KINETICS AND MECHANISM OF PROTEOLYTIC ENZYME CATALYSED REACTIONS

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by

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The most incomprehensible thing about the universe is its comprehensibility.

Einstein.

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Abstract

The enzyme actinidin has been purified and studied chemically and kinetically. The enzyme has many structural and kinetic similarities with ficin and papain. Specificity studies indicate a strong preference for a basic side chain in the S_1 site, and competitive inhibitor binding shows a preference for an aromatic group in the S_2 site. Inactivation studies show the presence of one active thiol group per enzyme molecule.

The hydrolysis of Nα-carbobenzoxy-L-lysine p-nitrophenyl ester by actinidin has been studied in detail. The Michaelis constant, K_m, is dependent on groups ionising at pH 3.75 and 8.1. The turnover number, k_{cat}, shows little pH dependence at low pH but an upward inflection dependent on a group ionising at pH 8.1. When the reaction is followed with enzyme concentration in excess of substrate concentration a biphasic reaction is observed. This is interpreted by a mechanism similar to that proposed for ficin and papain catalysed hydrolyses of this substrate. This mechanism is more complicated than the simple acylation-deacylation mechanism normally expected, involving an isomerisation of some kind. Microscopic rate constants for the reaction have been calculated.

The significance of various physico-chemical principles of catalysis has been discussed in relation to enzymic catalysis. From a study of the imidazole catalysed

hydrolysis of N,O-diacetylserinamide, it has been concluded that general base catalysis could play a much greater part in enzymic catalysis than had previously been estimated.

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