Na^+ Ion as Activator of α-Amylase

It is more or less universally accepted that one of the main properties of α-amylase is chloride dependence[1-4]. MYRBACK[1] demonstrated the effect of certain anions on mammalian α-amylase and indicated that the enzyme is activated to a maximum by chloride ions. HAWK[5], however, opines that the activation of α-amylase by chloride ion is not specific, and may be due at least in part to the effect of strong electrolytes in general on the solubility of proteins. BERNFELD[6], on the other hand, reports Cl^- requirement for a full activation of human and pig pancreatic α-amylase.

The present work deals with pancreatic α-amylase of an amphibian, reptile, bird and mammal and demonstrates the effect of Na^+ on the activity of the enzyme. The findings are in great contrast to previous reports and show that the activation of the enzymes is not due to chloride ion but due to the sodium ion.

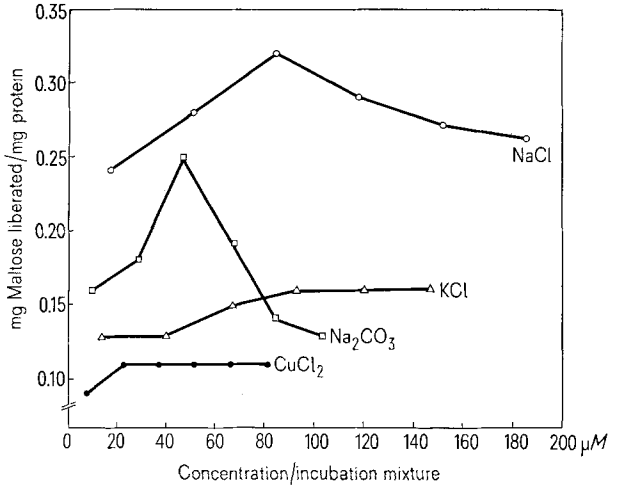

Fig. 1. Effect of NaCl (-○-○-), KCl (-△-△-), Na_2CO_3 (-□-□-) and $CuCl_2$ (-●-●-) on the activity of the pancreatic α-amylase of *Bufo*.

Acclimated, adult, healthy toads (*Bufo melanostictus*), reptiles (*Calotes versicolor*), pigeons (*Columba livia*) and white rats were selected for the study. The pancreas was taken out from freshly sacrificed animals and homogenized in Potter-Elvehjem homogeniser and a 0.5% homogenate was prepared in glass distilled water for each case. The homogenate was then subjected to differential ultracentrifugation in a Beckman Model L preparative ultracentrifuge to obtain $105{,}000 \times g$ supernatant and this fraction in each case was used as the enzyme.

A 1.0 ml aliquot of properly diluted enzyme solution was separately incubated with 2.0 ml, 1% buffered (Na-phosphate buffer, 0.02 *M*, pH 7.0) starch solution and increasing concentrations of NaCl or KCl or Na_2CO_3 or $CuCl_2$ solutions, at 37 °C in an atmosphere of air for 10 min. The amylase activity was measured following the method of BERNFELD[6] and expressed in terms of mg maltose liberated per mg protein. Proteins were measured by the method of LOWRY et al.[7] using bovine serum albumin as the standard.

The effect of NaCl, KCl, Na_2CO_3 and $CuCl_2$ in increasing concentration on the pancreatic amylase activity of toad, calotes, pigeon and white rat is demonstrated in Figures 1 to 4 respectively. The optimum concentrations of NaCl per incubation mixture were 85 μ*M* in *Bufo*, *Columba* and white rat and 51 μ*M* in *Calotes*. In case of Na_2CO_3 the optimum concentrations per incubation mixture were 47 μ*M* in *Bufo* and *Calotes*, 71 μ*M* in *Columba* and 61 μ*M* in white rat. None of the concentrations of KCl and $CuCl_2$, however, showed a significant high activity.

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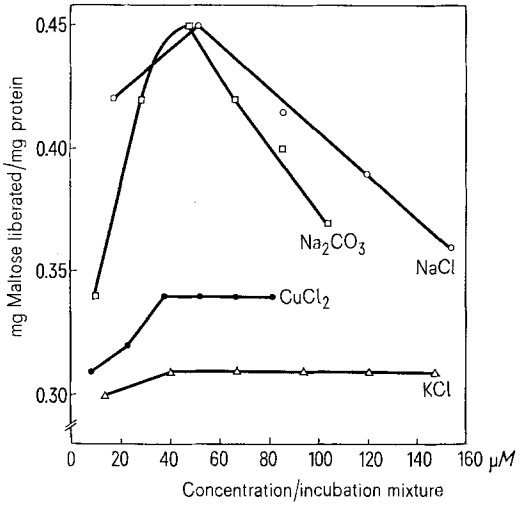

Fig. 2. Effect of NaCl (-○-○-), KCl (-△-△-), Na_2CO_3 (-□-□-) and $CuCl_2$ (-●-●-) on the activity of the pancreatic α-amylase of *Calotes*.

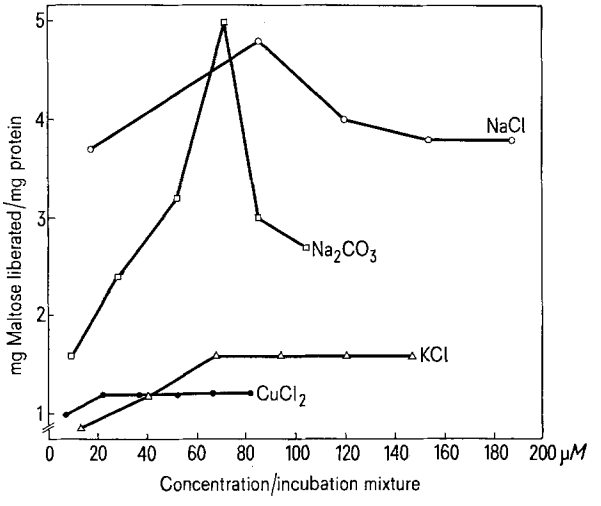

Fig. 3. Effect of NaCl (-○-○-), KCl (-△-△-), Na_2CO_3 (-□-□-) and $CuCl_2$ (-●-●-) on the activity of the pancreatic α-amylase of *Columba*.

The present data from Figures 1 to 4 indicate that the pancreatic α-amylase activity is stimulated by Na^+ ion, as NaCl or Na_2CO_3 in every case is found to be stimulatory, though excess of these ions inhibited the enzyme activity. If Cl^- ion had been the activator as suggested by earlier workers, a similar result would have been obtained when KCl or $CuCl_2$ were used in the incubation mixture. But the results show a completely different picture. The enzymic reaction towards Na_2CO_3 is similar to that obtained when NaCl was added to the incubation mixture in increasing concentrations. To observe whether or not the alteration in pH due to the addition of NaCl or KCl or Na_2CO_3 or $CuCl_2$ could influence the stimulation of α-amylase activity; 2 sets of experiments, one using buffer and another without buffer, were run concurrently. In incubation mixtures with NaCl, KCl or $CuCl_2$ there was no significant change in pH and amylase activity in the non-buffered system. With the addition of Na_2CO_3 a rise in pH occurred in the non-buffered assay medium, inspite of which there was no considerable change in amylase activity when compared to that of the buffered system. The probable reason behind this phenomenon is the ability of sodium ion to activate the α-amylase to a great extent.

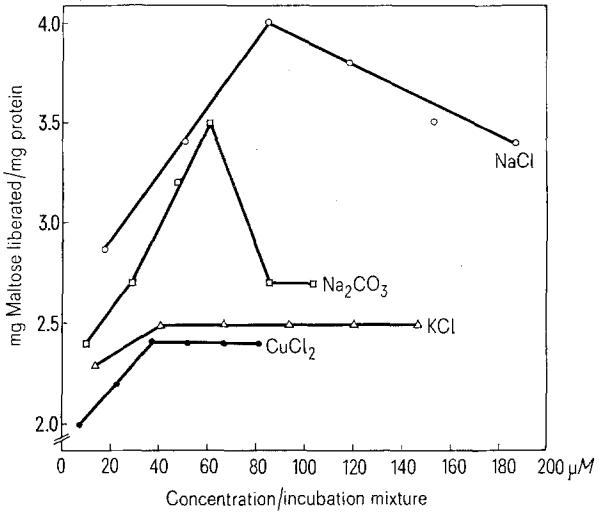

Fig. 4. Effect of NaCl (-○-○-), KCl (-△-△-), Na_2CO_3 (-□-□-) and $CuCl_2$ (-●-●-) on the activity of the pancreatic α-amylase of white rat.

The major function of the sodium ion in the animal body appears to be in connection with osmotic pressure regulation and acid base balance. Although a large amount of sodium is present in the food, it has never received any attention regarding its role in enzymic reactions. HAWK[5] summarizes that a catalytic effect of sodium on enzyme reaction cannot be totally excluded. The involvement of Na^+ ions in the activation of β-galactosidase has recently been demonstrated by WALLENFELS et al.[8]. Previous workers in this field, who supported the chloride dependence of α-amylase, could not avoid the presence of Na^+ in their reaction mixtures in the form of NaCl, assuming that Cl^- was the activator. In contrast the present data clearly shows that Cl^- of KCl or $CuCl_2$ could not stimulate the α-amylase activity. On the other hand, Na^+ without Cl^- (Na_2CO_3) ion stimulated α-amylase activity in all cases. From the above findings it may be suggested that the Na^+ ion has an important role in the α-amylase activation.

Zusammenfassung. Im Gegensatz zu früheren Berichten wird gezeigt, dass die Aktivierung der α-Amylase nicht durch Chloride sondern durch Natrium-Ionen verursacht wird. Weiter wurde gefunden, dass nur NaCl und Na_2CO_3 die Aktivität steigert, nicht aber $CuCl_2$ und KCl. Alle Lösungen, die Na^+ enthalten, haben ebenfalls eine aktivierende Wirkung.

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New Chromosome Numbers in Australian Leptodactylidae (Amphibia, Salientia)

The Australian frogs of the family Leptodactylidae include the living forms phenetically closest to the stem stock that is probably ancestral to all or most of the 'advanced' families of the order[1,2]. This evolutionarily interesting group of Anurans has been subdivided by PARKER[3] into 2 subfamilies (Cycloraninae and Myobatrachinae) whose reciprocal relationships and phyletic roles are still uncertain[4]. The recent discovery in Queensland of a primitive genus *Rheobatrachus*, tentatively included in the Leptodactylidae (but showing other, especially pelobatid, characters)[5], and the anatomical and biological affinities shown by some Cycloraninae (e.g. *Cyclorana*) with the Hylidae[6,4], seem further to complicate the present taxonomic definition of these frogs.

The few fata collected on the chromosomes of the Myobatrachinae (4 species belonging to the genera *Ranidella*[7], *Uperoleia* and *Pseudophryne*) and of the Cycloraninae (3 species of *Limnodynastes*) show that these frogs are karyologically very homogeneous (all have $2n = 24$ and similar karyotype morphology)[8]. They also appear to be generally more differentiated than some groups of Neotropical and African Leptodactylidae with $2n = 26$ (this higher chromosome number seems to be basic for this and for most of the advanced families[8]).

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