cellulase" have been shown to contain specific β-xylosidases which can be separated from the β-glucosidase activity which is also present[6].

The function of β-xylosidase in the kidney requires investigation. It could, however, account for the production of acid mucopolysaccharides which occur in normal urine by releasing them from the protein complexes found in the plasma.

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Competitive inhibition of adenosine deaminase by urea, guanidine, biuret, and guanylurea

Many enzymes are inhibited by low concentrations of urea and guanidine[1–3]. The most extensive study is that of RAYAGOPALAN, FRIDOVICH AND HANDLER[1] who have studied a number of enzymes; according to their findings urea can be a competitive or a non-competitive or an uncompetitive inhibitor of different enzymes. When the inhibition is competitive 1 or 2 molecules of urea per molecule of enzyme are involved in the formation of enzyme–urea complexes.

Adenosine deaminase (EC 3.5.4.4) from calf intestinal mucosa is not irreversibly denatured by urea at concentrations lower than 6 M (ref. 4). However, urea at relatively low concentrations inhibits the enzyme activity. A kinetic analysis of the inhibition of adenosine deaminase by urea is reported in this paper. The inhibition of the enzyme is competitive and 2 molecules of urea are involved in the formation of an enzyme–urea complex. We have also observed that the enzyme is inhibited by guanidine, biuret, and guanylurea.

Adenosine deaminase was purified from calf intestinal mucosa[5]; enzymatic activity was determined spectrophotometrically according to KALCKAR[6].

The reversibility of the inhibition by the reagents assayed was demonstrated by means of a procedure whereby the enzyme was first exposed to a given concentration of inhibitor which was then lowered by dilution. Prolonged incubation (60 min) of the enzyme in 3 M urea or guanidine sulphate, as well as in 10 mM biuret or guanylurea sulphate, at pH 7.5 and 25° resulted in no detectable inactivation of the enzyme.

The effect of varying the adenosine concentration on the extent of urea inhibition was investigated. Lineweaver–Burk plots of the inhibition data (Fig. 1) indicated that urea is formally competitive with respect to adenosine. However, when the data are formulated in a Dixon plot, curves are obtained instead of straight lines. A deviation of this type is observed when more than one molecule of inhibitor reacts with one molecule of enzyme. Otherwise it may be associated with a reaction between inhibitor and substrate, which seems unlikely in this case; moreover, the reaction of inhibitor with substrate also leads to deviation from linearity in the Lineweaver–Burk plots[7], which is not observed here.

From an analysis of the inhibition data by the method of JOHNSON, EYRING AND WILLIAMS[8] it appears that 1.84 molecules of urea per molecule of enzyme are involved in the formation of the enzyme–urea complex and that this leads to the ob-

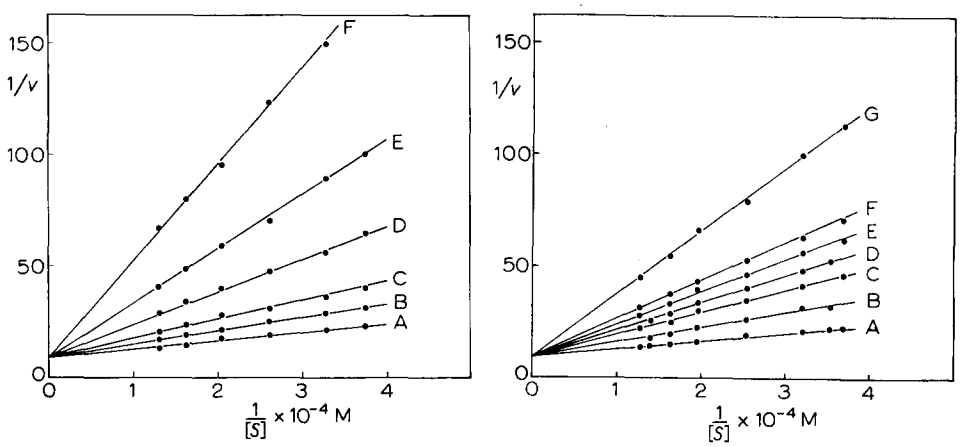

Fig. 1. Competitive inhibition of adenosine deaminase by urea. The reaction was carried out in 0.1 M phosphate buffer (pH 7.5) at 25°. Velocity (v) is expressed as absorbance change per min at 265 mμ. A, no inhibitors; B, 0.2 M urea; C, 0.3 M urea; D, 0.5 M urea; E, 0.7 M urea; F, 1 M urea.

Fig. 2. Competitive inhibition of adenosine deaminase by guanidine, biuret, and guanylurea. Conditions were the same as in Fig. 1. A, no inhibitors; B, 3.3 mM biuret; C, 0.8 M guanidine; D, 10 mM biuret; E, 0.5 mM guanylurea; F, 1.5 M guanidine; G, 1.25 mM guanylurea.

served inhibition. The inhibition constants for the two molecules of urea have been estimated assuming that only the enzyme with no inhibitor bound can form the enzyme–substrate complex[2]. The inhibition constants have been found to be 1.4 M and 0.07 M for the first molecule of urea and for the second one, respectively.

The study has been extended to guanidine which has been found to be a far more powerful inhibitor than urea for several enzymes[1].

Guanidine acts as a competitive inhibitor of adenosine deaminase but it is less effective than urea (Fig. 2); one molecule of inhibitor is involved in the formation of the enzyme–guanidine complex and the K_i is 0.4 M. The sulphate ion does not affect the enzyme activity.

In view of these results we have tested the effect of biuret and guanylurea on the enzyme activity. These two reagents have been found to be far more powerful

competitive inhibitors than urea and guanidine (Fig. 2). The inhibition constants for biuret and guanylurea are, respectively, 3.7 mM and 0.22 mM. One molecule of either biuret or guanylurea per molecule of enzyme is involved in the formation of enzyme–inhibitor complexes.

The competitive nature of the inhibition of adenosine deaminase by the compounds assayed suggests that the inhibition results from interference with the formation of the appropriate enzyme–substrate complex. The ratio of moles of inhibitor bound per mole of enzyme (2 where the inhibitor is urea and 1 in the other cases) indicates that the effect of the inhibitors is fairly specific within a small region of the enzyme molecule. To test if these inhibitors act at the same site or at independent sites on the enzyme molecule, mixed inhibition studies[9] using urea together with each of the other inhibitors were undertaken. The results obtained show that urea, guanidine, biuret, and guanylurea all compete for an identical site on the enzyme molecule. Moreover, mixed inhibition studies using each inhibitor plus 2,6-diaminopurine, which is a competitive inhibitor and a substrate analog ($K_I = 1.7 \cdot 10^{-4}$ M), showed that each inhibitor competes with 2,6-diaminopurine for the same site. This site is probably the binding site of the purine moiety of adenosine.

A possible explanation to account for the differences observed between the inhibitory capacities of the various compounds which have been tested, and the nature of the interaction between inhibitors and the active site of adenosine deaminase are under investigation.

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