1-AMINOGLYCOSIDES, A NEW CLASS OF SPECIFIC INHIBITORS OF GLYCOSIDASES[+]

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SUMMARY: 1-Aminoglycosides represent a new class of specific and relatively potent inhibitors of glycosidases. These compounds are specific against those enzymes which act upon glycosides that correspond to glycone of the inhibitor. Thus α- and β-D-glucosidases are inhibited by D-glucosylamine but not by D-galactosylamine and D-mannosylamine. α- and β-D-galactosidases are inhibited by D-galactosylamine but not by the other two glycosylamines. D-Mannosylamine inhibits mannosidase.

It is well known that free sugars which are the products of glycosidase reactions are competitive inhibitors of the enzyme. Tris is also known to be a competitive inhibitor of several glycosidases (1). Studies made with variously hydroxylated alkyl amines have indicated that the inhibitory nature of Tris may be due to the combined influence of the amino group and the polyhydroxy constellation (2). Ammonium ion alone is not inhibitory. We have reasoned that a compound possessing, at once, the essential configuration of a specific glycone as well as an amino group, might act as an effective inhibitor of the corresponding glycosidase. Glycosylamines meet these specifications and accordingly several have been prepared and tested against a variety of glycosidases with encouraging results.

MATERIALS AND METHODS

Chemicals - p-Nitrophenyl α-D-glucoside and p-nitrophenyl α-D-galactoside were obtained from Aldrich; p-nitrophenyl β-D-glucoside and o-nitrophenyl

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β-D-galactoside were from Calbiochem and from Mann Research Laboratories. β-D-Glucosylamine, α-D-galactosylamine and β-D-mannosylamine were prepared by the method of Isbell et al. (3). All inhibitor solutions were freshly prepared in 0.067 M phosphate buffer, pH 6.8, before use.

Enzymes - Maltase and α-methylglucosidase from Saccharomyces oviformis were purified from freshly harvested cells by ammonium sulfate fractionation, chromatography on DEAE-sephadex and chromatography on hydroxyapatite C.[1] These enzymes were homogeneous by disc gel electrophoresis. β-Glucosidase and β-galactosidase from Kluyveromyces fragilis and α-galactosidase from Saccharomyces carlsbergenesis were obtained from crude extracts of freshly harvested cells by repeated freezing and thawing. Bacterial β-galactosidase was obtained from Escherichia coli (strain W3110) grown in single strength minimal medium (4) with 0.2% lactose as inducer. Emulsin, purchased from Calbiochem, also served as a source of β-glucosidase and α-galactosidase. α-Galactosidase from Vicia faba was prepared as described by Dey and Pridham (5). α-Mannosidase was obtained as a crude extract from hog kidney.

Enzyme Assays - Glycosidase activities were measured by a slight modification of Halvorson's method (6). The assay reaction mixture contained 2.7 ml of 0.0667 M phosphate buffer, pH 6.8, 0.1 ml of 3.25 mM reduced glutathione, 0.1 ml of nitrophenyl glucoside and 0.1 ml enzyme. The concentration of the added substrate was 10 mM except for yeast α-galactosidase when 30 mM was used because of its high K_M with this enzyme. Hydrolysis was allowed to proceed for 2 to 30 minutes depending on the enzyme activity. The reaction was stopped by the addition of 1 ml of 16% (w/v) Na_2CO_3. The liberated p-nitrophenol or o-nitrophenol was measured at 400 nm or 420 nm, respectively.

RESULTS AND DISCUSSION

The K_I values for D-glucosylamine, D-galactosylamine and D-mannosylamine against several glucosidases are given in Table I, together with the K_M values

[1] H. Ashe, T. Fukui and B. Axelrod, manuscript in preparation.

TABLE I

K_M values for p-nitrophenyl α-D-glucoside, p-nitrophenyl β-D-glucoside, p-nitrophenyl α-D-galactoside and o-nitrophenyl β-D-galactoside for various glycosidases and the corresponding K_I values for glycosylamines.

Substrate concentrations were 0.16--0.67 mM for p-nitrophenyl α-D-glucoside, 0.13--2.67 mM for p-nitrophenyl β-D-glucoside, 0.26--4 mM for p-nitrophenyl α-D-galactoside, 0.43--2.15 mM for o-nitrophenyl β-D-galactoside and for p-nitrophenyl α-D-mannoside. Inhibitor concentrations were 0.57--40 μM for glucosylamine, 30--800 μM for α-galactosylamine and 3.7--37 μM for mannosylamine. Assays were done in 0.067 M phosphate buffer, pH 6.8. Values for K_M and K_I were calculated by the method of Lineweaver and Burk.

Enzyme	Source	K_M (mM)	K_I (mM)		
			Glucosyl-amine	Galactosyl-amine	Mannosyl-amine
Maltase	Yeast	0.162	0.027	----	----
α-Methylglucosidase	Yeast	0.368	0.0318	----	----
β-Glucosidase	Yeast	0.143	0.00227	----	----
α-Galactosidase	Yeast	16.7	----	0.156	----
β-Galactosidase	Yeast	1.66	----	0.0488	----
α-Galactosidase	*Vicia faba*	0.512	----	0.0725	----
β-Galactosidase	*E. coli*	0.572	----	0.225	----
α-Mannosidase	Hog kidney	0.635	----	----	0.0042

for the substrates employed. Although the substrates used in each case have relatively favorable K_M values, it will be noted that the K_I values are substantially smaller. The Lineweaver-Burk plots from which these values were obtained showed the inhibition to be competitive in all cases.

The results summarized in Table II permit a comparison of the relative effects of the three aldosylamines on any given enzyme. An apparent exception to the rule that only the corresponding glycosylamine is a good inhibitor is seen in the cases of β-galactosidase and β-glucosidase from emulsin. Each enzyme is almost equally inhibited by both D-glucosylamine and D-galactosyl-

amine. However, this anomaly is readily resolved in light of the demonstration that a single enzyme in emulsin possesses both activities (7).

The glucosylamines thus afford a new class of competitive glycosidase inhibitors of high specificity and with a moderate to powerful inhibitory potency depending on the enzymes. When acting on β-glucosidase from yeast, D-glucosylamine has a K_I value of 2.2×10^{-6} M making it one of the most potent competitive glycosidase inhibitors reported. The corresponding free carbohydrates are from one to three orders of magnitude less effective.

With respect to the anomeric specificity of the inhibition, the D-glucosylamine as ordinarily prepared and as used in these experiments is the β form and is known to be stable in solution (3). This inhibitor proved effective against both α- and β-glucosidases. D-Galactosylamine and D-mannosylamine are both known to mutarotate rapidly in solution (3). Hence no final conclusion can be drawn from these experiments as to anomeric requirements for inhibition.

A common, albeit speculative, mechanism proposed for glycosidase action invokes general acid catalysis promoted by an acidic group in the active center of the enzyme (8). The reaction, as visualized, proceeds via a glycosyl carbonium ion, presumably in a half-chair conformation. Indirect support for this proposal arises from studies of non-enzymic catalysis (9) and from enzymic studies with inhibitors such as aldonolactones (10, 11) and glycals (12). Recent crystallographic and chemical studies made with lysozyme, which is the only glycosidase whose structure is known, offers more tangible support for the above proposal and strongly suggests that the free carboxylic acid groups of the aspartic and glutamic residues located in the proximity of the glycosidic bond undergoing scission, participate in the formation and stabilization of an intermediate glycosyl carbonium ion, by charge interactions (13). Moreover, steric considerations are consistent with the idea that the transition state of the substrate, namely the glycosyl carbonium ion, must be in the half-chair form to be accommodated in the available space.

TABLE II

Inhibition of Glycosidases by Aldosylamines

Enzyme	Source	Glucosylamine		Galactosylamine		Mannosylamine	
		Conc.	Inhibition	Conc.	Inhibition	Conc.	Inhibition
		mM	%	mM	%	mM	%
α-Glucosidase (maltase)	Yeast	0.02	50	15.0	50	10.0	50
β-Glucosidase	Yeast	0.002	50	2.9	50	13.3	0
α-Galactosidase	Yeast	1.8	0	0.09	50	7.8	0
β-Galactosidase	Yeast	2.0	0	0.04	50	3.8	0
β-Galactosidase	*E. coli*	12.0	5	0.6	50	7.2	0
β-Glucosidase	Emulsin	1.0	50	1.0	50	12.3	5
β-Galactosidase	Emulsin	0.95	50	1.3	50	9.6	12
α-Galactosidase	Emulsin	3.2	0	0.09	50	5.8	0
α-Galactosidase	*Vicia faba*	5.0	0	0.062	50	8.5	0

It is attractive to speculate, if one may generalize from the lysozyme model, that glycosylamines exhibit a strong affinity for the glycosidases because they are bound not only at the site that determines the glycone specifity, but also through their glycosidic amine at the acidic site present in the catalytic center. Whether the glycosylamine is more prone to assume the half-chair conformation than the free sugar remains to be established.

It is worth noting that 2-amino-2-deoxy-D-glucose is not an inhibitor of yeast glucosidase. On the other hand, the antibiotic nojirimycin (5-amino-5-deoxy-D-glucose) is a highly effective inhibitor of α- and β-glucosidases. It has been suggested that nojirimycin, as the N-analog of D-glucopyranose, is effective because it forms an imine by elimination of H_2O between N and C-1. The imine, presumably resembling the glycosyl cation, acts as an inhibitor (14).

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