Conformational Changes of IRC-50 Arvin at High pH

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IRC-50 Arvin is the purified coagulant enzyme from *Agkistrodon rhodostoma* venom; this enzyme has many properties in common with the mammalian serine proteinases, possessing both coagulant and esterase activities that are inactivated by di-isopropyl phosphorofluoridate and phenylmethane-sulphonyl fluoride (Collins *et al.*, 1971; Collins & Jones, 1972). At high pH values both the esterase activity and rate of inactivation of the enzyme by di-isopropyl phosphorofluoridate diminish, owing to the ionization of two residues on the protein.

IRC-50 Arvin is inactivated by nitrous acid. At pH 4.35 and 0°C, in the presence of a constant concentration (excess) of nitrous acid, the loss of esterase activity is first-order with respect to enzyme concentration and proceeds at the same rate as the loss of enzyme active sites as determined by titration with *p*-nitrophenoxy-*p'*-guanidinobenzoate. The loss of coagulant activity was biphasic, an initial fast inactivation being followed by a slower loss of coagulant activity at approximately the same rate as the loss of esterase activity. Determination of *N*-terminal groups by the cyanate method (Stark & Smyth, 1963; Gertler & Hofmann, 1967) showed that the loss of enzyme active sites proceeds in parallel with loss of the *N*-terminal valine residue. Further, the destruction of *N*-terminal valine, esterase activity and enzyme active sites by nitrous acid are all prevented by the substrate α -*N*-benzoyl-L-arginine ethyl ester. The coagulant activity of IRC-50 Arvin is only partially protected under the same conditions.

When IRC-50 Arvin is preincubated at pH values above 8.5 and then assayed for esterase activity at pH 8.0, a lag is observed before the maximal steady-state rate of hydrolysis of substrate is attained. This phenomenon can be interpreted in terms of a transition between an inactive (alkaline) and active (neutral) form of the enzyme (cf. chymotrypsin; Garel & Labouesse, 1971). The pH-dependence of the amplitude of this lag shows that the transition is controlled by a single ionizing group on the protein with $pK(\text{app.}) \approx 10.0$.

These results show that the catalytically active conformation of IRC-50 Arvin is maintained by the protonated form of a group with $pK(\text{app.}) \approx 10.0$; this group is probably the *N*-terminal valine residue, which has been shown to be essential for catalytic activity.

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Studies on the Mode of Action of Anilino-*propionamide* Herbicides

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The anilino-*propionamides*, e.g. 2-(4-methyl-2,6-dinitroanilino)-*N*-methyl-*propionamide*, are a group of experimental pre-emergence herbicides (Yates, 1968). They do not cause auxin-type growth abnormalities, nor do they interfere with photosynthesis or oxidative phosphorylation. Their mode of action is therefore different from that of most common herbicides.

2-(4-Methyl-2,6-dinitroanilino)-*N*-methyl-*propionamide* has two optical isomers, since it contains an alanine moiety. The D-form is herbicidally active, whereas the L-form is virtually inactive.

A number of these compounds were tested in an assay (D. E. Moreland, personal communication) that measured their effect on the incorporation of [¹⁴C]leucine into protein by soya-bean hypocotyl segments. Concentrations causing 50% inhibition of leucine incorporation, measured over a 6h incubation period, were as low as 4 μ M and correlated well with herbicidal activity. D-2-(4-Methyl-2,6-dinitroanilino)-*N*-methyl-*propionamide* was at least 100 times as active as the L-isomer in this test.

Soya-bean hypocotyl tissue was incubated under similar experimental conditions, but with 20 μ M-DL-2-(4-methyl-2,6-dinitroanilino)-*N*-methyl-*propionamide* ([1-¹⁴C]alanine-labelled) replacing the [¹⁴C]leucine. This concentration would cause approx. 80% inhibition of leucine incorporation, but did not affect respiration of the tissue.

The tissue was then macerated and filtered, and the filtrate was subjected to gel filtration on a Sephadex G-25 column. Five radioactive fractions were obtained. One was of particular interest, being associated with the macromolecular (mol.wt. > 5000) fraction and representing up to 10% of the radioactivity in the tissue. The radiolabel in this fraction was shown to be bound to the protein, since removal of nucleic acid and lipids etc. by the method of Peterson &