BIOCHEMICAL LETTERS

Chymotrypsin compensation plots: a cautionary note

This letter has been prompted by the recent paper of Adams (1980). We have no wish to fault his arguments, but we do want to caution readers that, for the reasons to be given, conjectures based on α -chymotrypsin compensation plots are problematic at this time.

Although the uncertainty in compensation plots ($\Delta H^\ddagger$ versus $\Delta S^\ddagger$) due to experimental errors is well known, there is a particular hazard when utilizing α -chymotrypsin-catalysed reactions for the construction of such plots. Although well documented (Baggott & Klapper, 1976, and references therein), it is not well recognized that many α -chymotrypsin reactions yield nonlinear Arrhenius plots, with linearity and nonlinearity obtained in a single class of substrates. Thus, in a series of hydrolyses of *p*-nitrophenyl-2-(5-*n*-alkyl)furoates, Arrhenius plots were nonlinear when $n = 0, 2$ and 3 , but were linear when $n = 1, 4$ and 5 (Baggott & Klapper, 1976). For this reason, activation parameters obtained on the assumption of Arrhenius linearity may be in error.

Even when Arrhenius nonlinearity is taken into account, the compensation plots that result depend heavily upon interpretation of the data. We originally analysed our results assuming two linear curves joined through a discontinuity found in the range of 20 to 30°C. Using high- and low-temperature data yielded two reasonable compensation plots with two different values for T_c (obtained as the slope of the $\Delta H^\ddagger$ versus $\Delta S^\ddagger$ plot), 233 and 354 K. We now know that this simple interpretation was incorrect (Wang *et al.*, 1981). Repeating some of the experiments has led us to conclude that the Arrhenius plots do not contain a discontinuity, but are smooth curves. The simplest model which can account for our new, more precise, data is that of a rapid, temperature-dependent equilibrium between two active forms of the acylated enzyme. With this model we have analysed the nonlinear Arrhenius data for the transesterification of α -chymotrypsinyl-2-(5-*n*-propyl)furoate by ten different alcohols. The activation parameters for the postulated high-temperature enzyme form yield a reasonable compensation plot with $T_c = 316$ K.

Clearly, until an unequivocal explanation of the temperature dependence is available, little can be concluded from the value of the compensation temperature.

A further complication exists. There is strong evidence that acylation of chymotrypsin proceeds through one or more kinetically significant intermediates (e.g. Fink, 1977). From a recently observed $^{16}\text{O}/^{18}\text{O}$ kinetic isotope effect, we have suggested that the same may be true in the deacylation reaction (Wang *et al.*, 1980). Thus, even were it possible to account correctly for observed Arrhenius non-linearities, the activation parameters so obtained would in all likelihood refer to a combination of primary processes and would not be suitable for simple interpretations.

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Interpretation of thermodynamic compensation plots for acyl α -chymotrypsin systems

In the preceding letter, Klapper *et al.* (1981) refer to the interpretation of thermodynamic compensation plots for acyl α -chymotrypsin systems which exhibit 'gross' Arrhenius plot anomalies. In this case I fully agree that interpretation of anomalous characteristic temperatures, obtained from compensation plots, for such systems cannot be made in terms of a variable $\Delta C_p^\ddagger$, since such Arrhenius plots