Biochimica et Biophysica Acta, 483 (1977) 63—69

BBA 68171

HYPOXANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE FROM RAT LIVER

EFFECTS OF MAGNESIUM 5-PHOSPHORIBOSYL 1-PYROPHOSPHATE ON THE CHEMICAL MODIFICATION AND STABILITY OF THE ENZYME

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(Received December 22nd, 1976)

Summary

1. The homogeneously purified hypoxanthine-guanine phosphoribosyltransferase (IMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.8) from rat liver was strongly inactivated by the sulfhydryl reagents, *p*-chloromercuribenzoate and 5,5′-dithiobis(2-nitrobenzoic acid). The addition of magnesium 5-phosphoribosyl 1-pyrophosphate, the substrate, almost completely protected the enzyme and magnesium pyrophosphate, the product, partially protected the enzyme from the inactivation by SH reagents.

2. The time course of 2,4,6-trinitrobenzenesulfonic acid-inactivation of hypoxanthine-guanine phosphoribosyltransferase revealed that the enzyme activity was initially increased and then decreased. The enzyme was also completely protected against the 2,4,6-trinitrobenzenesulfonic acid-inactivation in the presence of either magnesium 5-phosphoribosyl 1-pyrophosphate or magnesium pyrophosphate.

3. Hypoxanthine-guanine phosphoribosyltransferase was substantially protected against heat- and trypsin-inactivation by the addition of phosphoribosylpyrophosphate plus $MgCl_2$. The sucrose density gradient centrifugation showed that phosphoribosylpyrophosphate yielded the enzyme species with a larger *s* value than that of control.

4. These results suggest that the thiol and/or amino groups of the enzyme could be responsible for the binding of 1-magnesium pyrophosphoryl group of magnesium phosphoribosylpyrophosphate, and the binding of magnesium phosphoribosylpyrophosphate induces the more rigid conformation of the enzyme molecule somewhat different from those caused by the other substrates, products and inhibitors.

Abbreviations: PRPP, 5-phosphoribosyl 1-pyrophosphate; Rib-5-P, ribose 5-phosphate; Nbs_2, 5,5′-dithiobis(2-nitrobenzoic acid); $N_3bSO_3^-$, 2,4,6-trinitrobenzenesulfonic acid

Introduction

Hypoxanthine-guanine phosphoribosyltransferase (IMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.8) catalyzes the transfer of the 5-phosphorylribose moiety of 5-phosphoribosyl 1-pyrophosphate to hypoxanthine and guanine to form their corresponding mononucleotides.

The importance of hypoxanthine-guanine phosphoribosyltransferase in purine metabolism in relation to the overproduction of uric acid makes this enzyme an interesting subject for enzymological and physicochemical studies from the regulatory point of view. Although the kinetic and structural properties vary widely depending on the sources and the purification of hypoxanthine-guanine phosphoribosyltransferase [1—7], the substrate, PRPP has been shown to protect the enzyme against inactivation by heat [5—8] or freezing and thawing [9] in vitro, suggesting that PRPP may induce a conformational change of the enzyme molecule to a more stable form. However, no information on the active center of the enzyme molecule is available as far as we know.

In this paper, the effect of some ligands on chemical modification by SH reagents or $N_3bSO_3^-$ and inactivation by heat treatment or trypsin digestion were investigated. Furthermore, the conformational change in the enzyme molecule by binding of the substrate, MgPRPP was demonstrated by sucrose gradient centrifugation.

Materials and Methods

Materials. 5,5′-dithiobis(2-nitrobenzoic acid), *p*-chloromercuribenzoate, various nucleotides, trypsin, type III, from bovine pancreas and trypsin inhibitor, type I-S, from soybean were obtained from Sigma. 2,4,6-trinitrobenzene sulfonic acid came from Wako Pure Chemical Industries. [8-^{14}C]hypoxanthine (41.6 mCi/mmol) was purchased from Daiichi Pure Chemicals. Tetrasodium salt of 5-phosphoribosyl 1-pyrophosphate was obtained from Kyowa Hakko Kogyo. All other chemicals were of the highest purity commercially available.

Enzyme assay. The enzyme assay was as described by Gutensohn and Guroff [10]. One unit was defined as the amount of enzyme catalyzing the formation of 1 μmol of IMP per min at 37° C.

Enzyme preparation. Hypoxanthine-guanine phosphoribosyltransferase was purified from rat liver as previously described [7]. A final recovery of 28.6% resulted in an apparently homogeneous enzyme preparation as judged by polyacrylamide disc gelelectrophoresis. The specific activity of the purified enzyme was 6.08 units/mg and was almost equivalent to those of the enzymes purified homogeneously from other sources [3,5,6].

The purified enzyme was concentrated to 2 mg/ml of protein in 0.05 M Tris · HCl buffer (pH 7.4) containing 10 mM 2-mercaptoethanol, 5 mM $MgCl_2$ and 5 mM PRPP and stored at 4° C. The stored enzyme retained at least 90% of its activity after 2 months. Prior to the experiments, the stored enzyme was usually applied to a Sephadex G-25 column (1 cm × 25 cm) equilibrated with 0.05 M Tris · HCl buffer (pH 7.4) to remove MgPRPP and 2-mercaptoethanol. The ligand-free enzyme retained at least 90% of its activity at 0° C or 37° C after 150 min.

Results

Inactivation of the enzyme by sulfhydryl reagents

The purified preparation of enzyme from rat liver was strongly inactivated by incubation with *p*-chloromercuribenzoate at a final concentration of 1 mM for 6 min in an ice bath (Table I). In the presence of PRPP plus $MgCl_2$, this inactivation was considerably reduced. However, PRPP was less effective without $MgCl_2$. Although PP_i showed the accelerative effect of this inactivation, PP_i plus $MgCl_2$ protected the enzyme slightly. On the other hand, GMP accelerated the inactivation either in the presence or the absence of $MgCl_2$ under the experimental conditions.

In the experiments using Nbs_2 as the sulfhydryl reagent, the effects of some ligands were compatible with the data obtained with *p*-chloromercuribenzoate (Table II). The enzyme was completely protected by PP_i plus $MgCl_2$ as well as by PRPP plus $MgCl_2$ against Nbs_2-inactivation, an effect which was enhanced by GMP or PP_i. These results suggest that the thiol groups may be closely related to the binding site(s) of the substrate, MgPRPP and the product, $MgPP_i$.

Inactivation of the enzyme by $N_3bSO_3^-$

The effect of $N_3bSO_3^-$ on the enzyme activity was investigated. When hypoxanthine-guanine phosphoribosyltransferase was incubated with 2 mM $N_3bSO_3^-$ in 0.05 M Tris · HCl buffer (pH 7.4) at 37°C, the enzyme activity initially increased and then decreased as shown in Fig. 1. The addition of PRPP plus $MgCl_2$ slightly activated the enzyme with the $N_3bSO_3^-$ treatment, while PRPP without $MgCl_2$ did not show any effect. Although PP_i promoted this inactivation, PP_i plus $MgCl_2$ in addition to PRPP plus $MgCl_2$ caused a slight activation of the enzyme. In the presence of IMP, the enzyme activity did not increase but decreased slowly (Fig. 1). On the other hand, neither $MgCl_2$, hypoxanthine, inosine nor ribose 5-phosphate had any effect.

TABLE I

EFFECTS OF SOME LIGANDS ON INACTIVATION OF HYPOXANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE BY *p*-CHLOROMERCURIBENZOATE

The enzyme solutions were incubated with *p*-chloromercuribenzoate at a final concentration of 1 mM in an ice bath in the presence of the additions indicated. After standing for 6 min, enzyme activity was assayed under the standard conditions except that bovine serum albumin was added to the reaction mixture at a final concentration of 2 mg/ml.

Additions	Controls (not treated) (munits/ml)	*p*-chloromercuribenzoate-treated (munits/ml)	Residual activity (%)
None	6.02	2.38	39.5
$MgCl_2$, 5 mM	6.08	2.66	43.8
GMP, 0.01 mM	5.80	0.90	15.5
GMP, 0.01 mM + $MgCl_2$, 5 mM	5.86	0.96	16.4
GMP, 1 mM	2.54	0.29	11.4
GMP, 1 mM + $MgCl_2$, 5 mM	2.34	0.10	4.3
PP_i, 10 mM	2.78	0.20	7.2
PP_i, 10 mM + $MgCl_2$, 20 mM	5.08	3.10	61.0
PRPP, 1 mM	6.28	3.48	55.4
PRPP, 1 mM + $MgCl_2$	6.31	5.19	82.3

TABLE II

EFFECTS OF SOME LIGANDS ON INACTIVATION OF HYPOXANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE BY Nbs_2

The enzyme solutions were incubated with Nbs_2 at a final concentration of 1 mM in an ice bath in the presence of the additions indicated. After standing for 60 min, enzyme activity was determined by the standard assay conditions except that bovine serum albumin was added to each reaction mixture at a final concentration of 2 mg/ml.

Additions	Controls (not treated) (munits/ml)	Nbs_2-treated (munits/ml)	Residual activity (%)
None	5.23	3.39	64.8
Hypoxanthine, 0.5 mM	4.81	3.07	63.8
Guanosine, 1 mM	4.86	2.71	55.8
GMP, 1 mM	1.79	0.32	17.9
PP_i, 5 mM	4.82	1.15	23.9
PP_i, 5 mM + $MgCl_2$, 10 mM	5.03	5.03	100.0
PRPP, 1 mM + $MgCl_2$, 5 mM	5.30	5.35	101.0

Heat- and trypsin-inactivation of the enzyme

It was of interest to examine the effects of heat treatment and trypsin digestion on the enzyme activity in the presence of various ligands in order to shed more light on the nature of hypoxanthin-guanine phosphoribosyltransferase stability. Table III shows the effects of some ligands on heat-inactivation at 70°C for 6 min. The enzyme was completely protected in the presence of PRPP plus $MgCl_2$, while neither PRPP, $MgCl_2$, PP_i, PP_i plus $MgCl_2$, IMP nor GMP had any effect.

When the enzyme was treated with trypsin at a final concentration of 1 mg/

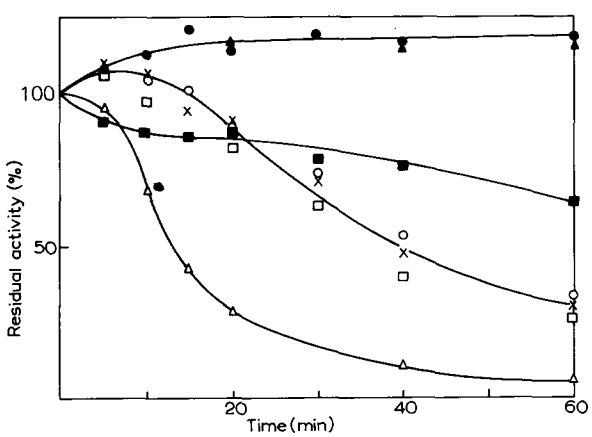

Fig. 1. Effects of some ligands on inactivation of hypoxanthine-guanine phosphoribosyltransferase by $N_3bSO_3^-$. The enzyme solutions were incubated with $N_3bSO_3^-$ at a final concentration of 2 mM at 37°C in the absence (X———X) or the presence of either 1 mM PRPP (○———○), 1 mM PRPP + 5 mM $MgCl_2$ (●———●), 5 mM PP_i (△———△), 5 mM PP_i + 10 mM $MgCl_2$ (▲———▲), 0.5 mM IMP (■———■) or 0.5 mM hypoxanthine + 1 mM ribose 5-phosphate (□———□). At the indicated times, aliquots (5 μl) were removed from the incubation mixtures and assayed for hypoxanthine-guanine phosphoribosyltransferase activity. The residual activity was represented as percent of the original activity.

TABLE III

EFFECTS OF SOME LIGANDS ON INACTIVATION OF HYPOXANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE BY HEAT TREATMENT

The enzyme solutions were heated at 70°C for 6 min in the presence of the additions indicated. Enzyme activity was assayed under the standard conditions.

Additions	Controls (not treated) (munits/ml)	Heat-treated (munits/ml)	Residual activity (%)
None	7.71	0.77	10.0
Hypoxanthine, 0.5 mM	7.51	0.92	12.3
Inosine, 0.5 mM	7.61	0.98	12.9
IMP, 0.5 mM	4.67	0.48	10.3
Rib-5-*P*, 1 mM	7.24	0.85	11.7
Hypoxanthine, 0.5 mM + Rib-5-*P*, 1 mM	7.22	0.59	8.2
$MgCl_2$, 5 mM	7.68	0.81	10.5
PP_i, 5 mM	5.48	0.42	7.7
PP_i, 5 mM + $MgCl_2$, 10 mM	6.32	0.70	11.1
PP_i, 5 mM + Rib-5-*P*, 1 mM	5.32	0.39	7.3
PP_i, 5 mM + Rib-5-*P*, 1 mM + $MgCl_2$, 10 mM	6.13	0.74	12.1
PRPP, 1 mM	7.66	0.92	12.0
PRPP, 1 mM + $MgCl_2$, 5 mM	7.94	8.02	101.0

ml at 37°C for 10 min, the enzyme was completely inactivated. However, 65% of the enzyme activity was retained after this treatment in the presence of PRPP plus $MgCl_2$. Neither GMP nor hypoxanthine prevented the enzyme from the trypsin-inactivation (Fig. 2).

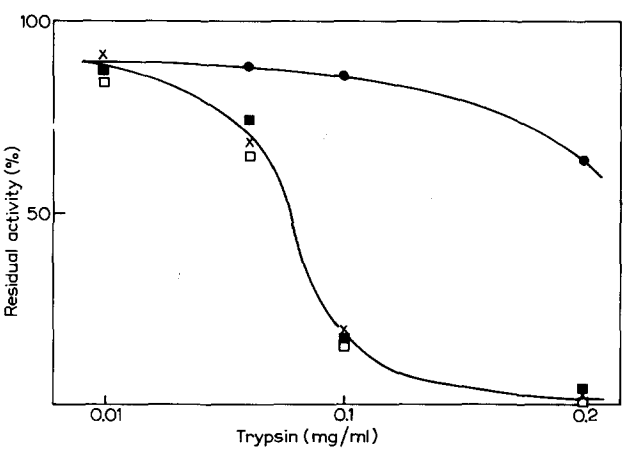

Fig. 2. Effects of some ligands on inactivation of hypoxanthine-guanine phosphoribosyltransferase by trypsin treatment. The enzyme solutions were treated by various concentrations of trypsin at 37°C in the absence (X——X) or the presence of either 1 mM PRPP + 5 mM $MgCl_2$ (●——●), 0.05 mM GMP (■——■) or 0.3 mM hypoxanthine (□——□). After incubation for 10 min, the trypsin digestion was terminated by addition of the equimolar trypsin inhibitor. The enzyme activity was assayed under the standard conditions immediately after trypsin treatment and the residual activity was represented as percent of the original activity.

Effects of ligands on the s value of the enzyme

Centrifugation of rat liver hypoxanthine-guanine phosphoribosyltransferase in a linear 5—25% sucrose gradient containing 0.05 M Tris · HCl buffer (pH 7.4) was performed as described by Martin and Ames [11] with bovine serum albumin as a marker. Each preparation yielded a single peak of the enzyme activity. The *s* values (means ± S.E. of 5 determinations) were 5.1 ± 0.1, 5.4 ± 0.1 and 5.2 ± 0.1 in the absence and the presence of 1 mM PRPP plus 5 mM $MgCl_2$ and 1 mM GMP, respectively. The *s* value of the enzyme with MgPRPP was slightly but significantly larger than control ($P < 0.01$). The difference of the values between the control and the GMP added groups was statistically insignificant.

Discussion

Studies on the chemical modifications of rat liver hypoxanthine-guanine phosphoribosyltransferase by SH reagents and $N_3bSO_3^-$ were performed in order to investigate the structural properties of the catalytic site(s) of the enzyme. It should be noted that the modification of the purified enzyme by SH reagents was almost completely protected by the addition of MgPRPP. This result is in good agreement with the result of Krenitsky and Papaioannou using a crude preparation of hypoxanthine phosphoribosyltransferase from human erythrocytes [12]. PRPP and PP_i have been shown also to protect the enzyme against the inactivation by $N_3bSO_3^-$ as well as SH reagents in the presence of $MgCl_2$. On the other hand, the products, GMP and IMP, to which 5-phosphorylribose moiety of MgPRPP was transferred, did not prevent the enzyme from these inactivations. Since $N_3bSO_3^-$ has been generally known to react with α- and ϵ-amino groups and thiol groups of various proteins [13], these observations suggest that the thiol and/or amino groups may be closely related to the binding site(s) of magnesium pyrophosphoryl group of MgPRPP, i.e. the binding site(s) of the product, $MgPP_i$. Magnesium ion is now known to be required for the protection of the enzyme against SH- or $N_3bSO_3^-$-inactivation as well as for its catalytic activity [7]. In the absence of $MgCl_2$, PRPP was a less effective protector and PP_i conversely promoted these inactivations, suggesting that PP_i and $MgPP_i$ have distinct binding sites: the binding of PP_i to sites distinct from the $MgPP_i$ sites may induce a conformational change in the enzyme so that the essential thiol and amino residues of the enzyme protein become more sensitive to SH reagents or $N_3bSO_3^-$.

MgPRPP has been shown to have a pronounced protective effect on the enzyme against heating and trypsin treatments, while neither free PRPP, the other substrates nor products has any effect. These findings were compatible with the sucrose gradient centrifugation experiments: the *s* value of the enzyme in the presence of MgPRPP was demonstrated as a larger one in comparison with control, suggesting that the binding of MgPRPP induces a conformational change of the enzyme molecule to a more compact form, which is more resistant to the heating and trypsin treatments. However, for the more detailed information on the conformational change of hypoxanthine-guanine phosphoribosyltransferase molecule, further studies are necessary.

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