CONTROL OF PHOSPHORIBOSYLPYROPHOSPHATE SYNTHESIS IN HUMAN LYMPHOCYTES

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Summary

The regulation of phosphoribosylpyrophosphate synthetase purified from human peripheral blood lymphocytes has been studied. Enzyme activity was stimulated by inorganic phosphate, IMP and GMP and inhibited by ADP and, to a lesser extent, by AMP. The results suggest that phosphoribosylpyrophosphate synthetase may play an important role in the mechanism of lymphocyte proliferation.

Introduction

Human lymphocytes are able to synthesize purines de novo (1,2) and the importance of this pathway is emphasized by the defective responses to antigenic and mitogenic stimulation observed with lymphocytes from patients with congenital defects affecting de novo purine synthesis (3). The last common step leading to both purine and pyrimidine ribonucleotides is catalyzed by phosphoribosylpyrophosphate synthetase (ATP:D-ribose 5-phosphate pyrophosphotransferase, EC 2.7.6.1). The intracellular concentration of phosphoribosylpyrophosphate[1] seems to play an important role in the regulation of the purine metabolism in microorganisms (4) as well as in lower animal cells (5) and human cells (6), both as the substrate for PribosePP[1] amidotransferase (7) and through reaction with free purine bases in salvage pathways (8). Hence the regulation of PribosePP synthesis is a problem relevant to the overall control of purine nucleotide metabolism and, as a consequence, basic to the understanding of diseases in which disorders of this pathway are involved (3).

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[1] PribosePP, phosphoribosylpyrophosphate

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PribosePP synthetase from several sources has been reported to be inhibited by some purine nucleotides (9-13). This is generally interpreted as a mechanism of feedback control exerted by the pool of nucleotide end products. The present study of the PribosePP synthetase from human lymphocytes shows some differences in the regulatory properties from those described for enzymes isolated from other sources. As with other PribosePP synthetases, we have found inhibition by ADP and under some circumstances AMP; however, the lymphocyte enzyme is activated by IMP and to a lesser extent by GMP, as well as by inorganic phosphate. The possible relevance of these observations in lymphocyte activation is discussed.

Materials and Methods

Materials: [8-^{14}C]hypoxanthine was purchased from the Radiochemical Centre, Amersham, U.K. D-ribose 5-phosphate, nucleosides and nucleotides were from Sigma Chemical Co. or BDH Chemicals Ltd. Sephadex G-25 and G-100 were obtained from Pharmacia and DEAE-cellulose DE 23 from Whatman Biochemicals Ltd. The buffy coat residues were provided by the North London Blood Transfusion Centre, Edgware, Middlesex. All other reagents were of analytical grade.

PribosePP synthetase purification: Human peripheral blood lymphocytes were obtained from buffy coats of healthy donors by a modification of the Ficoll-Hypaque technique (14). After washing them with saline, they were resuspended in the following buffer: 20 mM Tris-HCl, 5 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM sodium phosphate, 20% glycerol, pH 7.4. This buffer was used throughout the preparation. After freeze-thawing four times, the suspension was centrifuged and the 30,000xg supernatant was filtered through a Sephadex G-25 column and chromatographed on a DEAE-cellulose column. The enzyme was eluted with 0.2 M KCl. The eluted activity was measured in the presence of 10 mM sodium phosphate, pH 7.4.

HGPRTase[2] preparation: HGPRTase was purified from human erythrocytes (15) up to the Sephadex G-100 filtration step. No PribosePP synthetase activity was detected.

Assay of PribosePP synthetase activity: The mixtures contained, in a total volume of 0.1 ml, 40 mM Tris-HCl buffer, pH 7.4, 6 mM $MgCl_2$, 0.5 mM ribose-5-phosphate, 0.1 mM [8-^{14}C]hypoxanthine (20 μCi/μmol), sodium phosphate buffer, pH 7.4, as indicated in each case, ATP at the concentrations specified, PribosePP synthetase preparation and excess of HGPRTase so as to convert not less than 100 pmoles of PRPP to IMP per minute. The reactions were carried out at 37°C for various times and stopped by addition of 5 μmoles of EDTA and 0.5 μmoles of IMP. 50 μl aliquots were subjected to electrophoresis on Whatman 3MM paper in 0.02 M sodium lactate, pH 3.6, at 6 kV for 30 minutes. The IMP spots were located under U.V. light, cut out and counted for radioactivity in a liquid scintillation spectrometer. In

[2] HGPRTase, hypoxanthine-guanine phosphoribosyltransferase

all the cases time courses were plotted and initial velocities of PribosePP formation were calculated.

Results

PribosePP synthetase from human lymphocytes was inhibited by 1 mM but not by 0.1 mM AMP (Table 1, Expt.1). ADP proved to be a strong inhibitor at low ATP concentrations, whereas at saturating ATP the effect was observed only with 1 mM concentration. Sometimes the inhibition by AMP was not even observed at a high concentration (Table 1, Expt.2), although ADP inhibited the same enzyme preparation as usual. This raises the possibility that AMP does not act by itself but through conversion to ADP by AMP kinase which is eliminated during the purification procedure. This assumption is supported by the fact that using a less purified PribosePP synthetase, i.e. a Sephadex G-25 eluate (see Methods), the inhibition by 0.1 and 1.0 mM AMP was 29 and 85% respectively, at saturating ATP concentrations. As ATP concentrations were lowered the inhibition by AMP considerably increased. Another possible explanation for the purification-dependent effect of AMP is loss of enzyme sensitivity to inhibition during the purification procedure. On the other hand, ADP is an activator of PribosePP formation with many non-purified enzyme preparations, possibly through inhibition of ATPases that consume one of the substrates for PribosePP synthetase.

Adenosine had no effect on PribosePP synthetase activity (Table 1, Expt.1) in concentrations from 10^{-6}M to 10^{-3}M, even when used with non-DEAE cellulose-purified enzyme preparations.

The most remarkable finding was considerable stimulation of PribosePP synthetase activity by IMP (Table 2, Expt.1). PribosePP formation was relatively more enhanced at low ATP and Pi concentrations (Table 2, Expt.2). Pi by itself activates PribosePP synthetase (Tables 2 and 3). The effect of IMP was dependent on the presence of ribose 5-phosphate in the incubation mixture, excluding the possibility of IMP pyrophosphorylase activity being responsible for the increased PribosePP formation. Inosine and hypoxanthine

Table 1 : Effect of adenosine and adenosine phosphates

Expt.	Additions	PribosePP synthetase activity (pmoles PribosePP/min)		
		50 mM Pi		
		0.009 mM ATP	0.015 mM ATP	0.300 mM ATP
1	None	3.1		12.9
	1 mM Adenosine	2.8		11.8
	0.1 mM AMP	3.4		12.7
	1 mM AMP	1.6 (48%)		4.6 (64%)
	0.1 mM ADP	0.3 (90%)		13.6
	1 mM ADP	0.3 (90%)		1.3 (90%)
2	None		5.3	11.2
	0.1 mM AMP		5.2	10.7
	1 mM AMP		6.1	12.4
	0.1 mM ADP		0.6 (89%)	8.2 (27%)
	1 mM ADP		0.3 (94%)	1.1 (90%)

The figures in parenthesis are percentages of inhibition of enzyme activity.

had no significative effects on PribosePP synthetase activity.

Regarding guanine nucleosides and nucleotides (Table 3), GMP was found to activate PribosePP synthetase better at low ATP and Pi levels. GDP had a much smaller effect, leaving GDP to GMP conversion as a possible explanation. Cyclic GMP did not activate PribosePP synthetase at all even in the presence of theophylline. It had no effect in concentrations within the range 10^{-6}- 10^{-4}M, at low ATP levels, using purified or non-purified enzyme preparations from resting or phytohemagglutinin-stimulated lymphocytes. Guanosine was inhibitory, presumably because of its partial degradation to guanine and interference with [^{14}C]IMP formation from [^{14}C]hypoxanthine. GMP could stimulate through conversion to IMP: some experiments showed a greater effect of IMP than of GMP using the same enzyme preparation at the same time.

Discussion

Our experimental findings on the regulation of the PribosePP synthetase activity

Table 2 : Effect of inosine monophosphate, inosine and hypoxanthine

Expt.	Additions	PribosePP synthetase activity (pmoles PribosePP/min)				
		0.2 mM Pi		10 mM Pi		50 mM Pi
		0.015 mM ATP	0.180 mM ATP	0.015 mM ATP	0.180 mM ATP	0.009 mM ATP
1*	None					3.0
	1 mM Inosine					4.4 (+ 47%)
	1 mM IMP					10.8 (+260%)
	1 mM Hypoxanthine					3.9 (+ 30%)
2	None	0.3	0.6	2.2	4.3	
	0.1 mM IMP	2.7 (+800%)	3.8 (+530%)	8.0 (+264%)	9.5 (+121%)	

* The PribosePP synthetase assay was performed in two stages in Expt. 1. The first incubation was carried out as described in Methods but in the absence of [^{14}C]hypoxanthine and HGPRTase. The reactions were stopped by adding 1 μmol EDTA and heating at 100°C during 1 minute. Activated charcoal was then added and the tubes were left 30 minutes at 0°C and centrifuged. The PribosePP content of the supernatants was determined by adding HGPRTase, [8-^{14}C]hypoxanthine and $MgCl_2$ in the usual amounts.

The figures in parenthesis indicate the percentages of activation.

Table 3 : Effect of guanosine and guanosine phosphates

Additions	PribosePP synthetase activity (pmoles PribosePP/min)			
	0.2 mM Pi		10 mM Pi	
	0.015 mM ATP	0.180 mMATP	0.015 mM ATP	0.180 mM ATP
None	0.5	1.9	4.0	7.6
0.1 mM Guanosine	0.2	1.0	1.0	2.9
0.1 mM GMP	3.3	3.6	9.3	9.0
0.1 mM GDP	1.9	2.2	6.3	7.0
0.1 mM cGMP[a]	0.1	-	3.9	6.3
0.1 mM cGMP + 1 mM Theophylline	0.5	1.1	3.4	5.7

[a] cGMP, guanosine 3':5' cyclic monophosphate

from human peripheral lymphocytes indicate some differences from reported results using the same enzyme from other sources (10-13). Cyclic GMP was not found to be an activator of PribosePP synthetase, as reported by Green and Martin (16). Instead an enhancing effect by IMP and GMP was observed. The effect of GMP may occur through deamination to IMP. The inhibitory effects of ADP and possibly AMP are similar to those described for the enzyme from bacteria (9,10), Ehrlich ascites tumor cells (11), human erythrocytes (12,13) and rat hepatoma cells (17).

The regulatory effects here reported could be involved in the mechanism of lymphocyte proliferation, which is thought to require de novo purine synthesis (18-20). PribosePP synthetase does not seem to be induced soon after mitogenic stimulation of the lymphocytes (our unpublished results), so that a regulatory change rather than a change of total enzyme content could account for the activation process. We suggest that in the resting lymphocyte PribosePP synthetase activity is low because the concentrations of Pi, IMP and GMP, which activate the enzyme, are low, whereas the concentration of ADP, which inhibits the enzyme, is relatively high. An early increase in the level of Pi after stimulation of lymphocytes activates PribosePP synthetase. Faster PribosePP

formation is followed by de novo purine synthesis, increasing the levels of IMP and GMP in the cells; these further stimulate PribosePP synthetase so that end-product stimulation results in self-amplification. Thus the PribosePP which is required for purine and pyrimidine synthesis is formed. In accordance with this hypothesis, in resting lymphocytes much more hypoxanthine is incorporated into adenine than guanine nucleotides, whereas in growing lymphoblasts the ratio is close to equality (8). In lymphocytes deficient in adenosine deaminase, levels of AMP and ADP would rise while those of IMP and GMP fall. Hence the rate of synthesis of PribosePP and of purines by the de novo pathway would be markedly decreased, and the cells would not respond to stimulation.

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