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EFFECTS OF SOLUTES ON THE TEMPERATURE DEPENDENCE OF CHYMOTRYPTIC HYDROLYSIS

DAVID M GLICK
Biochemistry Department, Medical College of Wisconsin, Milwaukee, Wisc 53233 (U S A)

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SUMMARY

Contrary to published reports, chymotrypsin is sensitive to the nature of the solute accompanying it in solution This is seen in the temperature dependence of its hydrolysis of *N*-acetyl-L-tyrosine ethyl ester in 9 different solutions Despite the variation in temperature dependence as a function of the solute, there exists a temperature, near 25°, where the rate is insensitive to the solute This constitutes an example of linear entropic compensation for enthalpy in the rate determining step of catalysis The results are considered in relation to the known thermally-induced conformational change that chymotrypsin undergoes at 25°

INTRODUCTION

Some salts are known to enhance the stability of globular proteins, others to denature them[1] Previous studies with chymotrypsin (EC 3 4 4 5), mainly at 25°, showed little difference among salts in their effect on catalytic activity[2–7] However, the present work shows that solutes, when they are studied over a range of temperatures, have profoundly different effects on the maximal rate of chymotryptic hydrolysis

MATERIALS AND METHODS

α-Chymotrypsin (three times crystallized) was purchased from Worthington Biochemical Corp (Freehold, N J) Its concentration was determined spectrophotometrically using ε_{mM} at 281 nm 52 05 (ref 8) *N*-Acetyl-L-tyrosine ethyl ester (ATEE) was purchased from Aldrich Chemical Co (Milwaukee, Wisc), pectin (grade II) from Sigma Chemical Co (St Louis, Mo), and polyvinylpyrrolidone (PVP) (40 000 av mol wt) from GAF Corp (New York, N Y)

Chymotryptic activity was assayed at pH 8 by measuring the rate of uptake of standard 0 05 M NaOH (Hellige, Garden City, N Y) by 5 0 ml of a solution containing 1–5 μg of chymotrypsin, 2 5–50 μmoles of ATEE, and 50 μl of acetonitrile (the

Abbreviations ATEE, *N*-acetyl-L-tyrosine ethyl ester, PVP, polyvinylpyrrolidone

TABLE I

THE TEMPERATURE DEPENDENCE OF CHYMOTRYPTIC HYDROLYSIS OF ATEE IN VARIOUS SOLUTIONS

Solution	*Turnover number (sec^{-1})*					
	10°	*15°*	*20°*	*25°*	*30°*	*40°*
0 5 M $CaCl_2$	112 4*	132 2	176 9	225 7	261 8	420 6
0 5 M glycerol	70 9	81 2	92 2	236 0	230 0**	925 5**
3% (w/v) pectin	52 8	90 1	111 0**	167 1**	172 0	258 7
3% (w/v) PVP	38 4	65 3	121 1	140 7	182 1	238 8
0 5 M $NaClO_4$	67 1**	75 5**	112 1**	151 2	186 2	265 3
0 5 M Na_2SO_4	66 0	87 3	133 2	179 8	198 3	312 2
1 0 M Na_2SO_4	64 6	96 8	149 3	178 2	222 4	316 6***
0 5 M sucrose	62 5	85 6	114 2	176 6	179 6	356 3
Water	58 2	81 9	97 0	140 4	197 5	492 1

* Number for 11°
** Average of 2 determinations
*** Number for 37°

solvent for the substrate) Other solutes were present as indicated The pH and temperature were held constant by a recording pH-stat (E H Sargent and Co , Chicago, Ill) The v_{max} for a series of assays at increasing ATEE concentrations was calculated by the method of LINEWEAVER AND BURK[9] The enthalpies ($\Delta H^{\ddagger}$) and entropies ($\Delta S^{\ddagger}$) of activation were calculated according to LAIDLER[10]

RESULTS AND DISCUSSION

The temperature dependence of chymotryptic hydrolysis of ATEE was determined in water and 8 aqueous solutions (Table I) chosen to represent extremes of the lyotropic series[1] a polyanion (pectin), non-ionic polar compounds (glycerol and sucrose), and a non-ionic polymer (PVP) Arrhenius plots of some of the data are presented in Fig 1 As has been found before[11], there is a bending (change in slope) in

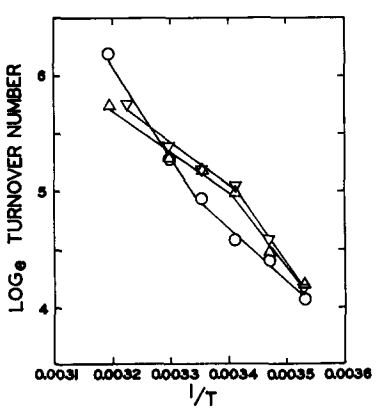

Fig 1 Arrhenius plots in 3 solutions (○—○), water, △—△, 0 5 M Na_2SO_4, and △—△, 1 0 M Na_2SO_4 The natural logarithm of the turnover number (sec^{-1}) is plotted against the reciprocal of the absolute temperature

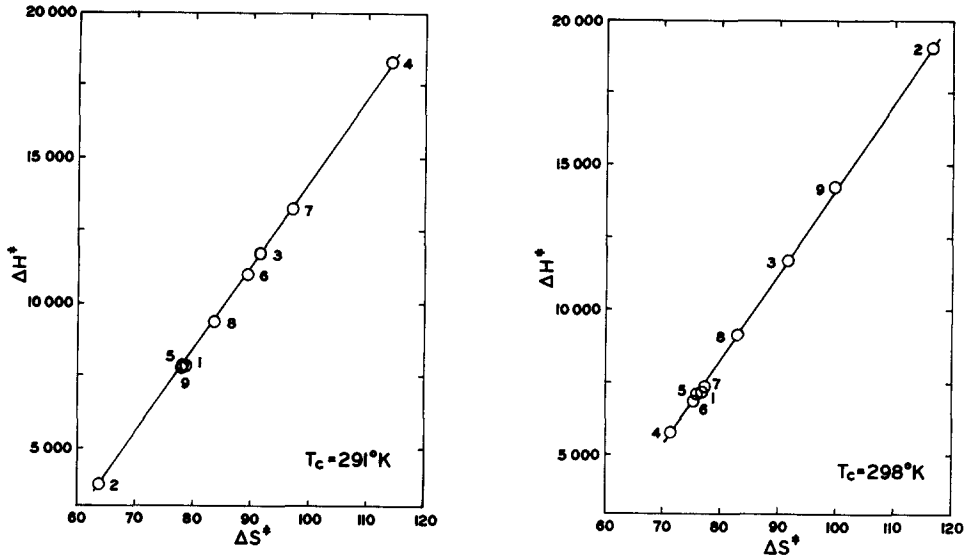

Fig 2 Compensation plot for the chymotryptic hydrolysis of ATEE in various solutions below about 20° $\Delta H^{\ddagger}$ (cal mole^{-1}) is plotted against $\Delta S^{\ddagger}$ (cal mole^{-1} degree^{-1}) (1) 0 5 M $CaCl_2$, (2) 0 5 M glycerol, (3) 3% (w/v) pectin, (4) 3% (w/v) PVP, (5) 0 5 M $NaClO_4$, (6) 0 5 M Na_2SO_4, (7) 1 0 M Na_2SO_4, (8) 0 5 M sucrose, (9) water T_c is 291°K

Fig 3 Compensation plot for the chymotryptic hydrolysis of ATEE in various solutions above about 25° $\Delta H^{\ddagger}$ (cal mole^{-1}) is plotted against $\Delta S^{\ddagger}$ (cal mole^{-1} degree^{-1}) Identification of points as in Fig 2 T_c is 298°K

the region corresponding to 290–295°K Arrhenius plots for all 9 solutions showed linearity in the upper (above 295°) and lower (below 290°) temperature ranges and these data were considered separately From the data for the lower temperature range were calculated $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ These values are plotted, one against the other, in Fig 2. The data for the upper temperature range were treated similarly, and for these data $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are presented in Fig 3 The values fall on straight lines whose slopes, the compensation temperatures (T_c), are 291°K (Fig 2) and 298°K (Fig 3) In comparing Fig 2 with Fig 3 several reversals can be noted Glycerol, which in Fig 2 has the lowest $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values, has the highest values in Fig 3 The converse is true of PVP Water and 1 0 M Na_2SO_4 also substantially reverse their positions in going from one temperature range to the other

It is apparent from Table I that solutes may affect rates of chymotryptic hydrolysis $\Delta H^{\ddagger}$ may vary by a factor of 5, yet there exists a temperature at which rates in all 9 systems converge That this is so means there is linear compensation of $\Delta H^{\ddagger}$ by $\Delta S^{\ddagger}$ That temperature at which the rates converge is the slope of the compensation plot, $\Delta\Delta H^{\ddagger}/\Delta\Delta S^{\ddagger}$, or T_c The fact that earlier studies did not reveal any striking effects of varying solutes on chymotryptic action is due to the accident of their being performed very near to T_c, where $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ exactly compensate to maintain a constant rate, unaffected by changes in the solution

Entropic compensation for enthalpy in chymotryptic hydrolysis has been described for a series of different substrates[12,13] Compensation behavior in the temperature region 250–315°K is not only characteristic of a wide range of protein phenomena,

but has been seen in non-protein systems as well[13] Although the physical basis of the phenomenon is unknown, the factor common to all examples of it is water, which, on the basis of such findings is suspected of undergoing some as-yet undiscovered thermally-induced change near 290°K Therefore discussions of compensation behavior have revolved around the role water plays in these systems Without attempting to unravel the mystery of compensation behavior, but assuming its basis in water's intimate involvement in protein structure, a few comments may be made about the case presented here VON HIPPEL AND SCHLEICH[1] analyze the effects of solutes according to three competing, therefore mutually dependent, forces acting on the shell of water surrounding a macromolecule (protein) the non-polar groups of the protein that, by extending into the water, organize water around themselves, the solute, that imposes its own order on the water, and the unperturbed water lattice If compensation behavior is basically a consequence of some property of water, it is not surprising that solute effects on chymotrypsin show compensation

The near coincidence of T_c with the temperature at which the Arrhenius plots curve suggests that the thermally-induced conformational change that accounts for these bends[14] has a necessary relation to the compensation behavior that is observed In this view, although at high and low temperatures the conformations of the enzyme-substrate complex may vary from solution to solution, they are most alike halfway through the thermally-induced change The transition state, another well-defined state, is raised above the complex by the same $\Delta F^{\ddagger}$ in each case (the rates at T_c are all the same) by a process that allows water structure to intervene[15] The individual differences in solutions do not affect $\Delta F^{\ddagger}$ at T_c, but do affect the activation process ($\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$), which is merely to restate the fact that there is compensation Those forces in the solution that below T_c drive the enzyme toward the most favorable conformation for catalysis, drive it away from that conformation above T_c, hence the reversal in passing through T_c from Fig 2 to Fig 3

It is entirely possible that the mid-point in the transition is only fortuitously close to T_c, yet the fact that they do nearly coincide, and that each phenomenon is influenced by water structure suggests that these two processes are linked These data presented here show that chymotrypsin is sensitive to the nature of the solution in which it acts, and they raise the possibility of using this sensitivity as a probe to study the dynamics of chymotryptic catalysis

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