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INHIBITORS OF GLYOXALASE ENZYMES

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SUMMARY

Both glyoxalase enzymes from various sources are inhibited by nucleotides, nucleosides and a series of structurally related compounds in an apparently cooperative manner. In general, aromatic compounds, which contain heterocyclic nitrogen and/or have amino groups on the aromatic ring, are effective inhibitors of these enzymes. Since intracellular concentrations of some of the nucleotides are significantly higher than the concentrations needed to inhibit both enzymes completely, the inhibitions observed may have physiological significance.

INTRODUCTION

The glyoxalase system, known since 1913 (1,2), catalyzes the disproportionation of α-ketoaldehydes into the corresponding α-hydroxycarboxylic acids. Reduced glutathione serves as cofactor (3-6). The physiological role of these reactions is still uncertain. The system consists of two enzymes, glyoxalase I and glyoxalase II (7). Glyoxalase I (S-Lactoylglutathione methylglyoxal-lyase (isomerizing), E.C. 4.4.1.5) acts upon the equilibrium adduct of methylglyoxal and GSH, a hemimercaptal, with the resultant formation of the corresponding thioester, S-D-lactoylglutathione. Glyoxalase II (S-2-hydroxyacylglutathione hydrolase, E.C. 3.1.2.6) hydrolyzes the thioester to regenerate GSH and liberate free D-lactic acid.

A number of roles have been suggested for the glyoxalase system such as protection against α-ketoaldehyde toxicity (8) and regulation of cellular growth (9-11). The glyoxalase system has been shown to affect cell-free microtubule assembly (12), and it is thought to be

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involved in a glycolytic bypass system from dihydroxyacetone phosphate to D-lactate via methylglyoxal synthase and glyoxalases (13).

In the literature, from 1957 on, there have been numerous reports on the inhibition of glyoxalase I by different GSH derivatives or substrate analogs (14-18); these compounds are typically competitive inhibitors. Recently, there have been reports on some glyoxalase I inhibitors which are not substrate analogs (19-21); both competitive and noncompetitive inhibitions were observed. Few reports are in the literature relating to inhibitions of glyoxalase II (22).

We report herein the inhibitions of both glyoxalase enzymes (glyoxalase I and glyoxalase II) by nucleotides, nucleosides and a series of structurally related compounds.

MATERIALS AND METHODS

General. For the studies involving mouse liver glyoxalases the enzyme preparations used were the homogeneous enzymes obtained in this laboratory with affinity chromatographic procedures (22,23). Pseudomonas fluorescens was grown on Trypticase soy broth, and extracts were prepared by mechanical homogenization. Yeast glyoxalase I and glutathione were purchased from Sigma Chemical Co., St. Louis, MO. Methylglyoxal was prepared by the method of Kellum et al. (16). S-D-lactoylglutathione was prepared and purified by the procedure of Uotila (25). Nucleotides, nucleosides and structurally related compounds were purchased either from Sigma Chemical Co., St. Louis, MO, or from Calbiochem, Los Angeles, CA. S-Octylglutathione was prepared by reacting 1-bromooctane with GSH employing Method A of Vince et al. (14). All other chemicals were the highest purity obtainable from commercial sources.

Enzyme assays. The details for the spectrophotometric assays for both enzymes, based on the absorbance of S-D-lactoylglutathione at 240 nm are explained elsewhere (22,23).

RESULTS AND DISCUSSION

Nucleotides, nucleosides and some structurally related compounds are potent inhibitors of both glyoxalase enzymes derived from different sources. Fig. 1 shows the activity response obtained with different nucleotides for pure mouse liver glyoxalase I. Similar results were

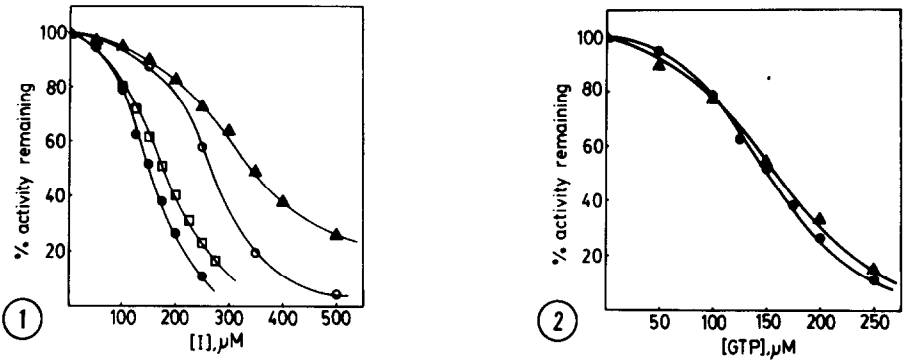

Fig. 1. Inhibition of Glyoxalase I by GTP, ATP, CTP and UTP.
The calculated hemimercaptal concentration is 0.2 mM. The free GSH concentration, after equilibration, is 1.55 mM. Glyoxalase I used in this study was the homogeneous enzyme preparation obtained from mouse liver (23). ●—●, GTP; □—□, ATP;○—○, CTP;▲—▲, UTP.

Fig. 2. Inhibition of both Glyoxalases by GTP.
The calculated hemimercaptal concentration is 0.2 mM. The free GSH concentration, after equilibration, is 1.55 mM. The S-D-lactoyl-glutathione concentration for the glyoxalase II assay is 0.4 mM. Both glyoxalases were obtained from mouse liver (22,23). ●—●, glyoxalase I; ▲—▲, glyoxalase II.

obtained for pure mouse liver glyoxalase II. As an example, Fig. 2 shows the effect of GTP on both enzymes; the inhibitions exhibit cooperativity.

S-Octylglutathione is a competitive inhibitor of the glyoxalase enzymes, and it has been used in this laboratory as an affinity ligand for the purification of mouse liver glyoxalase I (23) and also as an eluant of mouse liver glyoxalase II from an affinity chromatography column (22). The glyoxalase I inhibition response curve given by S-octylglutathione is quite different from the response curve given by nucleotides, as shown in Fig. 3 for GTP. When a Hill plot (log (%Inh./(100-%Inh.)) vs. log(GTP) (26)) is constructed with the data of Fig. 3, a Hill coefficient (slope) of 3.3 is obtained for the GTP inhibition.

Inhibitions of the mouse liver glyoxalase enzymes by some nucleic acid bases, nucleosides, nucleotides and a series of structurally related compounds are summarized in Table I. I_{50} values for each

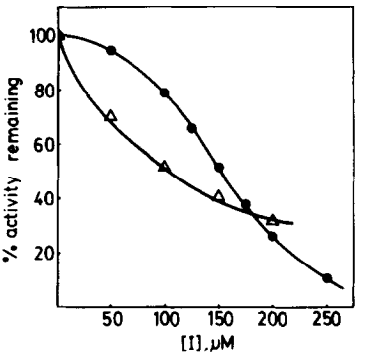

Fig. 3. Inhibition of Glyoxalase I by GTP and S-Octylglutathione. The calculated hemimercaptal concentration is 0.2 mM. The free GSH concentration, after equilibration, is 1.55 mM. The glyoxalase I used in this study was the homogeneous enzyme preparation obtained from mouse liver (23). ●—●, GTP; △—△, S-octylglutathione.

inhibitor are given; all compounds listed in the table inhibit both glyoxalase enzymes in a cooperative manner. Within a given family of nucleic acid bases, there is no difference in the extent of inhibitions given by the free base, the corresponding nucleoside, or the nucleoside 5'-mono-, di- or triphosphates. In addition to the inhibitors listed

TABLE I

I_{50} VALUES (μM) FOR SOME GLYOXALASE INHIBITORS

Inhibitor	Glyoxalase I[a,b]	Glyoxalase II[a,c]	Hill Coefficient
GTP, GDP, GMP, guanosine, guanine	150	160	GTP=3.3
ATP, ADP, AMP, adenosine, adenine	175	175	ATP=3.24
CTP, CDP, CMP, cytidine	250	250	
benzimidazole	275	250	
xanthine	300	290	
UTP, UMP, uridine, uracil	350	350	UTP=2.8
pyridoxamine	475	500	
pyridoxal	500	475	
theophylline	500	500	
thymine	550	550	
aniline	550	550	
caffeine	575	550	
theobromine	600	575	
2-aminopyridine	600	600	
trypthophan	900	800	

(a) Both enzymes used in this study were homogeneous enzyme preparations from mouse liver (22,23).

(b) For glyoxalase I assays the hemimercaptal concentration is 0.2 mM and the free GSH concentration after equilibration is 1.55 mM.

(c) For glyoxalase II assays the S-D-lactoylglutathione concentration is 0.4 mM.

in Table I, pyridine nucleotides, cAMP, and cGMP were studied. Significant inhibition (35-60%) of both glyoxalase enzymes was given by these nucleotides at 150 μM concentrations. In general, aromatic compounds, which contain heterocyclic nitrogen and/or have amino groups on the aromatic ring, are effective cooperative inhibitors of both glyoxalase enzymes. Compounds not having these structural features (e.g., tyrosine, phenylalanine, histidine, arginine, imidazole) do not inhibit either enzyme, even at concentrations above 2 mM.

Comparisons of the inhibitions (by nucleotides) of both glyoxalases from different sources were undertaken. There are no differences between the inhibitions of mouse liver glyoxalase I and the yeast enzyme. Nucleotides are comparatively better inhibitors of the glyoxalases from Pseodomonas fluorescens than of these enzymes from mouse liver. Interestingly, the inhibitions of glyoxalase II from the bacterial source do not exhibit sigmoidal kinetics.

Since intracellular levels of some of the nucleotides (27,28) (e.g., ATP) are significantly higher than the concentrations needed to inhibit both enzymes completely (0.5 mM), the inhibitions observed may have physiological significance. The glyoxalases are either completely inactive in vivo (there is experimental evidence to the contrary (29)), or they are active because of isolation from inhibitors through compartmentilization, or there is a variable endogeneous activator which reverses the inhibition of the enzymes, thereby regulating glyoxalase enzyme activity. The identification of such an activator would provide much information about the function of the glyoxalase enzymes.

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