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The interpretation of entropy/enthalpy compensation phenomena in the deacylation of acyl-α-chymotrypsins

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The thermodynamic-compensation law observed for the deacylation of a series of acyl-α-chymotrypsins has been re-examined. From consideration of the effect of small changes in $\partial\Delta H^{\ddagger}\partial T$ along a homologous series, it is suggested that the high T_c value of 420K observed for the process has its origin in the solvation of the acyl-group-catalyst transition state.

Studies on the α-chymotrypsin-catalysed hydrolysis of a series of *p*-nitrophenyl esters of the aliphatic fatty acids have produced comprehensive pseudo-thermodynamic-activation-parameter data for the deacylation of acyl-α-chymotrypsins (Martinek *et al.*, 1972; Marshall & Chen, 1973; Adams & Swart, 1977). A plot of $\Delta H^{\ddagger}$ against $\Delta S^{\ddagger}$ (enthalpy and entropy of activation respectively) for the series is found to be a straight line of slope (T_c) 420K.

The observed thermodynamic-compensation effect has been interpreted (Martinek *et al.*, 1972) in terms of a conformational change in a water–substrate (acyl) group–protein transition state; no explanation is offered, however, for the variance between the characteristic temperature of 420K observed in deacylation kinetics and the value of 280–300K associated with solvation processes in α-chymotrypsin catalysis (Lumry & Rajender, 1971), this latter value having been suggested as a ubiquitous consequence of the properties of liquid water (Lumry & Rajender, 1971).

In the present paper I compare results on the enzyme-catalysed deacylation process with those obtained for base (OH^-)- and imidazole-catalysed *p*-nitrophenyl acyl ester hydrolysis. The observations are consistent with a model involving different degrees of solvent reorganization in the solvated-acyl-group–catalyst transition state.

In Fig. 1 $\Delta H^{\ddagger}$ is plotted against $\Delta S^{\ddagger}$ for the enzymic deacylation process discussed to clearly demonstrate the observed thermodynamic-compensation behaviour. The data are the pooled literature results (Martinek *et al.*, 1972; Marshall & Chen, 1973; Adams & Swart, 1977) combined with my own results (Adams, 1974) for the base-catalysed hydrolysis of *p*-nitrophenyl acetate, propionate, butyrate, valerate and trimethylacetate. In addition, the results of Blyth & Knowles (1971) on the imidazole-catalysed hydrolysis of *p*-nitrophenyl acetate and *p*-nitrophenyl dodecanoate are included.

Values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for the base-catalysed hydrolysis were calculated from pseudo-first-order rate constants normalized to a catalyst (OH^-) concentration of 10^{-5}mol/dm^{-3}. This procedure means that both the enzymic and base-catalysed processes are referred to the same catalyst concentration as a relative standard state.

The solid line shown in Fig. 1 is that obtained by linear-regression analysis of the pooled enzymic-deacylation data. As can be seen, the results obtained from both the base- and imidazole-catalysed processes also lie on this regression line.

We thus have the following paradox: that any explanation involving the enzyme, put forward to explain the observed compensation effect, must equally well apply to the situation in which no enzyme is present.

Where then lies the origin of the observed enzymic compensation phenomenon? Two points require explanation: firstly, why should the deacylation process for a series of structurally similar substrates exhibit the compensation effect? and secondly, why do data for both enzymically and non-enzymically catalysed processes lie on the same compensation line?

In general, enzymes with a relatively broad substrate specificity tend to distinguish kinetically between different structural types of substrate. Thus catalytic reactions of the same substrate type are generally found to proceed at approximately the same rate. The free energy of activation ($\Delta G^{\ddagger}$) will thus be approximately constant for the same substrate type, since $\Delta G^{\ddagger}$ is relatively insensitive to

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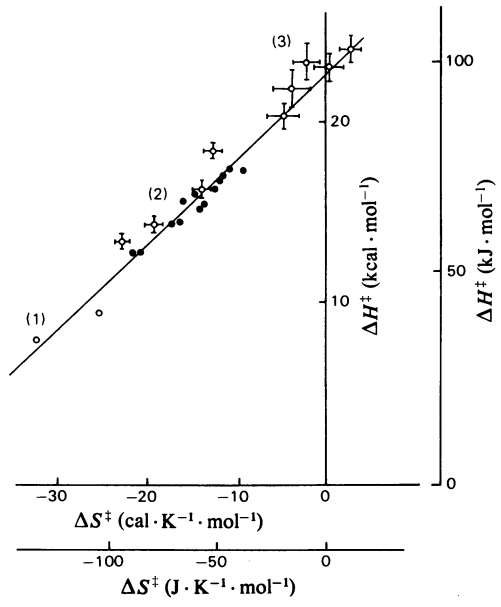


Fig. 1. *Compensation plot of entropy-/enthalpy-of-activation data for the catalysed hydrolysis of p-nitrophenyl acyl esters*

Group (1), base-catalysed hydrolyses; group (2), α-chymotrypsin-catalysed hydrolysis; group (3), imidazole-catalysed hydrolyses. Error limits are shown on the data of Adams (1974) [group (1)] and Adams & Swart (1977) [group (2)] and represent 95% confidence limits. Experimental conditions under which $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were evaluated were similar for the systems studied. For example, values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ in group (2) (the enzymic-deacylation data) were evaluated either from pH-independent rate constants (Adams & Swart, 1977; Martinek *et al.*, 1972) or were corrected for enzyme-active-site ionization (Marshall & Chen, 1973), a procedure that effectively gives rise to pH-independent parameters.

variations in rate constant. Thus if the temperature at which $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ is determined is constant over the substrate range, and if the temperature range is small compared with the mean temperature (in K), $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ will be linearly related by eqn. (1) (see, e.g., Banks *et al.*, 1972):

$$\Delta H^{\ddagger} = \Delta G^{\ddagger} + T\Delta S^{\ddagger} \qquad (1)$$

The mean values of $\Delta G^{\ddagger}$ (± total error) for the three groups of catalysed processes shown in Fig. 1 are: OH^--catalysed, 98.3 ± 2.1 kJ $(23.5 \pm 0.5$ kcal$)\cdot$mol^{-1}; enzyme-catalysed, 84.5 ± 3.3 kJ $(20.2 \pm 0.8$ kcal$)\cdot$mol^{-1}; and imidazole-catalysed, 73.2 ± 2.1 kJ $(17.5 \pm 0.5$ kcal$)\cdot$mol^{-1}. Therefore a linear relationship is not found between the three groups of catalysed data by virtue of constancy in $\Delta G^{\ddagger}$ values. The linear relationship with variable $\Delta G^{\ddagger}$ arises from the high value of T_c exhibited by the pooled enzymic data. Any explanation for this high value of T_c must, therefore, be sought in terms of some property common to all three catalytic processes.

The characteristic temperature for the compensatio shown is 420 K. This is considerably higher than the mean reaction temperature of about 300 K, which would be expected to be the slope from eqn. (1). An explanation for this increase lies in the fact that $\Delta H^{\ddagger}$ is not temperature-independent, as assumed in eqn. (1). To a first approximation the temperature variation of $\Delta H^{\ddagger}$ is shown in eqn. (2):

$$\Delta H^{\ddagger}_{obs.} = \Delta H^{\ddagger}_{0} + \Delta C^{\ddagger}_{P} \cdot T \qquad (2)$$

If the absolute value of specific heat of activation ($\Delta C^{\ddagger}_{P}$) now varies for a homologous substrate series, then, depending on the sign of $\Delta C^{\ddagger}_{P}$, $\Delta H^{\ddagger}_{obs.}$ will be raised above or lowered below the temperature-independent value by different amounts for each substrate. This will give rise to an apparent characteristic temperature greater or less than the mean reaction temperature as predicted in eqn. (1). The concept is shown graphically in Fig. 2, where an

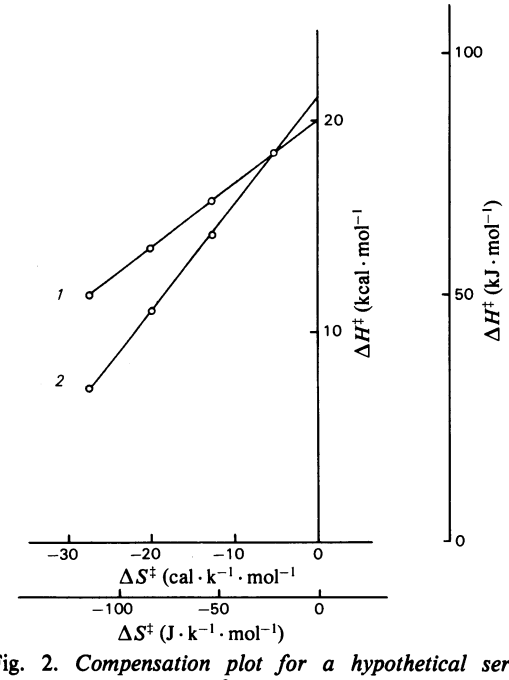
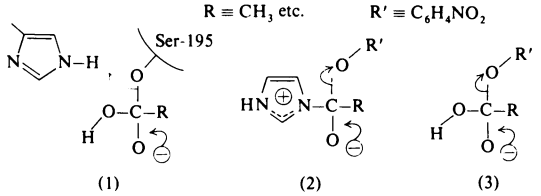


Fig. 2. *Compensation plot for a hypothetical series of reactions*

Line *1* shows the plot obtained for a temperature-independent $\Delta H^{\ddagger}$, whereas line *2* is obtained by assuming that the value of $\partial\Delta H^{\ddagger}/\partial T$ is 0, −21, −42 and −63 J (0, −5, −10, and −15 cal)$\cdot$K$^{-1}\cdot$mol^{-1} respectively as $\Delta S^{\ddagger}$ decreases right to left.

ideal compensation plot for four consecutive members of a hypothetical homologous substrate series is shown, assuming $T_{mean} = 300\,K$, $\partial\Delta H^{\ddagger}_{obs.}/\partial T = 0$ and then assuming that $\partial\Delta H^{\ddagger}_{obs.}/\partial T$ takes the values 0, −21, −42 and −63 J (0, −5, −10 and −15 cal)·K^{-1}·mol^{-1}. In the second case, an apparent T_c of 500 K is found. Clearly then, very small perturbations in $\partial\Delta H^{\ddagger}_{obs.}/\partial T$ can produce relatively large perturbation in T_c.

Negative specific heats of activation are generally regarded as measures of transition-state solvent reorganization in solvolysis reaction, $\Delta C_p^{\ddagger}$ for such reactions having always been observed negative, varying between 0 and −500 J (0 and −120 cal)·K^{-1}·mol^{-1} (Robertson, 1967). It is possible, therefore, that the compensation effect with $T_c = 420\,K$ noted for the catalysed (enzymic, base and imidazole) hydrolysis of the acyl esters as discussed, does in fact arise from solvation of the acyl–catalyst transition state. The similarity of the catalysed systems considered is evident on comparison of probable transition-state structures for the three types of reaction [(1) enzymic deacylation (Dixon & Webb, 1979); (2) imidazole-catalysed (Jencks, 1969); (3) hydroxyl-catalysed (Sykes, 1977)]:



In each case the rate-determining step in the reaction appears to be the attack of the nucleophile (H_2O, imidazole or OH^-) on the acyl carbon atom. The *p*-nitrophenyl portion of the substrates in catalytic groups (2) and (3) above must, if the proposed explanation is correct, play little part in the degree of transition-state solvation and solvent reorganization that gives rise to $\Delta C_p^{\ddagger}$. Interactions between the catalyst and the acyl regions of the catalyst–substrate complex must play the major role in deciding where (i.e. the relative $\Delta H^{\ddagger}/\Delta S^{\ddagger}$ values) on the compensation plot the data points lie. The same catalyst would be expected to make an approximately constant contribution to acyl-group–solvent reorganization in a homologous substrate series, and this explains the different catalyst groupings observed in Fig. 1 for hydrolysis of the same substrate series.

The effect of a varying $\Delta C_p^{\ddagger}$ on T_c has been clearly demonstrated, and it is interesting to speculate in reverse, i.e. whether experimental T_c found markedly different from the mean reaction temperature is in fact a consequence of varying degrees of transition-state solvation reorganization when homologous series of substrates are being compared.

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