The Purification and Properties of Shikimate Dehydrogenase

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Shikimate dehydrogenase (EC 1.1.1.25) is a key enzyme in the synthesis of many aromatic compounds by plants and micro-organisms. There is, however, no known function for the enzyme in mammalian systems and it was therefore considered to be a desirable site for the rational design of novel herbicides.

The enzyme was isolated from etiolated epicotyls of *Pisum sativum* and purified 400-fold to a specific activity of 2.25 units/mg. Analogues of dehydroshikimate were synthesized that were powerful reversible inhibitors, but surprisingly only in the direction shikimate → dehydroshikimate. This kinetic investigation of the reaction mechanism was made to try to explain these results.

The initial-velocity pattern at 25°C at pH 8.0 in the absence of products was determined for both directions of the reaction and when plotted according to Lineweaver & Burk (1934) gave a family of straight lines intersecting at a point to the left of the ordinate. The K_m values determined by the graphical method of Florini & Vestling (1957) were 0.34 and 0.07 mM for shikimate and $NADP^+$ respectively and 0.36 and 0.28 mM for dehydroshikimate and NADPH respectively. The dissociation constants for $NADP^+$ and NADPH obtained from the initial-velocity data (Frieden, 1957) were 0.11 and 0.017 mM respectively.

The equilibrium constant in the direction dehydroshikimate → shikimate calculated from the above values was in good agreement with that obtained experimentally (6.25 and 6.5 respectively).

In order to obtain more specific information about the kinetic mechanism the product inhibition pattern was determined. For the direction shikimate → dehydroshikimate NADPH gave linear competitive inhibition with respect to $NADP^+$ (K_i 0.033 mM) and dehydroshikimate was a non-linear competitive inhibitor with respect to shikimate (K_i 0.25 mM). In the reverse direction $NADP^+$ gave linear competitive inhibition with respect to NADPH (K_i 0.072 mM) and shikimate was a linear mixed inhibitor with respect to dehydroshikimate (K_i 0.75 mM).

The results are compatible with an ordered Bi Bi reaction mechanism (Cleland, 1963) in which only $NADP^+$ and NADPH can add to free enzyme, but including two dead-end complexes (eqns. 2a and 3a) (E·X·Y represents a reactive ternary complex):

$$E + NADP^+ \rightleftharpoons E \cdot NADP^+ \quad (1)$$

$$E \cdot NADP^+ + \text{shikimate} \rightleftharpoons E \cdot X \cdot Y \quad (2)$$

$$E \cdot NADP^+ + \text{dehydroshikimate} \rightleftharpoons E \cdot NADP^+ \cdot \text{dehydroshikimate} \quad (2a)$$

$$E \cdot X \cdot Y \rightleftharpoons E \cdot NADPH + \text{dehydroshikimate} \quad (3)$$

$$E \cdot NADPH + \text{shikimate} \rightleftharpoons E \cdot NADPH \cdot \text{shikimate} \quad (3a)$$

$$E \cdot NADPH \rightleftharpoons E + NADPH \quad (4)$$

The poor inhibition by dehydroshikimate analogues in the direction of shikimate formation can be explained by such a mechanism.

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A Latent Collagenase Released by Bone and Skin Explants in Culture

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Neutral collagenase activity was assayed on native trypsin-resistant collagen containing [^{14}C]-glycine by the release of soluble radioactivity from reconstituted fibres or by the prevention of gel formation from dispersed ^{14}C-labelled collagen (Nagai, Lapiere & Gross, 1966). In contrast with observations made on other systems (Gross, 1970), no overt activity was found in culture media of mouse bone explants; however, considerable activity could be elicited by exposure of the media to trypsin. Chymotrypsin was 20–40-fold less effective than trypsin as activator. Purified liver lysosomes were active between pH 5 and 7. Skin explants also released latent collagenase.

Activation of bone enzyme followed a sigmoidal course, apparently not explainable by autoactivation. Excess of trypsin inactivated the enzyme. Total latent collagenase was estimated by extrapolation to zero time of the exponential inactivation curve. In absence of substrate latent enzyme was stable at 37°C near neutrality, whereas activated enzyme was rapidly destroyed. Collagenase showed a flat optimum at pH 7.5, required