl-cAMP and cGMP on bovine liver glutamate dehydrogenase activity

Additions	Glutamate dehydrogenase NADPH-dependent		NADH-dependent	
	Specific activity	% activity	Specific activity	% activity
1. Control (without additions)	10.3	100	20.9	100
2. + 0.8 mM cAMP	29.3	284	39.1	187
3. + 0.5 mM Dibutyryl-cAMP	6.7	66	4.0	19
4. + 0.5 mM Dibutyryl-cAMP + 0.8 mM cAMP	10.6	103	12.4	59
5. + 0.5 mM Butyric acid	4.0	40	5.8	24
6. + 0.5 mM cGMP	4.8	47	14.5	69
7. + 0.5 mM cGMP + 0.8 mM cAMP	30.7	298	38.6	185

Protein content was determined by the standard Lowry procedure. Other details in text.

4. Discussion

It is well known that cAMP stimulates the activity of a number of enzymes such as muscle phosphorylase, epididymal fat pad lipase, phosphofructokinase and tyrosine aminotransferase, whereas it inhibits fructose 1,6-diphosphatase and glycogen synthetase [11]. We now find that 3′,5′-cyclic AMP activates in vitro both purified bovine liver glutamate dehydrogenase and the enzyme from rat liver, brain and testis mitochondrial preparations. However the concentrations of cAMP which affect glutamate dehydrogenase are much higher than those which occur in vivo. In this respect the physiological meaning of the phenomenon observed is not clear, but it is of interest from the enzymological point of view.

It is established that bovine liver glutamate dehydrogenase is an allosteric protein, being a tetramer with a mol. wt of 1 000 000 [3,12,13]. Upon dilution [14] and at high concentration of NADH [15] the enzyme dissociates to subunits with a molecular weight of 250 000. Frieden [15] has suggested that the allosteric effectors influenced the catalytic activity by changing the state of aggregation. It might be assumed that cAMP activates the enzyme in the same manner by altering the equilibrium between monomers and associated forms. At the same time, since cAMP activates only the reductive amination of oxoglutarate, it is possible that the enzyme–cAMP complex becomes the 'active enzyme' for oxoglutarate, NH_4^+ and NAD(P)H, but not for glutamate and $NAD(P)^+$, thus facilitating the formation of the enzyme–substrate complex at the substrate saturation concentrations. It is not surprising to find that cGMP has an inhibitory effect on account of the mutually opposite action of the two cyclic nucleotides. The fact that higher con-

Table 2
Activation of rat liver, brain and testis mitochondrial matrix glutamate dehydrogenase by cAMP

Mitochondrial matrix from	Additions	Glutamate dehydrogenase NADPH-dependent		NADH-dependent	
		Specific activity	% activity	Specific activity	% activity
Rat liver	None	0.22	100	0.03	100
	+ 1 mM cAMP	0.69	315	0.23	706
Rat brain	None	0.14	100	0.02	100
	+ 1 mM cAMP	0.34	303	0.13	670
Rat testes	None	0.010	100	0.001	–
	+ 1 mM cAMP	0.025	250	0.006	–

centrations of cAMP overcome cGMP inhibition probably shows a competition for the same binding site.

It is well known that adenyl cyclase is a component of the plasma membrane, but in some tissues this enzyme was shown to be bound to other intracellular membranes. In the brain it is localized predominantly in the microsomal and mitochondrial fractions [16]; in dog [17] and rat [18] testes it is also bound to the mitochondrial membranes. Sulimovici and Lunenfeld [19] have shown that both outer and inner rat testis mitochondrial membranes contain adenyl cyclase activity. This was the reason for testing the effect of cAMP in mitochondrial glutamate dehydrogenase from rat brain and testis tissues. The question of glutamate dehydrogenase activation by cAMP in the liver mitochondria is now open, because there are no data about adenyl cyclase activity of their membranes.

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