INFLUENCE OF ANIONS ON THE ACTIVITY OF PIG PANCREATIC ALPHA-AMYLASE

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ABSTRACT

Pancreatic α-amylase activity has been found to be inhibited by sodium fluoride, ammonium chloride, sodium sulphate, sodium nitrate and sodium bromide. The inhibition was reversible and noncompetitive in nature; each anion produced a substantial decrease in the maximum attainable velocity without having any detectable effect on the Michaelis–Menten constant. The values for 50% inhibition (I_{50}) were: sodium fluoride, 0.25 mM; ammonium chloride, 2.3 mM; sodium sulphate, 3.0 mM; sodium nitrate, 4.7 mM; and sodium bromide, 7.2 mM. The apparent inhibition constant (K_i) for ammonium chloride was found to be 0.5 mM. These observations provide additional evidence that salts alter enzyme activity by altering the organized structure of the protein.

INTRODUCTION

MOST studies of α-amylase have been focused on the amylase of mammalian digestion. Microbial and animal enzyme extracts have been used over many years as supplement in enzyme deficiencies of the pancreas and small intestine. Mammalian α-amylases are therefore of great importance. Anions play a vital role in the growth and development of tissues. The Hofmeister lyotropic series of ions describes the order of effectiveness of ions in influencing a very large number of chemical and physical properties of proteins. The series provides an ordering of both anions and cations for effectiveness in causing denaturation, in influencing activity coefficient, and in leading to structural transition in macromolecules[1]. It has been recently demonstrated that neutral salts at high concentration inhibit the activity of widely different enzymes[2]. Several heavy metal ions have been shown to inhibit mammalian amylases[3]. The crystalline α-amylase of fungal and pancreatic origin is inactivated by many high molecular ammonium quaternary salts, cationic dyes and sodium lauryl sulphate[4]. Several anions, like NO_3^-, Cl^-, Br^-, I^- and thiocyanate, increase the rate of salivary amylase inhibition[5]. Ammonium ion has been reported as an inhibitor of α- and β-amylase from rice. The extent of inhibition has been reported[6] to be 7 to 42%. In our studies on pancreatic α-amylase, the enzyme showed specific anion inhibition, which was strikingly noncompetitive. In this communication we report the effect of five salts, viz. sodium fluoride, sodium bromide, ammonium chloride, sodium nitrate and sodium sulphate on the activity of pancreatic α-amylase. Such studies have not been carried out earlier on pancreatic amylase.

MATERIALS AND METHODS

Pancreatic α-amylase was isolated and purified from pig pancreas according to the method of Fischer *et al.*[7] The enzyme was homogeneous with respect to size and charge as indicated by gel electrophoresis. All chemicals used were of analytical grade. Amylase activity was measured by using dialysed starch as substrate and measuring the amount of reducing sugar liberated by the method of Bernfeld[8]. Protein concentration was determined by the method of Lowry *et al.*[9] The inhibitory activity of anions was determined by incubating different concentrations of the respective salts (0–10 mM) with pancreatic amylase for 10 min, and measuring residual enzyme activity at 540 nm in a Hitachi spectrophotometer, model 200-20. The stoichiometry of anion binding was determined by plotting the data according to Johnson *et al.*[10], in the form of a $\log (V_0 - V_i)/(V_i)$ vs $\log$ (I) plot. The nature of inhibition was determined by the method of Lineweaver and Burk[11]. The apparent inhibition constant (K_i) was determined from a Dixon plot[12], with two different substrate levels (1 to 2%) and various inhibitor concentrations.

RESULTS AND DISCUSSION

Only sodium fluoride (NaF) had a pronounced inhibitory effect at the lowest concentrations (70%

at 1 mM). For each anion, the value of 50% inhibition (I_{50}) was read from the curves shown in figure 1. The following I_{50} values for the different anions were calculated: sodium sulphate, 3.0 mM; sodium nitrate, 4.7 mM; sodium bromide, 7.2 mM; sodium fluoride, 0.25 mM; and ammonium chloride, 2.5 mM. In each case, the anion inhibition was found to be reversible and could not be released by increasing the concentration of the substrate. The stoichiometry of anion binding was determined by plotting the data in the form of a $\log (V_0 - V_i)/(V_i)$ vs log (I) plot. Such a plot for sodium fluoride is shown in the inset of figure 1. The plots for the other anions were similar. In each case the relationship was linear. The slope was close to 1.0 for all the five anions studied, suggesting that cooperativity was not present[13]. Ammonium chloride had no effect on the Michaelis–Menten constant, but caused significant decrease in the maximum attainable velocity, V_{max} of the enzyme. These observations suggest that ammonium chloride inhibition is noncompetitive (figure 2). The apparent inhibition constant (K_i) for pancreatic amylase was found to be 0.5 mM.

Data on the effect of different salts on pig pancreatic α-amylase are given in table 1. Ammonium salts were more effective in inhibiting the amylase.

Meur and De[14] studied the activity of human salivary amylase in the presence of anions, and the effect of anions on the pH optimum of the enzyme. Alpha-amylase activity was determined in Tris–anion

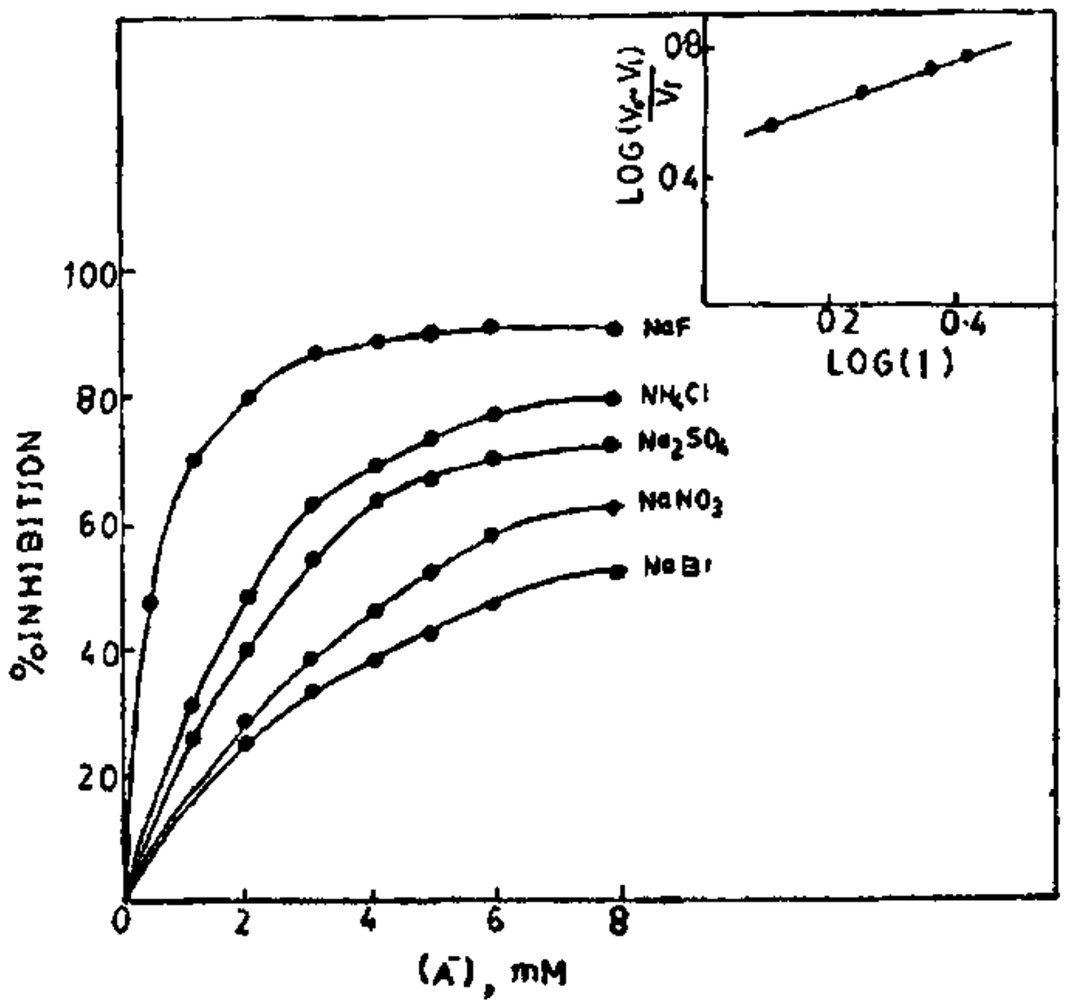

Figure 1. Inhibition of pig pancreatic α-amylase by anions at pH 6.9. Inset shows the plot of $\log (V_0 - V_i)/(V_i)$ vs log (NaF).

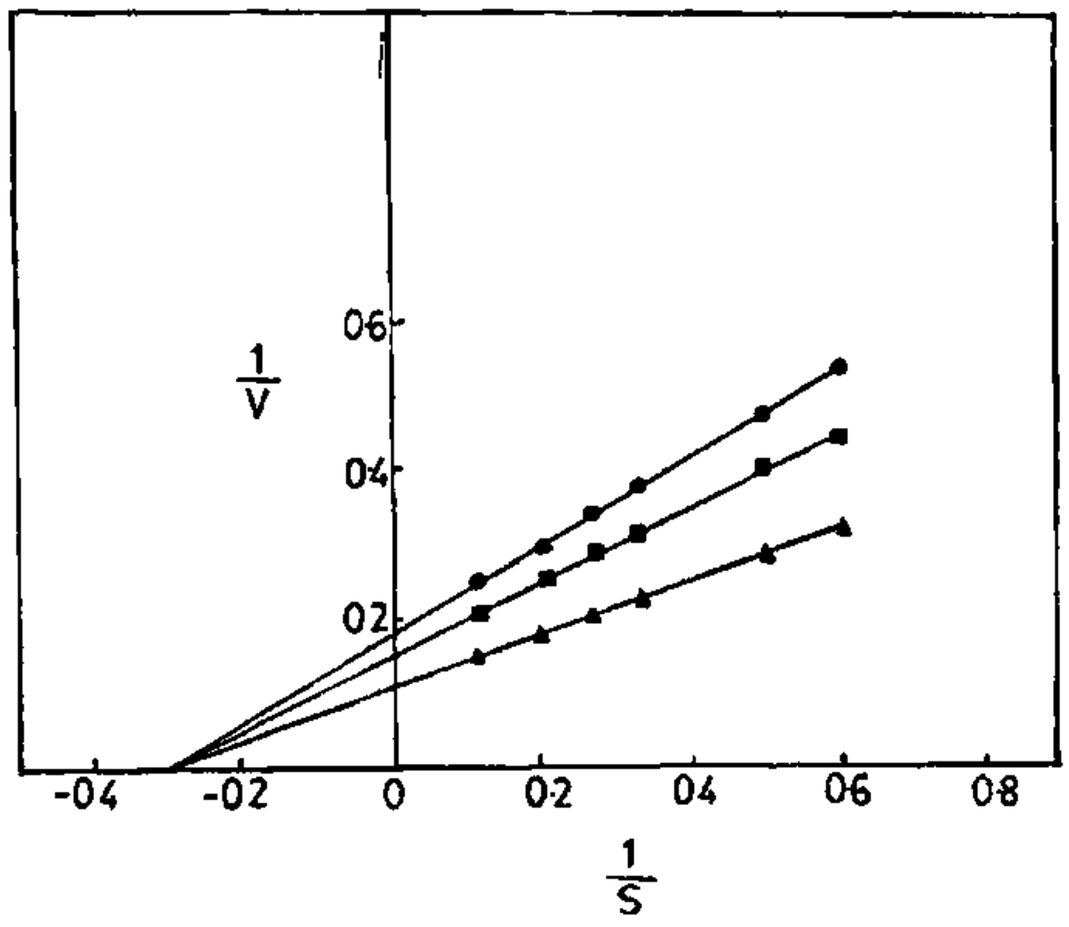

Figure 2. Lineweaver–Burk plot showing noncompetitive type of inhibition of pig pancreatic amylase by ammonium chloride. [●——● control, ■——■ 1 mM NH_4Cl, ▲——▲ 2 mM NH_4Cl.]

Table 1 *Inhibition by different salts on activity of pig pancreatic α-amylase*

Salt	Inhibition (%) 0.2*	0.4	0.6	0.8	1.0
Ammonium persulphate	20	40	50	100	100
Ammonium acetate	10	30	40	56	70
Ammonium ceric sulphate	65	100	100	100	100
Ammonium oxalate	20	75	50	75	100
Molybdic acid	25	35	61	70	71
Phosphomolybdic acid	75	51	91	95	100
Ammonium hydrogen sulphate	25	81	55	72	100
Ammonium sulphate	21	54	60	100	100

*Concentration, in mM.

buffer at pH 6.0, 6.9 and 7.0, and in 0.02 M phosphate buffer at pH 6.9 in the presence of various salts. At pH 6.0, different Tris-anion buffers had little effect on amylase activity. However, at pH 7.0, the activity was markedly affected by anions (aspartate < acetate < phosphate < oxalate < chloride)[14].

It has long been recognized that mammalian α-amylase requires chloride for maximum activity. Muss observed, with salivary amylase, that 1-10 mM NaCl gave maximum activity[15], and that the chloride ion depressed the solubility of the enzyme but enhanced its stability against inactivation by heat and by heavy metals. The optimum NaCl

concentration for porcine pancreatic α-amylase is 10 mM, with higher concentration producing an inhibitory effect. It has been demonstrated[2] that neutral salts at high concentration inhibit the activity of widely different enzymes in the order, of increasing effectiveness, Ac^-, Cl^-, NO_3^-, Br^-, I^-, SCN^-, ClO_4^-. The inhibition caused by neutral salts at high concentration is due to structural changes in the enzyme molecule. Such changes could be mediated by changes in solvent structure or could result from direct effects on the protein molecule. The inhibition of enzyme activity by neutral salts is probably associated with disruption of enzyme structure demonstrable at salt concentrations where partial activity remains.

Mediation of salt-induced changes in macromolecular structure by change in the organized structure of water has been suggested by Klotz[16]. Jencks[17] has interpreted on the basis of insensitivity of protein disruption to the nature of alkali cations. Robinson and Jencks[18] have presented evidence that salt effects are due to a direct action on peptide and amide groups, or possibly relocation of excluded ions at the polar–nonpolar surface, which would account for cation insensitivity[18].

It appears that the responsible mechanism is structure disruption of enzyme, resulting in accessibility of groups, which, in the absence of salts, are buried and hence nonreactive.

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