ARSENATE INDUCED ACTIVITY OF CERTAIN ENZYMES ON THEIR DEPHOSPHORYLATED SUBSTRATES

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Enzymes whose main substrates are phosphorylated sugars generally show little or no activity on their corresponding non-phosphorylated substrates [1,2]. Arsenate is known to replace phosphate in many phosphorolytic reactions [3]. An exploration was undertaken of possible activations by phosphate and/or arsenate of reactions of non-phosphorylated substrates. We found that arsenate, but not phosphate, either induces or greatly enhances enzyme activity on non-phosphorylated substrates in all cases examined, viz, an aldolase, two dehydrogenases, an isomerase, and a kinase. This activation may involve the spontaneous formation of sugararsenate compounds with properties mimicking those of the corresponding phosphoric esters.

The experimental conditions and main results are summarized in table 1. Enzyme activity on the non-phosphorylated substrates was measured spectrophotometrically by coupling to the reduction or oxidation of an appropriate pyridine nucleotide or chromogen. This was done directly in the case of dehydrogenases or through auxiliary enzymes able to couple a primary product to a dehydrogenase or peroxidase in the other cases.

After preliminary observations indicating that arsenate dependent activities increased with the concentration of both the arsenate and the non-phosphorylated substrate, concentrations of 0.1 M and 0.2 M, respectively, were routinely adopted whenever feasible, except for monophosphorylated derivatives of a diphosphorylated substrate.

No enhancement by inorganic phosphate specific for the activity on a non-phosphorylated substrate was observed, either at 0.1 M (table 1) or at lower or higher concentrations that were occasionally tested. In certain cases both phosphate and arsenate ions behaved as competitive inhibitors of the activity of the enzymes on their normal substrates. The nearly first order kinetics of the arsenate induction (or enhancement) of activity on the non-phosphorylated substrate analogues stands in contrast with apparent Ki values of arsenate (as well as of phosphate) for aldolase and glucosephosphate isomerase of about 1 and 10 mM, respectively.

Glucose-6-phosphate dehydrogenase has a small spontaneous activity on xylose similar to that on glucose. The activity on xylose is not significantly affected by either arsenate or phosphate (table 1). Furthermore, arsenate does not elicit ketose formation from xylose by glucosephosphate isomerase in contrast with its strong induction of ketose formation from glucose. These observations suggest that the inducing effects of arsenate may involve an interaction of the arsenate with the sugar giving an ester-like intermediate which, like the corresponding phosphoric ester, can bind specifically to the enzyme in a form susceptible to catalytic attack. A similar compound of arsenate with xylose would likely not be a suitable analogue. Transient formation of highly unstable arsenate esters of sugars has been demonstrated in phosphorolytic reactions [3]. Direct support for this hypothesis might be obtained with use of ^{18}O-labelled arsenate.

An arsenate dependent glucose isomerase of bacterial origin has been tentatively identified as a glucosephosphate isomerase by Natake [5]. This observation has been extended to glucosephosphate isomerase from peas [6]. We have also observed an arsenate dependent fructose to glucose isomerizing activity in skeletal muscle extracts, presumably ascribable to the high glucosephosphate isomerase content of this tissue. Arsenate dependent activity on free hexoses would then appear to be a feature common

Table 1
Arsenate-induced activity of certain enzymes on their dephosphorylated substrates.

Enzyme	Substrate	Auxiliary system	Control	Relative activities + phosphate 0.1 M	+ arsenate 0.1 M	Arsenate activation, times
FDP aldolase	FDP, 1 mM	TPI, αGPDH, DPNH	"100"	40	50	
	F1P, 20 mM	TPI, αGPDH, DPNH	4		0.7	
	F6P, 20 mM	TPI, αGPDH, DPNH	< 0.01		0.14	> 14
Glucose-6-phosphate dehydrogenase	G6P, 1 mM	TPN	"100"	150	180	
	Glucose, 0.2 M	TPN	0.12	0.44	1.9	16
	Xylose, 0.2 M	TPN	0.18	0.20	0.20	
Glucosephosphate isomerase	F6P, 1 mM	G6PDH, TPN	"100"	20	20	
	Fructose, 0.2 M	GO, PO, o-dianisidine	< 0.005	< 0.005	0.17	> 34
α-Glycerophosphate dehydrogenase	DHA-P, 1 mM	DPNH	"100"	280	330	
	DHA, 20 mM	DPNH	< 0.01	< 0.01	1.7	> 170
1-Phosphofructokinase	F1P, 1 mM	MgATP, ALD, TPI, αGPDH, DPNH	"100"	60	60	
	Fructose, 0.2 M	MgATP, GPI, G6PDH, TPN	< 0.01	< 0.01	10	> 1000

0.05 M imidazole, pH 7.0, was present in all cases. Arsenate and phosphate were used as sodium salts, adjusted to pH 7.0.

The enzymes were highly purified commercial preparations, as indicated below, except for the amebal 1-phosphofructokinase [4] that was supplied by Prof. R.E.Reeves. Muscle aldolase (ALD), glucose oxidase (GO), yeast glucose-6-phosphate dehydrogenase (G6PDH), and yeast glucosephosphate isomerase (GPI) were from Boehringer; muscle α-glycerophosphate dehydrogenase (αGPDH)-triosephosphate isomerase (TPI) was from Sigma; peroxidase (PO) was from Worthington. Main enzymes were generally used at 10 milliunits and 1 unit per ml for the phosphorylated and dephosphorylated substrates, respectively. Auxiliary enzymes were used at 0.2 to 1 unit per ml, except in the case of GO and PO that were used at 100 μg and 5 μg per ml respectively.

Coenzymes were used at 1 mM concentration (with 5 mM $MgCl_2$ in the case of kinase), except DPNH that was used at an initial concentration of 0.12 mM. o-Dianisidine was used at 50 μg per ml, with 0.2% Triton-X 100 (Rohm and Haas) and 5 mM EDTA.

Other non-standard abbreviations used are: FDP, fructose-1,6-diphosphate; F1P, fructose-1-phosphate; F6P, fructose-6-phosphate; G6P, glucose-6-phosphate; DHA-P, dihydroxyacetone-phosphate; DHA, dihydroxyacetone.

Enzyme activities were measured at 340 mμ when the reactions were coupled to a pyridine nucleotide, and at 420 mμ in the case coupled to o-dianisidine. They were referred to moles of substrate transformed per unit time and equal amounts of enzyme.

to glucosephosphate isomerases irrespective of their origin.

Fructosediphosphate aldolase is a particularly complex case, since its main substrate is a twice phosphorylated sugar. Muscle aldolase is known to be able to attack slowly fructose-1-phosphate [1]. Arsenate does not increase the activity of aldolase on fructose-1-phosphate, but it does elicit *de novo* a marked activity on fructose-6-phosphate.

All enzymes examined showed arsenate dependent activity on non-phosphorylated substrates one or several orders of magnitude greater than in the absence of arsenate. The generality of the observed phenomena suggests that other enzymes acting on phosphorylated sugars, and perhaps even on certain other types of phosphorylated compounds, might respond to arsenate in a similar way, i.e., might exhibit an arsenate dependent activity on non-phosphorylated substrates.

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