Allosteric regulation of NAD(NADP)-dependent glyceraldehyde-3-phosphate dehydrogenase from *Chlorella* by α-amino acids, dithiothreitol and ATP

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α-Amino acids (glycine, serine, histidine, aspartic acid and cysteine) and dithiothreitol (DTT) have been shown to activate both activities of the NAD(NADP)-dependent glyceraldehyde-3-phosphate dehydrogenase from *Chlorella*. The activation is allosteric and reaches 200–700%. The Hill coefficient values are close to 2 with all activators. ATP activates NADP-dependent but inhibits NAD-dependent activity, n_{app} and K values being the same for both enzyme activities. In this case positive cooperativity is also observed (n_{app} = 2.2). The present findings reveal the possible regulation of GAPD function in *Chlorella* with each of the coenzymes.

Allosteric regulation *Glyceraldehyde-3-phosphate dehydrogenase* Chlorella *α-Amino acid* *Dithiothreitol* *ATP*

1. INTRODUCTION

In photosynthetic tissues GAPD has two major functions. In addition to the central catalytic role in both carbohydrate breakdown by the glycolytic pathway and CO_2 assimilation by the Calvin cycle, the enzyme appears also to have an essential regulatory function. Thus, chloroplast GAPD is shown to be activated by light [1–3], as well as by some metabolites such as NADPH [4], ATP [5,6], 1,3-diphosphoglycerate [7]. Previous experiments in our laboratory indicate that α-amino acids (but not β- and γ-amino acids) also activate the enzyme in cell-free extracts from *Chlorella* [8], *Scenedesmus* and spinach leaves [9]. The activating effect of the amino acids has been proved with pig muscle GAPD in both directions, NAD^+ reduction [10,11] and NADH oxidation [12]. Other compounds such as DTT, EDTA, *o*-phenanthroline which, similarly to the α-amino acids contain heteroatoms (N, S or O) at two adjacent carbon atoms, are also activators of the enzyme [10,12,13].

In this work the effect of selected α-amino acids on purified NAD(NADP)-dependent GAPD from *Chlorella* has been studied and compared with those of DTT and ATP.

2. MATERIALS AND METHODS

NAD(NADP)-dependent GAPD was purified from *Chlorella pyrenoidosa* as in [14].

Enzyme activity was measured spectrophotometrically at 340 nm by oxidation of NADH in the following assay mixture: 25 mM Hepes–NaOH (pH 7.5), 3.5 mM 3-phosphoglycerate, 3.5 mM ATP, 10 mM $MgCl_2$, 0.125 mM NADH or NADPH, 2 units/ml 3-phosphoglycerate kinase

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Abbreviations: GAPD, D-glyceraldehyde-3-phosphate dehydrogenase; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; DTT, dithiothreitol

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and 9.4×10^{-8} M GAPD. The compounds under investigation were added to the assay mixture in varying concentrations and the enzyme was preincubated for 5 min at 25°C. The reaction was started by adding 3-phosphoglycerate kinase and ATP.

The dependence of enzyme activity on the concentration of each ligand was analyzed analogously to [5], assuming

$$\log \frac{\bar{Y}}{1-\bar{Y}} = \log K + n\log[L]$$

where L = ligand; K = apparent overall enzyme ligand association constant; and n = apparent Hill coefficient. The saturation function, $\bar{Y}$, is taken as the relative change of the enzyme activity at any point of the curve $\bar{Y} = (a - a_{min})/(a_{max} - a_{min})$, where a_{min}, a, and a_{max} denote enzyme activity without ligand, at any ligand concentration, and at saturation, respectively. Values of the apparent K and n of each ligand were determined from the plot of $\log \bar{Y}/(1 - \bar{Y})$ *vs* log[L].

3. RESULTS AND DISCUSSION

We here chose α-amino acids from different groups, namely glycine, serine, aspartic acid, histidine and cysteine. In accordance with our previous findings [8–10], all the amino acids activate both NAD-dependent and NADP-dependent

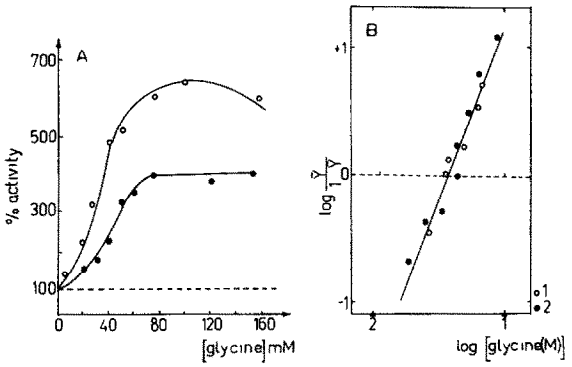

Fig.1. Dependence of NAD- and NADP-linked activities of GAPD on the concentration of glycine (A) and Hill representation of the data (B). (○, 1) NAD-linked activity, (●, 2) NADP-linked activity.

activity of GAPD from *Chlorella*. With both coenzymes sigmoidal curves of activation depending on the concentration of each amino acid were obtained. With glycine, cysteine and histidine the degree of activation of NAD-dependent activity is much higher than that of NADP-linked activity (fig.1 and table 1), while with aspartic acid and serine the activation of the reactions with both coenzymes is described by a common curve (fig.2). Nevertheless, in all cases by the Hill representation of the data (fig.1B,2B) a common straight line for both enzyme activities was obtained, which suggests that each amino acid is bound to the same

Table 1

Effect of α-amino acids, DTT and ATP on the NAD- and NADP-dependent activities of GAPD

Effector	Maximum of activation (inhibition)			n_{app}	K_{app} ($1/M^n$)
	At concentration (M)	Percent			
		NAD	NADP		
Glycine	8×10^{-2}	600	400	2.1	1.8×10^5
Serine	7×10^{-2}	250	250	2.2	2.3×10^5
Aspartic acid	6×10^{-2}	240	240	2.1	1.1×10^5
Cysteine	5×10^{-3}	700	450	2.2	2.7×10^7
Histidine	7×10^{-3}	650	300	2.3	1.1×10^7
DTT	8×10^{-3}	230	200	1.7	5.1×10^5
ATP	2×10^{-3}	50	160	2.2	7.2×10^6

NAD and NADP denote the enzyme activity with each coenzyme

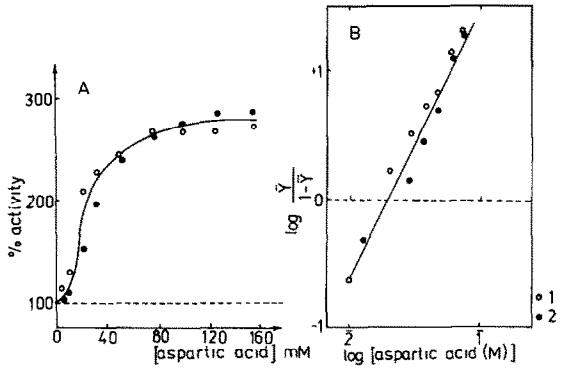

Fig.2. Dependence of NAD- and NADP-linked activities of GAPD on the concentration of aspartic acid (A) and Hill representation of the data (B). (○, 1) NAD-linked activity, (●, 2) NADP-linked activity.

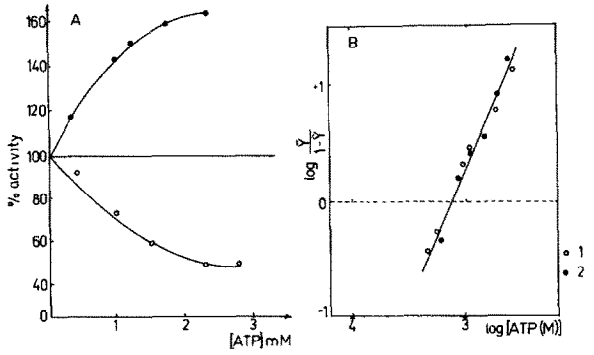

Fig.3. Dependence of NAD- and NADP-linked activities of GAPD on the concentration of ATP (A) and Hill representation of the data (B). (○, 1) NAD-linked activity, (●, 2) NADP-linked activity.

binding site activating either of the two reactions. With DTT the activation pattern is the same, the maximum of activation being about 200% for the NAD- and the NADP-dependent reactions (table 1).

As judged from the apparent K values obtained, the affinity of the enzyme towards cysteine and histidine is considerably higher compared to the other amino acids. The values of the apparent Hill coefficient determined for all amino acids and DTT are nearly 2 which reveals the allosteric character of the activation. It is noteworthy that in a similar analysis of the activating effect of amino acids on pig muscle GAPD gave apparent Hill coefficients equal to unity [11,12]. This indicates an essential difference between both enzymes in their activation by amino acids.

As mentioned above, other compounds with a similar structure containing at least two heteroatoms at adjacent carbon atoms also activate the enzyme [10,12], not due to their protective effect on the essential SH-groups. With pig muscle GAPD we found that EDTA, *o*-phenanthroline and aspartic acid, while activating both dehydrogenase [10,12] and diaphorase [16] reactions of GAPD, inhibit the phosphatase reaction [17], the deacylating step of the esterase reaction [18] and the arsenolysis of the *S*-acyl-enzyme (unpublished). The binding of these ligands to the enzyme is found to be in the active centre region close to Cys-153, as proved by difference spectroscopy and selective chemical modifications [19,20]. The binding of DTT to lobster muscle GAPD has been confirmed by crystallographic data [21].

ATP is also an allosteric effector of *Chlorella* GAPD. It activates the NADP-dependent but inhibits the NAD-dependent activity (fig.3) and the data from both curves in Hill coordinates fitted one straight line with a Hill coefficient equal to 2.2. This also demonstrates cooperative enzyme–ligand interaction. Since GAPD from *Chlorella* is a tetramer of 160 kDa and its activities with both coenzymes are nearly equal [14], it is hardly likely that the effect of ATP is to be explained by depolymerization of the molecule, as observed with higher-plant GAPD of 600 kDa by NADP, NADPH or ATP [22,23]. The *Chlorella* enzyme seems to possess separate sites for binding the coenzymes [24,25] located probably in different parts of the molecule even in different subunits or dimers inside the tetramer. It is likely that ATP, bound at a regulatory (allosteric) centre, affects these sites in a different manner.

Our results reveal the possible regulation of GAPD function in *Chlorella* by some important metabolites with each of the coenzymes and characterize NAD(NADP)-dependent GAPD as an allosteric enzyme.

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