Oulianoff & Milstone, 1965; Aronson & Ménaché, 1968). Limited information is available about the steady-state kinetics of reactions catalysed by thrombokinase (Sherry, Alkjaersig & Fletcher, 1965). We have studied the kinetics of reactions catalysed by factor Xa to establish its relationship with thrombokinase and to compare it with trypsin and thrombin. Judged by the magnitude of the specificity constant, k_0/K_m , Factor Xa is usually a much less efficient catalyst than trypsin or thrombin towards small synthetic substrates. For the hydrolysis of α -N-benzoyl-L-arginine ethyl ester at pH 8.0 and 25°C in 0.1M-sodium chloride K_m (0.45mM) is higher and k_0 (6.17s^{-1}) and k_0/K_m ($1.37 \times 10^4\text{s}^{-1}\cdot\text{M}^{-1}$) are lower with Factor Xa as catalyst than with β -trypsin ($K_m = 3.6\mu\text{M}$, $k_0 = 24.8\text{s}^{-1}$, $k_0/K_m = 7.0 \times 10^6\text{s}^{-1}\cdot\text{M}^{-1}$) or thrombin ($K_m = 10.4\mu\text{M}$, $k_0 = 10.5\text{s}^{-1}$, $k_0/K_m = 1.0 \times 10^6\text{s}^{-1}\cdot\text{M}^{-1}$). Surprisingly the k_0 value for the corresponding methyl ester is lower than for the ethyl and cyclohexyl esters. Like trypsin, Factor Xa undergoes substrate activation with α -N-toluene-*p*-sulphonyl-L-arginine methyl ester as substrate ($K_m = 0.74\text{mM}$, $K'_m = 9.8\text{mM}$, $k_0 = 6.45\text{s}^{-1}$, $k'_0 = 30.0\text{s}^{-1}$). α -N-Acetyl-L-arginine methyl ester ($K_m = 1.14\text{mM}$, $k_0 = 103\text{s}^{-1}$, $k_0/K_m = 9.01 \times 10^4\text{s}^{-1}\cdot\text{M}^{-1}$) is a better substrate than either the α -N-benzoyl or the α -N-toluene-*p*-sulphonyl analogues. Unlike trypsin and thrombin, Factor Xa catalyses the hydrolysis of lysine derivatives poorly. For α -N-benzoyl-L-lysine methyl ester $K_m = 24\text{mM}$, $k_0 = 0.99\text{s}^{-1}$ and $k_0/K_m = 41\text{s}^{-1}\cdot\text{M}^{-1}$, and for α -N-toluene-*p*-sulphonyl-L-lysine methyl ester $K_m = 23\text{mM}$, $k_0 = 6.1\text{s}^{-1}$ and $k_0/K_m = 270\text{s}^{-1}\cdot\text{M}^{-1}$. Factor Xa does not hydrolyse α -N-toluene-*p*-sulphonyl-L-homoarginine methyl ester, and methyl γ -guanidino-L- α -toluene-*p*-sulphonamido-butyrates is a very poor substrate. Clearly permissible variation of the type and length of the substrate side chain is more limited with Factor Xa than with trypsin or thrombin.

Roberts (1970) has reported that K^+ and, to a smaller extent, Na^+ inhibit thrombokinase. In the hydrolysis of α -N-benzoyl-L-arginine ethyl ester by Factor Xa, we find that K_m is increased by 0.1M-cations ($\text{Ca}^{2+} > \text{Mg}^{2+} \approx \text{Li}^+ > \text{Rb}^+ \approx \text{Cs}^+ > \text{K}^+ > \text{Na}^+$). Ca^{2+} and Mg^{2+} increase k_0 whereas the univalent cations, especially K^+ , decrease k_0 . The second-order rate constant for the acylation of Factor Xa by 4-methylumbelliferyl *p*-guanidinobenzoate (Roberts, Adams, Elmore, Jameson & Kyle, 1971) is also decreased by cations.

The kinetics parameters reported for thrombokinase (Sherry *et al.* 1965) show some qualitative similarities to our results, but identity of thrombokinase and Factor Xa has not been established.

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Water Surface Free Energy and its Potential in Biochemical Activities

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Surface free-energy considerations are normally applied to conditions where surface-active agents are involved, e.g. in adsorption reactions, rather than to reactions in the bulk solvent. However, the presence of hydrophobic groups in soluble biochemical entities, such as proteins, results in their enforced presence within the bulk solvent where they give rise to water/hydrophobic group interfaces to which interface free energy and interface entropy considerations apply. The contribution of water/hydrophobic group interface free energy to the adherence of hydrophobic groups has been computed according to the equation:

$$L = 3 \times 10^{-3} \cdot \gamma \cdot A \text{ kcal/mol per mol of hydrophobic group interactions}$$

where A is the actual hydrophobic area contact (in $\text{\AA}^2$) and γ is the surface tension concerned (Lewin, 1971a,b).

For an otherwise identical set of conditions, a decrease in the value of the interface tension would result in corresponding equilibrium-constant changes. For example, for an original equilibrium constant value of 1000 a decrease of the surface tension to one-third of its original value can be readily computed to result in a corresponding decrease of the particular equilibrium constant to one-hundredth of its original value, thereby enabling reversal of the original reaction. This conclusion can explain the observation made by Cebra, Givol & Katchalski (1962) that antigen-antibody complex-formation can be reversed on addition of sodium dodecyl sulphate (which is known to lower the surface tension of water from approx. 72 dyn/cm to over 30 dyn/cm), and also the disaggregation

of tobacco-mosaic virus by 10M-acetic acid but not by mineral acids (Fraenkel-Conrat, 1957); the acetic acid decreases the surface tension value to the region of over 30 dyn/cm (*International Critical Tables*, 1928). However, even smaller surface-tension value changes can have significant effects on the equilibrium. Thus a decrease of 2.5 dyn/cm in the value of 47 dyn/cm in blood serum results in a greater than 20% decrease in the equilibrium-constant value; such changes can be induced in human sera by freezing and thawing and by increased lipoprotein concentrations (Lewin, 1972). It is relevant that 6M-urea solution reduces the value of the surface tension from approx. 72 dyn/cm to 63 dyn/cm at 25°C while guanidinium chloride solutions reduce it even more (Lewin, 1972). Hence the contribution of high urea and of high guanidinium chloride concentrations to a lowering of interface tension, and therefore to de-adherence of hydrophobic groups, is sufficiently significant to be taken into account when considering helix $\rightleftharpoons$ coil transformations.

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Inhibition of δ -Aminolaevulate Dehydratase by Pyridoxal Derivatives and by Cyanide

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δ -Aminolaevulate dehydratase (EC 4.2.1.24) was purified from extracts of *Rhodospseudomonas spheroides* grown photosynthetically, and assayed as usual by incubating it with substrate (δ -aminolaevulate) in tris buffer, pH 8.5, and determining the product (porphobilinogen) with the Ehrlich reagent (Nandi, Baker-Cohen & Shemin, 1968; S. van Heyningen & D. Shemin, unpublished work). When 1 mM-pyridoxal phosphate was added to the incubation medium the enzyme was inhibited by 30%. At 0.05 mM-pyridoxal phosphate inhibition was almost negligible. Surprisingly the kinetics of inhibition were found to be almost perfectly non-competitive, the K_m for δ -aminolaevulate being

0.8 mM both in the presence and in the absence of inhibitor. The inhibition was not increased by prolonged incubation and activity was fully recovered when pyridoxal phosphate was removed by dialysis.

Nandi & Shemin (1968) showed inhibition of the enzyme by several keto acid analogues of the substrate (e.g. laevulate), but in these cases the inhibition was competitive and presumably due to the binding of the inhibitor at a lysine residue, which they suggested was at the active centre. The effect of pyridoxal phosphate seems to be a specific one of the pyridoxal molecule and not of a carbonyl, since pyridoxal and pyridoxamine phosphate both inhibited to about the same extent as pyridoxal phosphate; but pyridine, benzaldehyde and pyridine-4-carboxaldehyde did not inhibit significantly at 1 mM concentration.

Shemin (1970) has suggested that pyridoxal phosphate, which is a cofactor of δ -aminolaevulate synthetase, which precedes δ -aminolaevulate dehydratase in the pathway of porphyrin biosynthesis, may be involved in a concerted reaction *in vivo* between the two enzymes. Our current work does show some evidence for interaction between pyridoxal phosphate and δ -aminolaevulate dehydratase.

We have also found inhibition of the enzyme by 1 mM-sodium cyanide, and this inhibition shows competitive kinetics. Such competitive inhibition by cyanide has been said to be evidence for a Schiff-base mechanism of the type suggested for this enzyme by Nandi & Shemin (1968); but we consider that it is equally well explained by direct Schiff-base formation between cyanide and δ -aminolaevulate, thereby preventing reaction between the δ -aminolaevulate and the enzyme.

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The Primary Structure of Yeast Aldolase

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Fructose 1,6-diphosphate aldolases fall into two distinct classes that possess different molecular and catalytic properties (Rutter, 1964). Class 1 aldolases occur in animals and higher plants and are tetrameric molecules with molecular weights of about 160 000; they are not inhibited by chelating