THE ROLE OF P_i IN THE CONTROL OF GLYCOLYSIS IN ASCITES TUMOR CELLS

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Intracellular P_i has been shown to be an important factor in the control of glycolysis in Ehrlich ascites tumor cells (1-5). Glyceraldehyde-3-P dehydrogenase was then the only glycolytic enzyme known to be influenced by P_i. However, recent reports have indicated that P_i may have other important effects on glycolytic systems. Thus, in cell-free systems, Passonneau and Lowry (6) as well as Mansour (7) showed that P_i may activate phosphofructokinase by counteracting the inhibitory effect of high ATP concentrations; Rose et al (8) have reported that P_i may partially relieve the G-6-P inhibition of hexokinase in red blood cells. This information has been applied to the examination of intact ascites tumor cells in order to define more precisely the point of action of intracellular P_i in the control of glycolysis.

METHODS

The handling of the Ehrlich tetraploid ascites tumor cells, and the method of incubation and preparation of samples were the same as previously described (2, 12).

^{14}C-glucose uptake was determined by the method of Rose and O'Connell (9), modified as follows: 0.4 ml. of the neutralized sample was diluted twenty-fold with water and passed through a Dowex-1-chloride column (1 x 3

Abbreviations: G-6-P, glucose 6-phosphate; 3-PGA, 3-phosphoglyceric acid; HMP, hexose monophosphates; FDP, fructose 1,6-diphosphate; TP, triose phosphates.

cm.); after washing the column with 20 ml. of water followed by 30 ml. of 1 mM glucose solution, the counts retained by the column were eluted with 15 ml. of 0.5 N HCl. The eluate was neutralized, 1 ml. was added to 19 ml. of counting fluid, and the sample was counted in a scintillation counter. The recovery of the counts in each sample was determined subsequently by the addition of a known amount of ^{14}C-glucose solution, and the proper corrections applied (10). 3-PGA was measured fluorimetrically by following the disappearance of DPNH, employing the conditions described for a spectrophotometric assay (11). Procedures for the measurements of glycolytic intermediates and adenine nucleotides, for the determination of ^{14}C-lactate, and the colorimetric assay of lactate have been described previously (12).

RESULTS AND DISCUSSION

The participation of intracellular P_i in the control of glycolysis was examined by following the effects of inosine, imidazole, or high concentrations of P_i on the levels of intracellular intermediates. Results in Table I show that with increasing levels of P_i in the medium, the stimulation of both aerobic and anaerobic glycolysis paralleled a decrease in intracellular HMP and an increase in FDP levels, indicating that phosphofructokinase activity was accelerated. Stimulation of aerobic glycolysis by imidazole produced similar changes. An observed increase in glucose uptake (not shown here), which parallels the production of lactate, might be due to the lowering of the G-6-P level through faster utilization of HMP by phosphofructokinase. The direct effect of P_i, in increasing the K_i of G-6-P for hexokinase (8), may also be partly responsible for the faster rate of glucose uptake.

The presence of high concentrations of P_i also doubled the level of 3-PGA, in spite of the fact that the conversion of 3-PGA to lactate was also accelerated. The increase in the concentration of 3-PGA was due to the stimulation of glyceraldehyde 3-P dehydrogenase. The increased activity of this enzyme may be due to a direct stimulation of P_i, or an indirect

Table I

EFFECT OF INOSINE, IMIDAZOLE, AND HIGH LEVELS OF P_i

ON INTRACELLULAR INTERMEDIATES AND GLYCOLYSIS

Gas phase	P_i	Inosine	Imidazole	Total lactate prod.	Intracellular conc.					
					HMP	FDP	TP	3PGA	ATP	AMP
	(mM)	(mM)	(mM)							
Air	4			39	1.18	0.14	0.34	0.05	3.8	0.15
	25			58	0.92	0.50	0.60	0.10		
	40			64	0.90	0.95	0.77	0.11	4.3	0.20
	4		45	78	0.74	1.16	0.50		3.6	0.19
	4	3		30	1.52	0.07	0.20	0.026	5.0	0.20
N_2	4			103	0.60	0.60	0.66		3.5	0.13
	25			126	0.46	0.94	0.72			
	40			130	0.47	1.24	0.92		3.2	0.22
	4		45	136	0.50	2.34	1.22		2.5	0.28

Ascites tumor cells (20 mg. of cell protein) were incubated in 2 ml. of buffered medium as previously described (2). After 12 minutes of gassing, 12 μmoles of glucose were tipped from the double sidearm of the Warburg vessel into the main compartment. At the end of 30 minutes of incubation, 0.1 ml. of 10 N $HClO_4$ was tipped from the single sidearm into the main compartment to terminate the experiments. Lactate production is expressed as μmoles per ml. of packed cells (140 mg. of cell protein), and intracellular concentrations are expressed as μmoles per ml. of intracellular water (12).

effect of P_i in stimulating phosphofructokinase and increasing glyceraldehyde 3-P concentration, or a combination of both. In contrast, addition of inosine resulted in the lowering of P_i and 3-PGA, indicating that glyceralde hyde 3-P dehydrogenase was inhibited.

The effect of inosine and high concentrations of P_i on glycolysis was next traced in short-term experiments to determine the sequence of events. Under aerobic conditions (Table II), the presence of inosine during the pre-

Table II

EFFECT OF HIGH P_i OR INOSINE

ON THE KINETICS OF GLYCOLYSIS AND THE LEVELS OF INTRACELLULAR INTERMEDIATES

Addition	Conc.	Incubation time	CO_2-O_2						CO_2-N_2					
			^{14}C-glucose uptake	^{14}C-lactate prod.	Intracellular conc. HMP	FDP	TP	ATP	^{14}C-glucose uptake	^{14}C-lactate prod.	Intracellular conc. HMP	FDP	TP	ATP
	(mM)	(min.)												
		0			0.12			3.1		0	0.01			0.20
		1	2.3	2.6	1.4	0.50	0.40	3.2	3.0	3.2	0.30	0.8	0.50	0.70
		3	3.0	4.4	1.3	0.12	0.19	3.0	6.1	12.0	0.63	1.8	0.92	1.7
		10	5.4	9.5	1.0	0.09	0.14	3.1		31	0.51	0.6	0.40	2.0
Inosine	3	0			0.48	0.02	0.08	3.6		0	0.03	0.03	0	0.60
		1	1.2	1.1	1.7	0.10	0.06	3.4	4.4	5.0	0.42	1.2	0.70	1.0
		3	1.8	2.1	1.6	0.05		3.4	6.6	13	0.72	1.6	1.0	1.9
		10	3.6	5.3	1.4	0.05	0.05	3.5		29	0.51	0.6	0.45	2.3
P_i	38	0			0.16		0	3.2		0	0		0.04	0.24
		1	2.7	3.4	0.8	0.64	0.42	2.9	3.2	3.4	0.30	0.9	0.52	0.75
		3	3.6	5.6	0.9	0.66	0.43	2.8	7.5	13	0.60	2.4	1.1	2.1
		10	6.7	12.8	0.7	0.60		2.8		34	0.45	1.4	0.75	2.1

Experimental conditions were the same as in Table I, except that when indicated, inosine (3 mM) or additional P_i (38 mM) was present during the preincubation period. After 12 minutes of preincubation, one Warburg vessel of each set of experiments was terminated with $HClO_4$ (zero time sample), while ^{14}C-glucose (5 μmoles) was tipped into the main compartment of all other vessels. Incubation in the presence of ^{14}C-glucose was terminated after 1, 3 or 10 minutes. Glucose uptake and lactate production are expressed as μmole per ml. of packed cells.

incubation resulted in a 4-fold increase in the HMP level (at zero time). Upon addition of glucose, the HMP level was elevated and continued to be higher in the presence of inosine, while glucose uptake and the levels of intracellular FDP and 3-PGA were both decreased. These observations suggest that a drop in the level of intracellular P_i (5) and an increase in ATP levels (also see Table I) were responsible for the inhibition of phosphofructokinase, thus leading to an inhibition of lactate formation on the one hand, and an increase in the HMP level and an inhibition of hexokinase on the other hand. Experiments in our laboratory (14) with a reconstructed system of partial glycolysis have revealed a marked and coordinated stimulation of phosphofructokinase and hexokinase by P_i. Of particular significance is the observation that the effect on hexokinase was demonstrable only with the mammalian enzyme, which is inhibited by G-6-P, but not with yeast hexokinase.

Addition of large amounts of P_i also resulted in an immediate and almost constant effect in stimulating aerobic glycolysis, in lowering HMP and in increasing FDP levels. The levels of FDP and TP remained high and almost constant throughout the aerobic incubations, whereas in the control experiments with low levels of P_i there was a precipitous drop in FDP and TP after the first minute.

Under anaerobic conditions, inosine stimulated glycolysis over the control during the first minute, presumably by keeping the initial level of ATP higher than that of the control. Addition of large amounts of P_i resulted in a higher FDP level than in the control.

These results are consistent with the interpretation that in the presence of a high concentration of P_i there is an immediate and continuous stimulation of phosphofructokinase under both aerobic and anaerobic conditions. These results support the observations of Lonberg-Holm (13) who has emphasized the role of phosphofructokinase in the control of aerobic glycolysis.

In conclusion, the control of aerobic glycolysis in Ehrlich ascites tumor cells has been shown to be mediated mainly through the effects of

P_i on hexokinase, phosphofructokinase, and glyceraldehyde 3-P dehydrogenase. It should be emphasized that the stimulation of these three enzymes by P_i is interrelated. Thus, a primary stimulation of phosphofructokinase would increase the levels of FDP and TP to provide more substrate for glyceraldehyde 3-P dehydrogenase, and would decrease the level of HMP to minimize the inhibition of hexokinase.

The regulation of phosphofructokinase activity may be exerted mainly through P_i or AMP, or other activators which reverse the ATP inhibition. The evidence from the experiments with inosine or high concentrations of P_i seems to indicate that in ascites tumor cells intracellular P_i is of major importance in reversing the inhibition of phosphofructokinase. In contrast, in kidney slices, inhibition of this enzyme is relieved mainly by AMP (12). It is clear that the Pasteur effect in both tissues is associated primarily with a more pronounced inhibition of hexokinase and phosphofructokinase activities under aerobic conditions than under anoxia. In ascites tumor cells, P_i appears to be the most important factor in the regulation of the activities of these two enzymes as well as in the control of the Pasteur effect.

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