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INHIBITION OF VARIOUS MAMMALIAN AMYLASES BY BERYLLIUM AND ALUMINUM*

ROBERT L. MCGEACHIN, WANDA M. PAVORD and WILLIAM C. PAVORD

Department of Biochemistry,
University of Louisville School of Medicine, Louisville, Ky., U.S.A.

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Abstract—Beryllium and aluminum salts in concentrations from 10^{-2} to 10^{-5} M will inhibit amylases from man, hog, rat, and dog to varying degrees. Magnesium, barium, and strontium salts do not inhibit these amylases. Certain proteins or salts of various carboxylic acids reduce the inhibiting effects of beryllium and aluminum. It is postulated that beryllium and aluminum produce these effects by complexing the free carboxyl groups of the amylases in such fashion that the structure of the original molecule is deformed and the enzyme activity is reduced.

FISCHER *et al.*,[1] Oikawa and Maeda,[2] and others have shown that calcium is an integral part of all known purified α-amylases and that added calcium ion increases the stability of amylases in solution. In the course of investigating the effects of other group II elements on amylase, we have found that beryllium salts inhibit the activity of several mammalian amylases.[3] The inhibiting effect of beryllium on alkaline phosphatases is well known, [4, 5] although no mechanism for its action has been determined. Ono and Hiromi[6] mentioned beryllium as a weak inhibitor of a bacterial amylase but apparently did not study the effect very extensively. Yamamoto and Fukumoto[7, 8] have shown that *Bacillus subtilis* α-amylase, inactivated by ethylenediamine tetraacetate (EDTA), can be reactivated by adding calcium acetate. In testing the reactivating properties of alkali metal acetates and other alkaline earth metal acetates, they found that beryllium acetate produced only 20 per cent as much reactivation as that obtained from calcium acetate.

In this communication we have reported the inhibition of several mammalian amylases, particularly hog pancreatic and human salivary amylases, by beryllium and aluminium salts and have described the modifying influences of proteins and various carboxylic acids.

MATERIALS AND METHODS

Materials. The crystalline hog pancreatic amylase used was obtained from the Worthington Biochemical Corporation[9] and recrystallized once by us. The human salivary amylase was prepared from pooled fresh human saliva.[10] The other amylase-containing solutions used were obtained by homogenizing or diluting the appropriate tissues or fluids with 0·11 N KCl. Human urine used was first exhaustively dialyzed against water to remove phosphate.

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The beryllium salts used were: beryllium sulfate, CP grade, E. H. Sargent & Co.; beryllium acetate, highest purity, Amend Drug and Chemical Co. The aluminium sulfate was CP grade obtained from the J. T. Baker Chemical Company.

The proteins used were obtained from the following sources: crystallized bovine plasma albumin, The Armour Laboratories; gelatin, USP powder, J. T. Baker Chemical Company; gliadin and protamine sulfate, Nutritional Biochemicals Corporation.

Analytical methods. Amylase activities were determined by Van Loon's amyloclastic method[11] modified by using 0·11 N KCl instead of 0·9 per cent NaCl (so that the KCl concentration in the final reaction mixture would be 0·01 N) and 0·02 M, pH 7, Tris-acetate buffer instead of 0·02 M phosphate.

To test the effect of added beryllium or aluminium, experiments of the following type were performed: 5 ml of 0·44 M KCl was mixed with 5 ml of 0·44 M $BeSO_4$ and then 10 ml of the amylase-containing solution was added. 1-ml aliquots for amylase analysis were removed and added to 9 ml of the starch-buffer substrate at 0, 10, 30, and 60 min after the addition of the enzyme to the KCl-beryllium mixture. In this example the concentration of $BeSO_4$ in the final reaction mixture was 0·01 (10^{-2}) M. For other molarities the initial concentration of $BeSO_4$ was varied accordingly. With hog pancreatic amylase the solution added to the KCl-beryllium mixture contained 0·2–0·24 μg per ml. For other amylases the solutions used contained amylase activities equivalent to those with hog pancreatic amylase. Unless indicated otherwise, the values for amylase inhibitions reported in the tables are those obtained at the 60-min time.

The experiments for studying the modifying effects of acetate or protein on inhibition by beryllium or aluminium were carried out in two ways. In the first experiments the acetate or protein was added to the amylase-containing solution before the beryllium or aluminium was added. In later experiments the acetate or protein was added 30 min after the addition of beryllium in order to test the reversibility of the enzyme inactivation (see Table 3 for details).

RESULTS

Since the use of a phosphate buffer in the amylase analysis caused a turbidity when beryllium was present, pH 7·0 Tris buffer was substituted. We determined that the activities of hog pancreatic and human salivary amylases are not changed by the change in buffer.

It is apparent from Table 1 that beryllium salts have an inhibiting effect on amylases, although human salivary amylase is inhibited less than is hog pancreatic amylase. Trials with magnesium, barium, and strontium acetates showed that these metals do not inhibit amylase.

In the first experiments involving beryllium salts the acetate was used, but since some difficulty was encountered in getting beryllium acetate into solution, beryllium sulfate was tried. It was immediately apparent that, at equimolar concentrations, the sulfate inhibited amylase more strongly than did the acetate. When the modifying effects of calcium and magnesium acetates on inhibition by beryllium sulfate were tested, it was found that these salts reduced the inhibition. We found, however, that *sodium* acetate would also reduce beryllium sulfate inhibition so that the modifying effects were apparently due to the acetate and not to the cations. Furthermore,

addition of calcium chloride did not alter the inhibition by beryllium sulfate. Beryllium chloride at equivalent concentrations caused the same inhibition as did beryllium sulfate. Control experiments with sodium sulfate in the absence of added beryllium indicated that sulfate ion per se does not inhibit amylase. The presence of the sodium salts (adjusted to pH 7·0) of succinic, tartaric, and malic acids reduced the inhibition of hog pancreatic amylase by beryllium sulfate, at 10^{-3} M, to 40 per cent.

TABLE 1. INHIBITION OF AMYLASES BY BERYLLIUM SALTS

	per cent inhibition of amylase activity by				
Amylase	10^{-2} M $BeSO_4$	10^{-3} M $BeSO_4$	10^{-4} M $BeSO_4$	10^{-3} M $BeSO_4$ + 10^{-3} M Ac^-	10^{-3} M $BeAc_2$
Hog pancreatic	100	95	80	40	30
Human salivary	100	70	60	40	35
Human pancreatic	95	60		40	
Human serum	65	10		0	
Human urinary	95	55		35	
Dog pancreatic		95			

The concentrations indicated for the beryllium salts were those in the final reaction mixture in which the beryllium-amylase combination had been added to the starch-buffer mixture.

The inactivation by beryllium sulfate was not instantaneous and did not reach its maximum until about 30 min at 10^{-2} M and not until 60 min at 10^{-3} and 10^{-4} M. For example, percentage inhibitions of hog pancreatic amylase by 10^{-3} M beryllium sulfate were 35, 50, 80, and 95 at 0, 10, 30, and 60 min, respectively. As was previously indicated, all values of inhibition or inactivation given in the various tables, unless indicated otherwise, were those obtained with a 60-min reaction time of beryllium on amylase.

We discovered that the presence of certain proteins would also reduce the beryllium inhibition. Table 2 shows that both bovine plasma albumin and gelatin will protect

TABLE 2. EFFECT OF PROTEIN ON INHIBITION OF AMYLASE BY BERYLLIUM

	per cent inhibition of amylase activity by			
		10^{-3} M $BeSO_4$ + protein		
		Bovine plasma albumin		Gelatin
Amylase	10^{-3} M $BeSO_4$	0·7 mg	7 mg	0·7 mg
Human serum	15			
Human salivary	70	40	20	50
Human urinary	50	30	30	40
Hog pancreatic	95	40	40	

hog pancreatic and human salivary amylases against inhibition by beryllium. Human serum amylase (serum diluted 1 : 10) was inhibited only 15 per cent as compared with 70 per cent for human salivary amylase, but when the inhibition of human salivary amylase was tested in the presence of 7 mg (the amount of protein in 1 ml of a 1 : 10

dilution of human serum) of added bovine plasma albumin, the inhibition was reduced to 20 per cent.

To test the reversibility of the amylase inactivation by beryllium, protein or acetate was added in some experiments 30 min after mixing the amylase and beryllium. The data in Table 3 indicate that the beryllium inactivation may be reversed by plasma

TABLE 3. REVERSAL OF BERYLLIUM INHIBITION OF HOG PANCREATIC AMYLASE BY PROTEIN

Time of action of beryllium on amylase	per cent inhibition of amylase				
			Protein added		
	Control	Sodium acetate added	7 mg albumin	70 mg albumin	70 mg gelatin
0	30				
10	35				
30	50				
60	70	65	25	0	20

In these experiments the amylase was added to the beryllium sulfate-potassium chloride mixture at time zero, and 1-ml aliquots of this ternary mixture were added to 9 ml of the starch-buffer substrate at the times indicated. The reactants were prepared so that the final reaction mixture was 10^{-4} M beryllium sulfate, 10^{-2} M potassium chloride and contained 0·20–0·25 Van Loon units of (0·1–0·12 μg) hog pancreatic amylase. At the end of 30 min from time zero the $BeSO_4$-KCl-amylase mixtures were divided into two portions. To one portion, sodium acetate (final concentration 10^{-2} M) or protein was added, the second portion serving as the control. At 60 min from time zero, 1-ml aliquots of each mixture were removed for determination of amylase activity.

albumin or gelatin but not by acetate. The degree of reversal is dependent to some extent on the amount of protein added. When 7 mg of plasma albumin was added, the inhibition was reduced to 25 per cent, compared with 70 per cent with no added protein; but with 70 mg of added albumin the beryllium inhibition was completely reversed. Gelatin brought about some reversal of the inactivation but was not so effective as plasma albumin in this regard.

With protamine, the addition of amounts up to 1 mg (if larger amounts than 1 mg were added, there was coagulation of starch particles in the reaction mixture and interference with the amylase analysis) had no protecting effect on inhibition by beryllium of hog pancreatic amylase. In fact, the protamine alone caused inhibition of 80 per cent, and the combination of beryllium and protamine gave 95 per cent inhibition. Since gliadin is not very soluble in aqueous solution, we used saturated solutions of this protein in these studies and so could add only about 0·5 mg. As with protamine, gliadin did not protect against inhibition of hog pancreatic amylase by beryllium and was itself inhibitory.

In Table 4, which illustrates the inhibition of rat amylases by beryllium sulfate, it is apparent that the inhibition is lowest for serum, whose protein content is highest and, conversely, the inhibition is highest for the diluted saliva, whose protein content is lowest.

Efforts to reverse the beryllium inhibition of amylases by dialyzing with disodium EDTA were unsuccessful. Even with equimolar or greater amounts of calcium present in the dialyzing fluid, there was no reversal of inhibition but, rather, complete loss of activity. Surprisingly, dialysis of the amylase alone with EDTA and added calcium still resulted in loss of activity, even with 0·02 M EDTA and 0·06 M calcium

chloride, a threefold excess of calcium. Dialysis of the amylases against calcium chloride alone gave no loss of activity.

Aluminium sulfate inhibited both hog pancreatic and human salivary amylases (Table 5) as effectively as did beryllium sulfate, and this inhibition was reduced by added bovine plasma albumin.

TABLE 4. INHIBITION OF RAT AMYLASES BY BERYLLIUM SULFATE

Conc. of $BeSO_4$	per cent inhibition of amylase activity		
	Amylase		
	Salivary	Pancreatic	Serum
M			
10^{-2}	95	95	85
10^{-3}	90	70	65
10^{-4}	60	50	25
10^{-5}	45	15	5

These solutions containing rat amylases were a 1 : 100 dilution of rat serum with 0·11 M potassium chloride, a 1 : 30,000 dilution of rat saliva, and a 1 : 100,000 dilution of homogenized rat pancreas.

TABLE 5. INHIBITION OF AMYLASES BY ALUMINIUM SULFATE

Amylase	per cent inhibition of amylase activity by		
	Concentration of aluminum sulfate		
	10^{-3} M	10^{-3} M + 0·7 mg albumin	10^{-4} M
Hog pancreatic	95	40	75
Human salivary	90	35	75

The effects on hog pancreatic and human salivary amylases of sodium oxalate and the sodium salt of phytic acid (phytic acid is the hexaphosphoric acid ester of inositol) have been studied. Although both oxalate and phytate are known to precipitate calcium, it was found that, at 10^{-3} M, oxalate had no effect, and phytate caused only 20 per cent inhibition. Citrate at 10^{-3} M had no effect on either amylase.

DISCUSSION

Schneyer[12] found that uranium nitrate inhibited human salivary amylase and that this inhibition could be partially prevented by certain carboxylic acids. He postulated that the inhibition involved combination of electronegative groups on the enzyme.

We think it likely that beryllium (and aluminium) salts at least partially produce their inhibiting effects on amylases by complexing the free carboxyl groups in such fashion that the structure of the original molecule is deformed and the enzyme activity is thus reduced. The deforming power of beryllium and aluminium would be large compared with that for calcium, since the field strength of beryllium,[13] as measured by the ionic charge divided by the square of the ionic radius, is 17·3 (9·2 for aluminium) as compared with 3·3 for magnesium and 1·8 for calcium. This helps

explain the "diagonal" resemblance of beryllium in group II of the periodic table to aluminium group III. It is known that several gram-atoms of calcium per mole of amylase are loosely bound[14] in addition to the one calcium atom that is an integral part of the molecule. Beryllium, which very readily forms complexes[13] with carboxylic acids, has an attraction for carboxylate ions much greater than that of calcium. It is known[15] that amylases contain large amounts of aspartic and glutamic acids and have many free carboxyl groups in their side chains with which the beryllium could react. Our data show that the addition of either salts of carboxylic acids or certain proteins will partially prevent the inhibition of amylases by beryllium.

Furthermore, we have shown that such inhibition can be at least partially and, under some conditions, completely reversed by the addition of plasma albumin or gelatin. This also indicates that the inactivation is not due to an irreversible denaturation of the amylase by beryllium. Neither protamine nor gliadin, proteins without free carboxyl groups in their side chains, furnished any protection to amylase against inhibition by beryllium.

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