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HYDROGEN BOND VS HYDROPHOBIC BOND CONTRIBUTIONS TO BINDING BY ADENOSINE DEAMINASES

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Abstract—The effects of varying temperature and pressure upon adenosine deaminase binding of the competitive inhibitor, purine riboside, suggest that the enzyme–inhibitor, and by implication the enzyme–substrate, complex is stabilized predominantly by hydrogen bonding.

INTRODUCTION

A site for the accommodation of the adenosine moiety must be generated in all enzymes that possess a dinucleotide cleft and in all adenylate-linked kinases. However, obtaining direct information on the properties of such adenosine binding sites has been hampered by two problems. Firstly, free adenosine usually binds relatively weakly to such enzymes (McPherson, 1970), and secondly, since it is relatively insoluble in water, its interactions with typical NAD-linked dehydrogenases or adenylate-linked kinases are difficult to study quantitatively. We hoped to circumvent these problems and gain some further insight into how enzymes interact with this important moiety by examining an enzyme for which adenosine is a true substrate.

Adenosine deaminase (E.C. 3.5.4.4) catalyzes the reaction:

$$\text{adenosine} + H_2O \rightarrow \text{inosine} + NH_3.$$

Although the crystal structure of the enzyme has not been determined, many of the functional properties of the substrate binding site are known. Thus the binding behaviour of over 50 substrate analogues and inhibitors can be explained by a model of the substrate site consisting of a hydrophillic region (for positioning the ribose moiety) and of a hydrophobic region (for accomodating the purine base). The ribose end of the substrate presumably interacts with the enzyme by hydrogen bonds to the 5′ position (an absolute requirement for binding) and also to either the 2′ or 3′ hydroxyls, or to both (Bloch *et al.*, 1967). The enzyme–purine interactions, on the other hand, are thought to be stabilized in a relatively non-specific way by hydrophobic interactions (Zielke & Suelter, 1971), although a general deficiency of electrons around position 6 of the purine ring appears to facilitate tight binding (Wolfenden, 1969; Wolfenden *et al.*, 1969). This description of the adenosine binding site, derived from analogue studies, is in many ways similar to current models of the adenosine domain in the coenzyme binding site of dehydrogenases, derived from crystallographic studies (Holbrook *et al.*, 1975). In neither case, however, do we know which of these 2 weak bond contributions is of greater importance in stabilization of the enzyme–ligand complex. An inlet into this latter problem may arise from temperature and pressure studies with model compounds (Nemethy & Scheraga, 1962; Brandts, 1967; Suzuki & Taniguchi, 1972). These tell us that hydrogen bonding tends to be stabilized at low temperatures and high pressures, while hydrophobic interactions are disrupted under these conditions. We therefore reasoned that an indication could be obtained of the relative contributions of hydrophobic vs hydrogen bonding to enzyme–ligand complexing by examining the temperature–pressure dependence of the enzyme interaction with substrate analogues that act as competitive inhibitors.

As temperature and pressure were to be variables, we chose to study adenosine deaminase isolated from species living in widely differing environments. The enzyme was isolated from 4 sources: calf intestine, heart of the skipjack tuna, *Euthynnus pelamis*, and the mantle muscle of the octopus, *Octopus cyanea*, and of the squid, *Symplectoteuthis oualaniensis*. The temperature and pressure effects on the binding of purine riboside, a competitive inhibitor of adenosine deaminase, lead us to propose that the enzyme–inhibitor, and by implication the enzyme–substrate, complex was stabilized predominantly by hydrogen bonding.

MATERIALS AND METHODS

Purified calf duodenal adenosine deaminase was purchased from Sigma Chemical Co., St Louis, MO, U.S.A., and was appropriately diluted for use. The other enzymes were obtained from freshly captured animals. In each case, the tissue was homogenized in 0.1 M imidazole–HCl buffer, pH 7.0 with a Virtis homogenizer. The suspension was centrifuged at 20,000 *g* for 20 min and the pellet discarded. The supernatent was then treated with 0.3 g/ml cellulose phosphate and recentrifuged. The adenosine deaminase activity remained in the supernatant, which was then treated with solid ammonium sulphate to 60% of saturation. After at least 60 min of stirring at 4°C, the suspension was centrifuged and the pellet discarded. The supernatant was then brought to 80% saturation with ammonium sulphate, stirred, then centrifuged. The supernatant was discarded.

The pellet was resuspended in 0.1 M imidazole buffer, pH 7.0 and this preparation was used without further purification for all kinetic studies.

All enzyme assays were performed at controlled temperature and pressure in 0.1 M imidazole buffer, pH 7.0. Enzyme activity was measured by the decrease in O.D. at 265 nm, due to adenosine hydrolysis to inosine, using a Unicam SP1800 recording spectrophotometer equiped with a constant temperature cell holder. For high pressure studies, the spectrophotometer was fitted with a high pressure cell of 1 cm light path and with sapphire windows.

K_m values were determined by Lineweaver–Burk plots. K_i values were determined from Dixon plots of 1/velocity vs inhibitor concentration using at least 2 substrate concentrations. All biochemicals were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. All other chemicals were reagent grade.

RESULTS AND DISCUSSION

The apparent enzyme–substrate affinities of adenosine deaminase from all 4 species were remarkably similar, the K_m values for the calf, tuna, octopus, and squid enzymes being 0.02, 0.03, 0.02, and 0.015 mM, respectively, at 25°C and pH 7.5. In all cases, the K_m values dropped with a drop in temperature of the reaction medium; we did not further pursue this behaviour since it has been carefully examined previously (Harbison & Fisher, 1973, 1974).

We reasoned that a good indication of the way in which the physical environment affects the substrate binding site could be obtained by examining how the enzyme interacts with purine riboside. Purine riboside is a substrate analogue of adenosine and is an established competitive inhibitor (Wolfenden, 1969; Wolfenden *et al.*, 1969). In agreement with previous studies, we found that adenosine deaminase in all 4 species showed a high affinity for purine riboside, comparable to its affinity for the true substrate (Michaelis constants in the 0.02–0.04 mM range, depending upon conditions). For this reason, the effect of the physical environment on enzyme–riboside complexing should mimic that of enzyme–substrate formation.

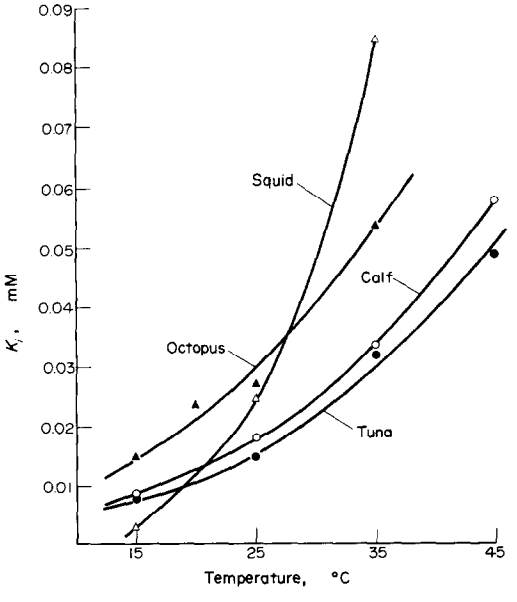


Fig. 1. The effect of temperature on the K_i for purine riboside for adenosine deaminases from 4 sources. Assay conditions: pH 7.0 imidazole buffer, 25°C, purine riboside varying from 0.01 to 0.1 mM, at adenosine concentrations varying between 0.01 and 0.05 mM. K_i values determined by Dixon plots.

The effect of temperature on adenosine deaminase binding of purine riboside is shown in Fig. 1. Although the squid enzyme appears to be the most temperature sensitive, the qualitative behaviour with respect to temperature is the same for all 4 enzymes studied. In each case, as temperature drops, the K_i for riboside drops; that is, low temperature appears to favour enzyme–riboside complexing. The thermodynamic parameters associated with the process are shown in Table 1. In each instance, the enthalpy and entropy changes occurring during enzyme–ligand association are large and negative, the values being highest for the squid enzyme. In the squid, such high temperature dependence of binding the substrate analogue (and by implication, also the substrate) may be an adaptive characteristic designed to take advantage of the organism's vertically migrating habit.

The effect of temperature on the binding process in all 4 cases suggests that of the 2 potential contributions to binding, the hydrogen bonding contribution greatly predominates the hydrophobic one, since low temperature tends to stabilize polar interactions such as hydrogen bonding but to disrupt hydrophobic ones (Nemethy & Scheraga, 1962; Brandts, 1967). The effect of increasing hydrostatic pressure on enzyme–ligand interactions further supports this interpretation. As evident in Table 1, high pressure stabilizes the enzyme–riboside complex and again the qualitative result is the same in all 4 cases. Ligand binding occurs with a negative volume change, the mammalian enzyme showing the greatest pressure sensitivity. Again, if the hydrophobic contribution to binding were dominant, the ΔV associated with the pressure would be positive and binding would be disrupted by high pressure, i.e. the reverse of our observed results. Hence, the effects of pressure on riboside binding are also consistent with the assumption that hydrogen bond formation, a process that is favoured by high pressure (Suzuki & Taniguchi, 1972), predominates over hydrophobic interactions in stabilization of the enzyme–riboside complex.

Table 1. Thermodynamic parameters for the association of adenosine deaminase with purine riboside and with inosine

Enzyme source	ΔG° (kcal/mole)	ΔH° (kcal/mole)	ΔS° (cal/deg mole)	ΔV (ml/mole)
Purine riboside				
calf	−6.5	−12.5	−20.1	−59
tuna	−6.6	−12.5	−19.8	−28
octopus	−6.3	−13.5	−24.2	−14
squid	−6.3	−25.0	−62.7	−18
Inosine				
squid	−4.9	+11.0	+53.0	+22.5

The ΔG° and ΔS° values are for 298 K. All values calculated from the effect of temperature (with pressure constant), or the effect of pressure (temperature being constant), on the K_i for the inhibitor

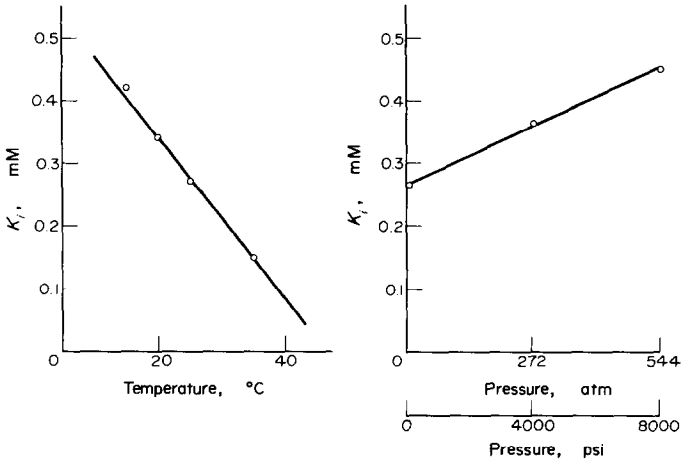
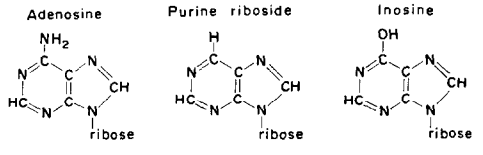


Fig. 2. The effect of temperature (left panel) and of hydrostatic pressure (right panel) on the K_i for inosine for squid muscle adenosine deaminase. Assay conditions as in Fig. 1, except in the pressure experiments, total volume of the reaction medium was 5 ml, and inosine concentrations ranging from 0.05 mM to 0.15 mM.

In contrast to these experiments utilizing purine riboside, studies with inosine as a competitive inhibitor indicate that for this substrate analogue, the hydrophobic contribution to enzyme–ligand association is the larger. Since inosine is a poor inhibitor, at concentrations required, it was rather difficult to study using our assay methods. For that reason, only the squid enzyme, the most inosine sensitive of the 4, was studied in detail. The data (Fig. 2) indicate that increasing temperature leads to a rise in enzyme–ligand affinity (a decrease in K_i), while increasing pressure leads to a drop in enzyme–ligand affinity, precisely the opposite to the situation with purine riboside. From the structure of inosine and purine riboside, given below in comparison with adenosine,



it is evident that the difference between the 2 substrate analogues is the presence of a polar group in inosine at position 6 of the purine ring. Its presence reduces by a factor of 10 the enzyme affinity for ligand, a result also observed by Wolfenden (1969) and Wolfenden *et al.* (1969). We presume the presence of a polar group in this position disrupts hydrogen bonding of the enzyme to the ribose hydroxyls. Under these conditions, then, the relative importance of the hydrophobic contribution to binding greatly increases, thus reversing the sensitivity of enzyme–ligand binding to changes in the physical environment.

SUMMARY

The effects of temperature and pressure upon adenosine deaminase (E.C. 3.5.4.4) binding of the competitive inhibitor, purine riboside, were determined in an attempt to evaluate the relative importance of hydrophillic and hydrophobic contributions to substrate binding. The enzyme was isolated from 4 sources: calf intestine, tuna heart, and the mantle muscle of squid and octopus. In all cases enzyme–ligand binding was accompanied by large and negative changes in entropy and enthalpy. The affinity of the enzyme for the inhibitor increased with decreasing temperature. Increased hydrostatic pressure stabilized the enzyme–riboside complex, enzyme–ligand binding occuring with a negative volume change. Both these results suggest that the enzyme–inhibitor, and by extension the enzyme–substrate, complex is stabilized predominantly by hydrogen bonding, a process which is favoured by low temperature and high pressure.

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