Effects of Dioxan on Thrombin and Trypsin Activities

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Summary Dioxan enhances the esterase activity of thrombin and increases both the apparent K_m and the K_{cat} values.

DIOXAN has been frequently used without question as a solvent in all phases of chemistry including enzymology. During the course of our work[1] to determine the number of exposed tryptophan residues in thrombin using dioxan and hydrogen peroxide[2] several discrepancies were noted and related to dioxan.

The esterase activities of thrombin and trypsin (Figure) are enhanced by the addition of dioxan. For thrombin the greatest increase, to *ca.* 150% of the control, occurs at 15% dioxan. The effect on trypsin is such that the activity can be increased to over 300% of the control.

Dioxan has the effect of inhibiting the primary enzymatic activity of thrombin, the clotting of fibrinogen. This effect is almost instantaneous and concomitant to the increase in esterase activity. Within 5 min of making a solution 10% in dioxan only one-fourth of the original clotting activity remains. This remaining fraction is gradually lost and after 24 h no activity remains. Dialysis of the dioxan–thrombin solution in 0·25M phosphate buffer, pH 6·5, for 24 h restores 64—80% of the original clotting activity, while the esterase activity returned to normal value (neglecting correction for small changes in concentration due to dialysis).

O.r.d. and c.d. studies indicate an increase in random structure due to the presence of dioxan and a return to native conformation upon dialysis.

Our kinetic studies show that the apparent Michaelis-Menten constant of thrombin for the ester substrate increases from $4{\cdot}13 \times 10^{-3}$M to $5{\cdot}97 \times 10^{-3}$M in the presence of 10% dioxan while K_{cat} increases from $1{\cdot}07 \times 10^{-3}$ s^{-1} to $1{\cdot}54 \times 10^{-3}$ s^{-1}. This phenomenon, while contrary to what is normally expected, is similar to the observation of Inagami and Sturtevant[3] on trypsin which we have

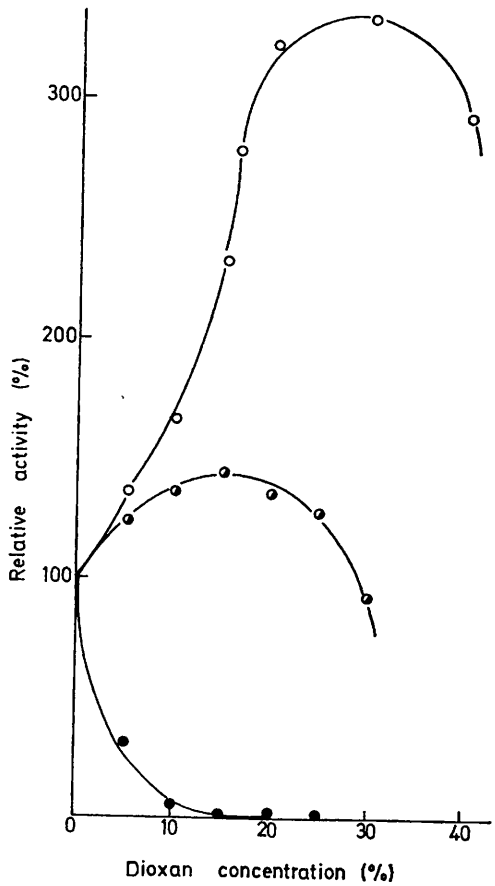

FIGURE. Effect of dioxan on the enzymatic activities of thrombin and trypsin. Esterase activities of trypsin (—○—○) and thrombin (—◒—◒—); clotting activity of thrombin (—●—●—).

repeated and confirmed. Attempts to obtain kinetic data on clotting activity were unsuccessful owing to the limited range of fibrinogen concentration that produced consistent clotting.

Bovine thrombin from Parke-Davis was purified by the method of Lundblad.[4] Bovine trypsin, twice crystallysed, from Sigma Chemical Company was used without further purification. Dioxan was distilled from sodium and stored under nitrogen.[5] Esterase activities were determined using *p*-tosyl-L-arginine methyl ester according to the method of Hummel,[6] except that for thrombin 0·3M phosphate buffer, pH 7·5, was used. The clotting activity was determined using a Becton fibrometer according to a previously described method.[7] Thrombin for these experiments was stored in polyethylene scintillation vials to prevent loss of activity due to adhesion to glass.

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