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Urease covalently coupled to porous glass

Enzymes have been insolubilized on or in organic polymers of several types These polymers include cellulose derivatives[1–3], polyamino acids[4], collodian membranes[5] and starch[6] Now we have prepared an insolubilized enzyme by covalently coupling urease (urea amidohydrolase, EC 3 5 1 5) to glass with an amino-functional silane coupling agent We refluxed a sample of a porous, 96% silica glass, 790 ± 50 Å pore size, approx 100-mesh (Corning Glass Works, Corning, N Y) in a toluene solution of γ-aminopropyltriethoxysilane[7] The aminoalkyl functional group was then converted to an aminoaryl functional group, by reaction with *p*-nitrobenzoic acid, and was reduced, diazotized[8] and added to a solution containing crystalline jack-bean urease (Worthington Biochemical Corp , Freehold, N J)

The resulting enzyme–glass derivative was assayed by the method of GORIN *et al*[9] The assay showed the glass to contain 1550 units of activity per g glass or 1 0 mg of active enzyme per g glass

The characteristics of the insolubilized enzyme were determined by several studies over a 30-day period, during which time no losses in enzymatic activity were detected The enzyme–glass derivative was packed into a chromatographic column, and urea at various concentrations and pH values was passed through at a constant flow rate of 2 5 ml/min The packed column was 1 0 cm in diameter × 10 0 cm in length For the NH_4^+ determinations, the substrate was dissolved in 0 5 M Tris–HCl to facilitate the use of a monovalent cation electrode (Corning Glass Works, Cat No 476220) and a Corning® Model-12 pH meter The electrode was calibrated against increasing concentrations of NH_4Cl and $(NH_4)HCO_3$ The results were identical (Fig 1) The response to NH_4^+ was nearly Nernstian over the range 10^{-4}–10^{-1} M and was unchanged from pH 3 to pH 8

Increasing concentrations of urea were passed through the column, and 20-ml fractions were collected and read with the cation electrode Fig 2 indicates that maximum velocity was reached at a urea concentration of approx 0 17 M At concentrations higher than 0 34 M, substrate was inhibitory

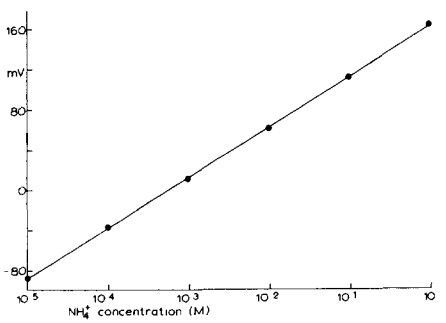

Fig 1 Standard curve for the monovalent cation electrode with NH_4Cl in 0 5 M Tris–HCl (pH 7 0) at 23°

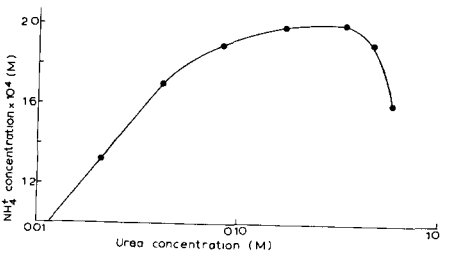

Fig 2 NH_4^+ production at various urea concentrations Flow rate was held constant at 2 5 ml/min Temperature was maintained at 23°

The pH optimum at 23° for the insoluble urease was found at pH 6.0 as compared to the published values of pH 6.4 to pH 7.6 (ref. 10).

We have shown that urease, covalently coupled to an inorganic carrier, retains enzymatic activity. This enzyme–glass derivative has been employed continuously in a column over long periods without detectable losses in enzymatic activity.

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Stereospecific aging of phosphonylated cholinesterases

The conversion, called aging, of phosphonylated cholinesterases into a form which cannot be reactivated implies the release of an alkyl group from the phosphorus moiety of the inhibited enzyme[1,2]. The rate determining step in this process is probably a unimolecular fission of the C–O bond in the alkoxy group RO (Eqn 1)[3].

$$RO(CH_3)P(O)O\text{–}E + H_2O \rightarrow HO(CH_3)P(O)O\text{–}E + ROH \quad (1)$$

In 1964 BERENDS[4] observed that the aging of butyrylcholinesterase inhibited with racemic alkyl methylphosphonofluoridates did not obey first-order kinetics and suggested that this effect was connected with both possible configurations around the phosphorus atom.

This paper describes the influence of the configuration around the phosphorus atom and around the α-carbon atom of the previously mentioned alkoxy group on the aging rates of acetylcholinesterase (acetylcholine acetyl-hydrolase, EC 3.1.1.7) and butyrylcholinesterase (acylcholine acyl-hydrolase, EC 3.1.1.8), inhibited with the stereoisomeric forms of cyclopentyl *S*-2-dimethylaminoethyl methylphosphonothioate, 1-methylheptyl methylphosphonofluoridate and 1,2,2-trimethylpropyl methylphosphonofluoridate (Compounds A, B and C of Table I, respectively).