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GUANOSINE 5'-PHOSPHATE REDUCTASE OF HUMAN ERYTHROCYTES

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SUMMARY

1. Human GMP-reductase [NADPH: GMP oxidoreductase (deaminating), EC 1.6.6.8] was purified from erythrocytes with a yield of 15% and a 1200-fold increase in specific activity.

2. The apparent K_m for NADPH and GMP is $8.5 \cdot 10^{-6}$ M and $4.9 \cdot 10^{-6}$ M, respectively. 1 molecule of IMP is formed for every molecule of NADPH oxidized to $NADP^+$.

3. The purified enzyme exhibits a rather sharp maximum of activity around pH 7.5 and is relatively thermostable, losing only 40% of its activity after heating at 67 °C for 15 min.

4. A sulfhydryl donor is not an absolute requirement for the enzymatic reaction. However, activity was decreased to 50% of normal when a sulfhydryl compound was omitted from the reaction mixture.

5. XMP is a potent inhibitor of GMP-reductase. The inhibition by XMP is competitive for GMP binding by the enzyme with a $K_i = 1.1 \cdot 10^{-6}$ M. The enzyme was also inhibited by all divalent metal ions tested.

INTRODUCTION

An enzyme catalyzing the conversion of GMP to IMP has been partially purified from extracts of *Aerobacter aerogenes*, *Escherichia coli*, and *Salmonella typhimurium*[1]. Bacterial GMP-reductase [reduced NADP:GMP oxidoreductase (deaminating), EC 1.6.6.8] has an absolute requirement for reduced triphosphopyridine nucleotide (NADPH) and yields 1 molecule of $NADP^+$ and 1 molecule of NH_3 for every molecule of GMP reduced. The enzyme does not catalyze the reverse reaction even in the presence of excess amounts of $NADP^+$ and $(NH_4)_2SO_4$. In addition, it was found that ATP was a potent inhibitor of GMP-reductase.

Conversion of guanine to hypoxanthine has been demonstrated in mammalian systems[2–4]. When extracts of rat liver were incubated with [^{14}C]guanine, radioactivity was found in subsequently isolated hypoxanthine. NADP was found to accelerate the

rate of transfer. The exact pathway of this conversion was not clarified; while one report claimed that it occurred at the nucleotide level[3], the other suggested a conversion of guanine to hypoxanthine *via* xanthine[2].

The ability of human and rabbit erythrocytes to convert guanine to hypoxanthine has also been reported[4]. The authors demonstrated that the conversion took place at the nucleotide level by a reductive deamination of GMP to IMP. The enzyme responsible for this conversion reportedly utilized NADPH and required a thiol compound.

This communication confirms the existence of a GMP-reductase in human erythrocytes with a NADPH-linked activity. It further reports on the partial purification and characterization of the enzyme.

MATERIALS AND METHODS

Chemicals

[8-^{14}C]GMP, [8-^{14}C]GDP, and [8-^{14}C]XMP were obtained from Amersham/Searle Corp. [8-^{14}C]Guanine, [8-^{14}C]guanosine, and [8-^{14}C]GTP were purchased from Schwarz BioResearch. The following coenzymes, thiol reagents, sulfhydryl compounds, and nucleotides were obtained from Sigma Chemical Co. and stored at − 20 °C except where noted: NADPH as the 95–99% pure tetrasodium salt was stored desiccated; β-NADH as the 98% pure disodium salt was stored in the dark at room temperature while desiccated; *p*-hydroxymercuribenzoate and 5,5′-dithiobis(2-nitrobenzoic acid); 2-mercaptoethanol, glutathione, dithiothreitol and mercaptoacetic acid; IMP, XMP, GMP, GDP and GTP were all 98–100% pure. Cysteine was obtained from Nutritional Biochemicals Corp. D-Penicillamine was purchased from Merck, Sharpe and Dohme. All other chemicals were commercially tested analytical grades.

Chromatography supplies

Dowex 1 (AG 50W-X12, 200–400 mesh in the hydrogen form), Chelex 100 (200–400 mesh in the sodium form), and hydroxylapatite (BioGel HTP) were supplied by Bio-Rad Laboratories. Diethylaminoethyl celluloses (DE-1 and DE-52) were purchased from W. and R. Balston. Copper-loaded Chelex was prepared as described previously[5]. Dowex 1 was converted to the formate form while Dowex 50 was used in the H^+ form. The DE-1 and DE-52 celluloses were precycled through 0.5 M HCl and 0.5 M NaOH and then equilibrated against their respective buffers.

Sheets of Polygram Cellulose MN 300 impregnated with polyethylenimine (PEI) on a 20 cm × 20 cm base were obtained from Brinkmann Instruments.

Enzyme preparation

Three different sources of enzyme were used in these studies. All steps in the preparations were carried out at 4 °C. Protein measurements were by the biuret method or by absorbance at 280 nm.

Intact red blood cells. Freshly drawn heparinized blood was centrifuged and plasma and buffy coat removed. Red blood cells were washed twice with 0.9% NaCl and then once with a phosphate–saline buffer (1 part of 0.1 M potassium phosphate buffer (pH 7.4) to 9 parts of 0.9% NaCl) supplemented with glucose at a concentration of 3 mM.

Hemolysate. Saline-washed red blood cells were lysed with 2 vol. of water. After dialysis overnight against 200 vol. of water, stroma was removed by centrifugation. The dialyzed solution was stored frozen at — 20 °C until used.

Partially purified enzyme. Saline-washed red blood cells were lysed with 2 vol. of water. 2 vol. of this hemolysate were added to 1 vol. of wet-packed DE-1 cellulose and allowed to stand 45 min with gentle manual stirring every 5 min. At the end of this time the DE-1 cellulose–hemolysate mixture was poured onto a Buchner funnel with a nylon net serving as a filter pad. The cellulose was washed with 25 vol. of 0.01 M potassium phosphate buffer (pH 6.9). The eluate was colorless by the final wash. The wet packed DE-1 cellulose was removed from the funnel and combined with 2 vol. of 1.0 M potassium phosphate buffer (pH 6.9), 0.001 M EDTA, 0.001 M 2-mercaptoethanol. This mixture was allowed to stand for 45 min with manual stirring every 5 min. The DE-1 cellulose was again poured onto the Buchner funnel. After most of the solution had passed through the funnel, the cellulose was gently pressed to remove residual solution. Solid $(NH_4)_2SO_4$ was added in small fractions to the eluate containing the enzyme with stirring until 0.65 satn had been reached. The resulting precipitate was collected by centrifugation and dissolved in a minimum of standard buffer (0.1 M potassium phosphate buffer (pH 6.9), 0.001 M EDTA, 0.001 M 2-mercaptoethanol). The solution was dialyzed against 40 vol. of standard buffer and then fractionated with $(NH_4)_2SO_4$. The precipitate collected between 0.35 and 0.55 satn was dissolved in a minimum volume of standard buffer, and dialyzed against 150 vol. of the same buffer. The dialyzed solution was then layered on a DE-52 cellulose column, 1.5 cm × 9 cm, which had been equilibrated with standard buffer. The enzyme was recovered by gradient elution with standard buffer containing increasing concentration of NaCl (from 0.05 to 0.5 M). The eluate was collected in 2.8-ml fractions and the absorbance measured at 280 nm. Fractions containing significant enzyme activity were collected and concentrated by solid $(NH_4)_2SO_4$ as described above. The concentrate was dialyzed against 200 vol. of standard buffer. The last step in the purification was chromatography on a hydroxylapatite column, 0.5 cm × 5 cm, that had been previously equilibrated with 0.01 M potassium phosphate buffer (pH 6.8), 0.001 M 2-mercaptoethanol. 1 volume of enzyme solution from the previous step was mixed with 4 vol. of 0.01 M potassium phosphate buffer (pH 6.8), 0.001 M 2-mercaptoethanol and loaded on the hydroxylapatite column. Elution was accomplished by passing 2-ml fractions of phosphate buffer of increasing strength, pH 6.8, through the column (0.04, 0.1, 0.2, 0.3, 0.4, and 0.5 M). The eluate was collected in 2-ml fractions in tubes that contained 2 μmoles of EDTA. The fraction eluted with 0.1 M phosphate containing the highest enzymatic specific activity was stored at — 70 °C.

1 unit of enzyme activity is defined as the amount of enzyme that converts one nmole of GMP to IMP per h at 37 °C.

Assay procedures

Assay of purine compounds from whole cell or hemolysates reaction mixtures was done in several ways. Following the incubation of intact red blood cells, these were washed twice with saline and lysed by addition of 2 vol. of water. Deproteinization of hemolysate was accomplished by submersion of the sample in a boiling water bath for 50 s. The precipitated protein was removed by centrifugation.

The relative distribution of ^{14}C-labeled nucleotides, nucleosides and purine

bases in the supernatant reaction mixtures was determined after chromatographic separation on Chelex and Dowex 1 columns. Free purine bases are strongly adsorbed onto Chelex, while nucleosides and nucleotides are not. The latter two can be readily separated on columns of Dowex 1 in the formate form which retains nucleotides.

Nucleotides were separated by thin-layer chromatography using PEI-cellulose. Development was by ascending two-dimensional stepwise chromatography[6]. A mineralight model S-L lamp was used for location of spots which were cut out for radioactivity determinations in a liquid scintillation counter. Autoradiography of thin-layer chromatograms was done by stapling the plastic sheet to Kodak RP Royal X-Omat Medical X-ray film. The chromatogram and film were wrapped in aluminum foil and stored in the dark during exposure.

The distribution of base residues in the reaction mixture was also determined after hydrolysis of purine compounds in 1 M HCl at 100 °C for 1 h in sealed tubes. Following hydrolysis, the sample was diluted with water to 0.5 M HCl and passed through a column of Dowex 50 in the hydrogen form, which retains bases. Hypoxanthine and guanine were separated by stepwise elution with 0.5 and 1.5 M HCl.

The conversion of GMP to IMP by partially purified enzyme was assayed by one of three methods.

Aliquots of the reaction mixtures containing ^{14}C-labeled purine nucleotides were hydrolyzed in 1 M HCl and then analyzed on Dowex 50 as described for the whole cell and hemolysate incubations.

Alternatively, the conversion of GMP was followed by determining the decrease in absorbance at 340 nm due to NADPH oxidation. Suitable controls were included to correct for oxidation of NADPH by the small amount of NADPH-oxidase activity that co-purified with the GMP-reductase.

The last method was separation of ^{14}C-labeled nucleotides by direct chromatography of an aliquot of the reaction mixture on thin-layer PEI-cellulose. One-dimensional development in 0.5 M sodium formate buffer (pH 3.4), separated GMP and IMP for subsequent radioactivity determination.

RESULTS

Enzyme purification

Table I is a summary of the purification steps. By this scheme an approx. 1200-

TABLE I

PURIFICATION OF GMP-REDUCTASE FROM HUMAN ERYTHROCYTES

Step	*Volume (ml)*	*Total protein (mg)*	*Total activity (units)*	*Spec. act. (units/mg)*	*Yield (%)*
Hemolysate	450	27 300	6730	0.25	
DE-1 cellulose					
(65% $(NH_4)_2SO_4$)	28	182	3650	20.2	54
35–55 % $(NH_4)_2SO_4$	4.5	52	2600	50	39
DE-52 cellulose					
(65 % $(NH_4)_2SO_4$)	3.1	13.2	1820	138	27
Hydroxylapatite	6	3.2	985	310	15

TABLE II

INCUBATION OF [^{14}C]GUANINE WITH HUMAN RED BLOOD CELLS

The reaction mixture contained 139 mM NaCl; 10 mM potassium phosphate buffer (pH 7.5); 3 mM glucose; 1 μCi [8-^{14}C]guanine (31.2 Ci/mole) and 500 μl packed red blood cells in a final volume of 1 ml. The control had 500 μl of water substituted for cells. Samples were incubated for 2.5 h at 37 °C. Chelex and Dowex 1 were used to separate purine bases, nucleosides and nucleotides. Individual nucleotides were isolated on thin-layer chromatograms. Purine bases released during hydrolysis were separated by gradient elution from Dowex 50.

Sample	% ^{14}C *recovered in nucleotides*	% ^{14}C *recovered in nucleosides*	*Percentage distribution of* ^{14}C *in nucleotides*						*Percentage distribution of* ^{14}C *in purine bases after hydrolysis*	
			AMP	*IMP*	*XMP*	*GMP*	*GDP*	*GTP*	*Hypoxanthine*	*Guanine*
Erythrocytes	97	0	0	21	0	2	18	59	17	82
Control	< 1	0	0	0	0	0	0	0	3	95

TABLE III

INCUBATION OF [^{14}C] GMP WITH HEMOLYSATE AND PARTIALLY PURIFIED ENZYME

The reaction mixture contained 33 mM potassium phosphate buffer (pH 7.5); 4.8 mM 2-mercaptoethanol; 33 μM [^{14}C]GMP (5 Ci/mole); 3.3 mM NADPH (hemolysate) or 0.33 mM NADPH (purified enzyme); and enzyme preparation, which was either 6 mg hemolysate protein or 0.0265 mg purified enzyme, in a final volume of 300 μl. The controls were the same except for omission of NADPH. Samples were incubated at 37 °C for 3 h (hemolysate) or 1 h (purified enzyme).

Sample	% ^{14}C *recovered in nucleotides*	% ^{14}C *recovered in nucleosides*	*Percentage distribution of* ^{14}C *in nucleotides*				*Percentage distribution of* ^{14}C *in purine bases after hydrolysis*	
			GMP	*GDP*	*GTP*	*IMP*	*Guanine*	*Hypoxanthine*
Hemolysate (+ NADPH)	99	0	24	2	0	74	31	68
Hemolysate (− NADPH)	97	0	98	2	0	0	99	0
Enzyme (+ NADPH)	95	0	40	0	0	60	42	58
Enzyme (− NADPH)	97	0	100	0	0	0	99	1

fold increase in specific activity was obtained. The final enzyme preparation contained slight amounts of NADPH-oxidase activity which was corrected for in the spectrophotometric assays. Omission of EDTA at the last step in purification resulted in total loss of activity. Storage for 2 months at — 70 °C of the enzyme from the hydroxylapatite column resulted in a loss of no more than 20 % of the activity.

Purine incubation products of red blood cells with guanine

Table II indicates the distribution of ^{14}C in purine compounds within the red cells. Practically all of the [^{14}C]guanine that entered the cells had been converted to nucleotide at the end of the incubation. This conversion is mediated by hypoxanthine–guanine phosphoribosyltransferase (inosine monophosphate: pyrophosphate phosphoribosyltransferase, EC 2.4.2.8) since patients with a deficiency of this enzyme fail to reutilize guanine[7]. No ^{14}C was found in nucleosides. Analysis of nucleotides on thin-layer chromatography indicated that GMP and GDP kinases are present in intact red cells. 21% of the guanine that entered the erythrocytes had been converted to IMP.

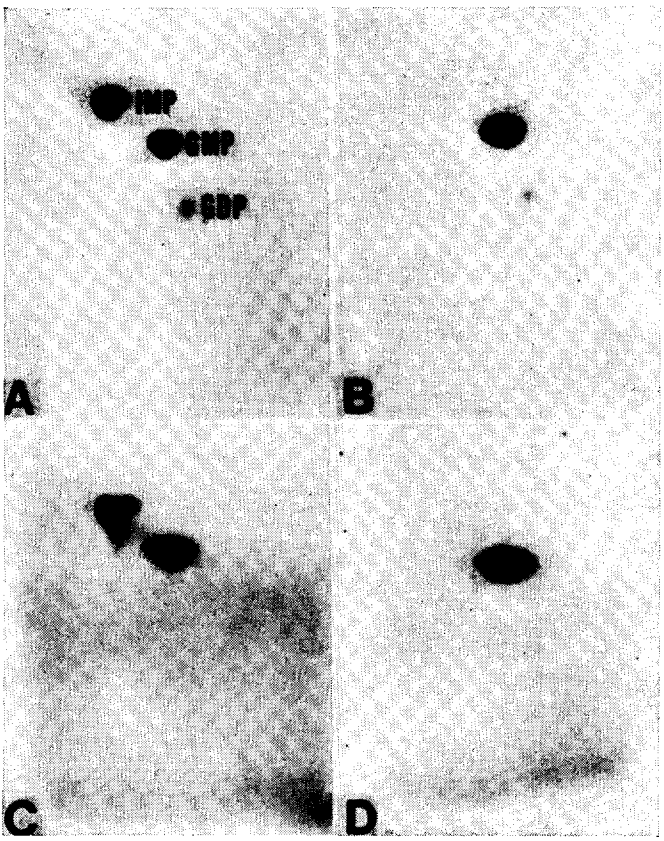

Fig. 1. Autoradiograms of the products of the GMP-reductase reaction using hemolysate and partially purified enzyme. The thin-layer chromatograms used to determine the distribution of nucleotides in Table III were analyzed by autoradiography. The autoradiograms were exposed for 1 week. Reaction mixtures are given in the experimental detail for Table III. A, hemolysate (+ NADPH); B, hemolysate (— NADPH); C, enzyme (+ NADPH); D, enzyme (— NADPH).

This figure is in good agreement with the percentage of total radioactivity found in hypoxanthine after hydrolysis of purine compounds in the reaction mixture.

Purine incubation products of hemolysate and purified enzyme with GMP

The products formed during incubation of [^{14}C]GMP with erythrocyte hemolysate or partially purified enzyme are shown in Table III. These studies indicate that IMP is formed from GMP at the nucleotide level, and that transfer of radioactivity to IMP has an absolute requirement for NADPH (Fig. 1). This confirms the report of Hershko *et al.*[4] on the conversion of GMP to IMP by human red cell hemolysate.

No formation of IMP occurred when [^{14}C]XMP was incubated with purified enzyme under the same conditions. This fact together with the absence of any other labeled intermediate in the reaction mixture suggest that GMP is converted to IMP in a single reductive step. The data confirm previous studies in this laboratory showing that breakdown of GMP to nucleoside or base does not take place (MacKenzie, J., unpublished observations). The enzymatic reaction catalyzing the reduction of GMP to IMP was found to be irreversible and NADH could not be substituted for NADPH.

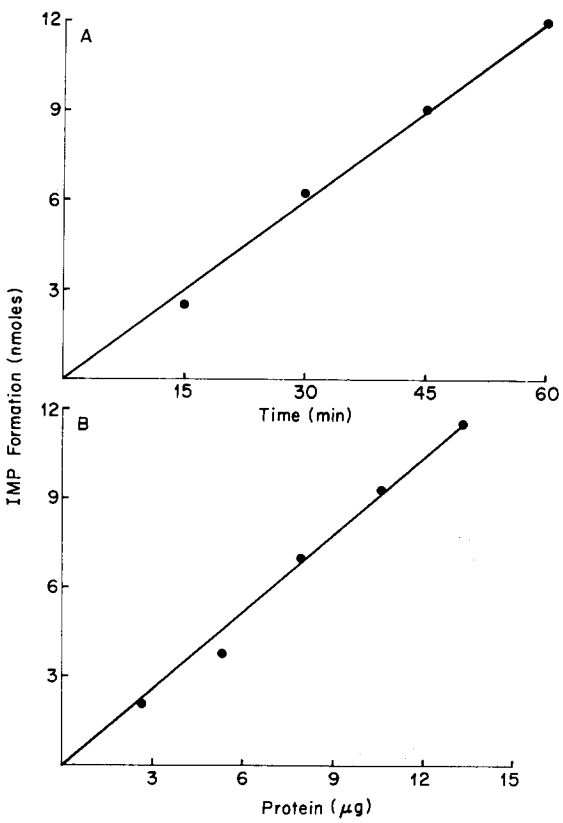

Fig. 2. Characteristics of assay system. The concentrations of reactants are given in Table III. Samples were analyzed on Dowex 50 as described under Materials and Methods. A. Time course of IMP formation from GMP. The incubation mixture contained 39.75 μg of purified enzyme in a volume of 450 μl. B. Formation of IMP from GMP by different amounts of enzyme. Final volume was 150 μl. Incubation was 60 min at 37 °C.

A [^{14}C]hypoxanthine-containing compound was never found in the reaction mixture when partially purified enzyme was incubated with [^{14}C]guanine, [^{14}C]-guanosine, [^{14}C]GDP, or [^{14}C]GTP.

Characteristics of assay system of partially purified enzyme

The time course of conversion of GMP to IMP was linear with time as shown in Fig. 2A. At 60 min the reaction was still first order with 80% of the total GMP having been converted.

Variation of enzyme concentration in the presence of a set amount of GMP yielded a constant proportional amount of IMP product per μg of enzyme (Fig. 2B).

Apparent K_m and stoichiometry for GMP and NADPH

The velocity of formation of IMP was determined as a function of GMP or NADPH concentration under saturating conditions of the other substrate. All kinetic reaction measurements were taken during the initial linear portion of the reaction. The results are shown in Fig. 3. The apparent K_m was $8.5 \cdot 10^{-6}$ M and $4.9 \cdot 10^{-6}$ M for

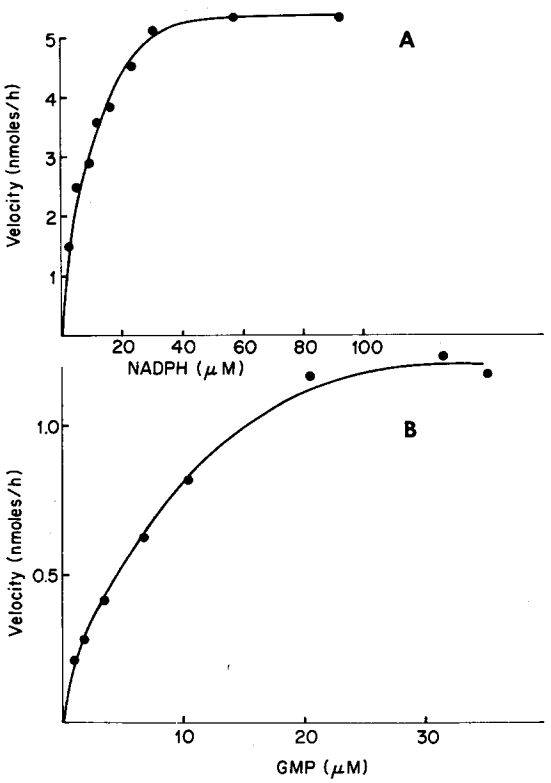

Fig. 3. Apparent K_m for GMP and NADPH. Each reaction mixture contained 33 mM potassium phosphate buffer (pH 7.5); 4.8 mM 2-mercaptoethanol; GMP and NADPH as indicated, in a final volume of 150 μl. A. Each reaction tube contained 4.15 μg of purified enzyme and NADPH at 270 μM. Nucleotides were assayed directly on PEI-cellulose as described under Materials and Methods. B. Each reaction tube contained 10.5 μg of purified enzyme and GMP at 330 μM. IMP formation was monitored by decrease in absorbance at 340 nm as described in Materials and Methods.

NADPH and GMP, respectively. The kinetics for GMP departed from the usual hyperbolic form. The reason(s) for this is not clear.

Absolute amounts of NADPH oxidized and IMP formed during the course of a reaction are shown in Fig. 4. The data show that for every molecule of NADPH oxidized to $NADP^+$, one molecule of IMP is formed. The other product of the reaction, NH_3, was liberated in too small amounts to be reliably determined. The stoichiometry of NH_3 awaits the further purification of the enzyme.

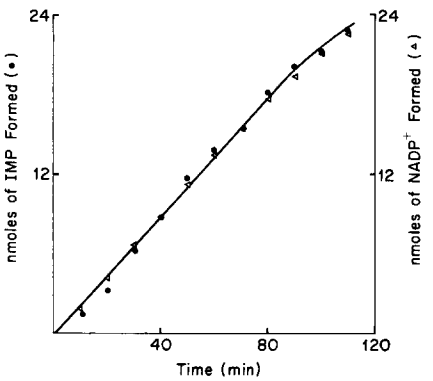

Fig. 4. Stoichiometry of GMP and NADPH in the GMP-reductase reaction. The incubation mixture contained 33 mM potassium phosphate buffer (pH 7.5), 4.8 mM 2-mercaptoethanol, 0.11 mM NADPH, 0.19 mM [^{14}C]GMP, and 0.0415 mg of purified enzyme in a total volume of 300 μl. IMP was assayed as described in Fig. 3A. NADPH oxidation was determined as outlined in Fig. 3B. A control without GMP was used to correct for the small amount of NADPH-oxidase activity in the enzyme preparation.

Effect of temperature on GMP-reductase

The enzyme retained 100% activity after heating at 47 °C for 15 min. Heating at 67 °C for the same period of time resulted in a reduction of the activity to 60% of the control value. The enzyme was 98 % inactivated by heating at 80 °C for 15 min.

Effect of pH on GMP-reductase

The activity of the enzyme at different pH values is shown in Fig. 5. The enzyme exhibited a rather sharp maximum of activity around pH 7.5. This compares with the reported pH range for maximum activity of 7.5–8.2 and 7.6–8.5 for bacterial[1] and human erythrocyte[4] enzyme, respectively.

Requirements for sulfhydryl compounds

A sulfhydryl donor was not an absolute requirement for the enzymatic reaction. However, activity was decreased to 50% of normal when 2-mercaptoethanol at a concentration of 4.8 mM was omitted from the reaction mixture. Storage of the enzyme in the absence of 2-mercaptoethanol caused activity to be lost twice as fast as enzyme preparations stored in the presence of this sulfhdryl donor.

Dithiothreitol, cysteine, and thioglycolic acid can be substituted for 2-mercaptoethanol in the reaction mixture at 15 mM concentration with little loss of enzymatic activity.

Two agents that react with thiol groups, *p*-hydroxymercuribenzoate and 5,5′-

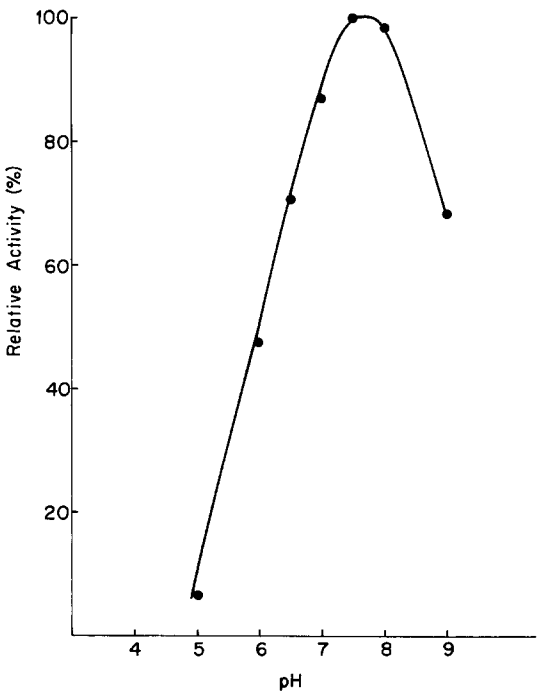

Fig. 5. Activity of GMP-reductase as a function of pH. Citrate–phosphate buffers were used at pH 4 and 5. Potassium phosphate buffers at 0.1 M were used in the range pH 6–8. A 0.1 M Tris–HCl buffer was used at pH 9. Incubation was at 37 °C for 1 h. The enzyme had been dialyzed against 0.01 M potassium phosphate buffer (ph 6.8); 1 mM 2-mercaptoethanol; and 1 mM EDTA prior to assay of activity. Other reactants as in Table III.

dithiobis(2-nitrobenzoic acid), were pre-incubated with the enzyme at 0.66 mM for 30 min at 37 °C. This treatment caused a reduction of enzyme activity of 18 and 33%, respectively.

Effect of metal salts on GMP-reductase activity

All divalent metal ions tested at 10 mM concentration inhibited the activity of the enzyme. The inhibition was greater than 90% for Mg^{2+}, Fe^{2+}, and Zn^{2+}. Mn^{2+}, Ni^{2+} and Cu^{2+} showed intermediate inhibition, while Ca^{2+} showed only 54% inhibition. The inhibition was reduced to 12% when the concentration of Mg^{2+} was lowered to 1 mM.

Nucleotide inhibition of GMP-reductase activity

The effect of various nucleotides on enzymatic activity are summarized in Table IV. XMP is a potent inhibitor of GMP-reductase. At equimolar concentrations of XMP and GMP, the former inhibited the reaction by approx. 90%. The inhibition of enzyme activity caused by different levels of XMP at a constant GMP concentration is shown in Fig. 6. The concentration of GMP for this experiment was chosen to closely approximate the apparent K_m for GMP under the reaction conditions. A 50% inhibition was observed when the concentration of XMP was 16% of the GMP K_m. The inhibition is competitive with respect to GMP with a $K_i = 1.1 \cdot 10^{-6}$ M.

TABLE IV

INHIBITION OF GMP-REDUCTASE BY PURINE NUCLEOTIDE

The reaction mixture contained 33 mM potassium phosphate buffer (pH 7.5), 4.8 mM 2-mercaptoethanol, [^{14}C]GMP at the indicated concentration, 0.33 mM NADPH, inhibiting nucleotide as shown, and 0.021 mg purified enzyme in a total volume of 150 μl. Incubation was at 37 °C for 1 h. The activity was determined as in Table IV.

GMP (μM)	*Nucleotide*	*Concn (μM)*	*Inhibition (%)*
3.3	XMP	3.3	85
33	XMP	3.3	5
33	XMP	33	93
33	XMP	198	98
33	IMP	33	30
33	IMP	330	70
33	IDP	33	0
33	ITP	33	0
33	AMP	33	5
33	ADP	33	7
33	ATP	33	11
33	GDP	33	8
33	GTP	33	3

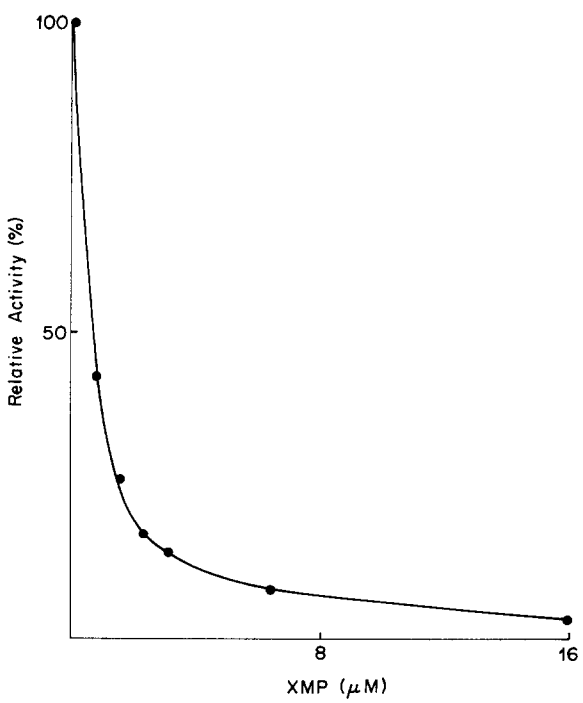

Fig. 6. XMP inhibition of GMP-reductase activity. The concentration of reactants are given in Table IV, except for [^{14}C]GMP which was 5 μM. Activity was assayed by the amount of IMP formed during a 30-min incubation at 37 °C. IMP was determined by chromatography on PEI-cellulose as described in Fig. 3A.

DISCUSSION

Partially purified GMP-reductase from human erythrocytes differs significant from the bacterial form. The human enzyme does not have an absolute requirement for a sulfhydryl donor, while *E. coli* enzyme reportedly does[1]. On the other hand, partially purified GMP-reductase from extracts of *A. aerogenes* did not have an absolute requirement for thiol[8], although a thiol was required for maximal activity as is the case in the present studies of human GMP-reductase. The stability of our enzyme toward sulfhydryl reagents suggests that thiol groups are not intimately associated with the active site(s). Another difference is seen in the inactivation of human GMP-reductase by divalent metal ions; the bacterial enzyme is not affected by such ions[1].

The most startling contrast between the two enzymes is their response to nucleotides. ATP is an effective inhibitor of bacterial GMP-reductase[1]. Our data demonstrate that ATP exercises little if any direct regulatory control on human GMP-reductase. XMP, on the other hand, is a potent inhibitor of the human enzyme. The importance of this regulating role for XMP remains to be elucidated, awaiting determination of the relative intracellular concentrations of XMP and GMP.

Guanyl ribonucleotides are important feedback inhibitors of the first committed reaction in the *de novo* synthesis of purines [9], and, in addition, are involved in the complex regulation of nucleotide interconversion and reutilization of guanine for GMP formation. IMP is a precursor of guanyl as well as adenyl ribonucleotides and thus occupies a pivotal position in the synthesis of purine nucleotides. The cell, responding to a number of factors, utilizes IMP according to its immediate requirements. This response is effected through the activity of various nucleotide interconversion enzymes, some of which are regulated by nucleotide levels existent in the cell. One of these nucleotide interconversion enzymes is IMP-dehydrogenase[10], which converts IMP to XMP and is inhibited by GMP[1]. This inhibition, along with XMP inhibition of GMP-reductase, might provide a means of controlling the relative levels of IMP, XMP, and GMP.

A high level of GMP would inhibit the conversion of IMP to GMP and thus lower the level of XMP intermediate. This in turn would result in a release of GMP-reductase inhibition and the production of IMP from GMP. Conversely, a low level of GMP would promote XMP formation. The XMP so formed would inhibit GMP-reductase and so serve to increase the concentration of guanine nucleotides at the expense of IMP (Fig. 7).

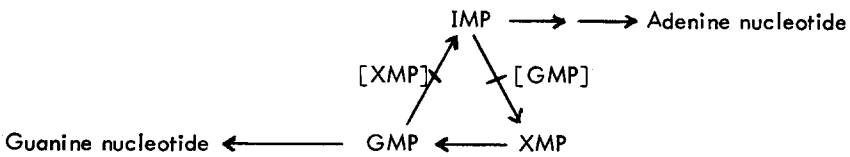

Fig. 7. Nucleotide interconversion. Crossed pathway indicates inhibition by nucleotide in brackets

Besides being converted to GMP, XMP can also be degraded to xanthine. The possibility that the degradation of XMP to xanthine is a major metabolic pathway[11] may be important in the regulatory properties of XMP.

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