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Temperature Adaptation of Enzymes: Roles of the Free Energy, the Enthalpy, and the Entropy of Activation

(rabbit/lobster/chick/tuna/halibut/cod)

PHILIP S. LOW, JEFFREY L. BADA, AND GEORGE N. SOMERO

Scripps Institution of Oceanography, University of California, La Jolla, Calif. 92037

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ABSTRACT **The enzymic reactions of ectothermic (cold-blooded) species differ from those of avian and mammalian species in terms of the magnitudes of the three thermodynamic activation parameters, the free energy of activation ($\Delta G^{\ddagger}$), the enthalpy of activation ($\Delta H^{\ddagger}$), and the entropy of activation ($\Delta S^{\ddagger}$). Ectothermic enzymes are more efficient than the homologous enzymes of birds and mammals in reducing the $\Delta G^{\ddagger}$ "energy barrier" to a chemical reaction. Moreover, the relative importance of the enthalpic and entropic contributions to $\Delta G^{\ddagger}$ differs between these two broad classes of organisms.**

Because all organisms conduct many of the same chemical transformations, certain functional classes of enzymes are present in virtually all species. It is, therefore, axiomatic that interspecific (homologous) variants of a particular type of enzyme must conduct their catalytic and regulatory functions at widely different temperatures. Since temperature is known to have profound effects on the activities and structures of enzymes, it is logical to ask whether a particular enzyme variant is especially well-adapted for function under the thermal regime it normally experiences. For example, are enzymes of ectothermic species, such as fishes, more effective catalysts at low temperatures than the homologous enzymes of warm-blooded birds and mammals?

In studies of enzymic adaptation to temperature two aspects of enzyme function have been of particular interest. First, since temperature is known to affect the higher orders of protein structure and the interactions of proteins with low molecular weight ligands, several recent studies have focused on the effects of temperature on the formation of enzyme–substrate complexes (1–3). Indeed, this step in the catalytic process is generally very temperature-sensitive, and enzymes of ectothermic species often display optimal substrate-binding properties at temperatures that approximate the normal habitat temperatures of the species (1–3).

The next event in the catalytic process is the conversion of the enzyme–substrate complex into an "activated complex," a high-energy complex that can decay into product(s) and free enzyme. To generate the active complex, free energy—the free energy of activation ($\Delta G^{\ddagger}$)—must be added to the enzyme–substrate complex. The magnitude of $\Delta G^{\ddagger}$ is, in effect, the "energy barrier" to the reaction, and by significantly reducing the $\Delta G^{\ddagger}$ values of chemical reactions, enzymes enable metabolic reactions to occur at high rates at biological temperatures. From the standpoint of adaptation to different environmental temperatures, it has been proposed that enzymes of cold-adapted organisms, i.e., most ectotherms, would be especially well-suited for low-temperature function if they were capable of reducing the $\Delta G^{\ddagger}$ characteristic of their reactions more than were the homologous enzymes of more warm-adapted species, i.e., birds or mammals.

In this paper, we report that the values of $\Delta G^{\ddagger}$ are indeed slightly lower for enzymic reactions catalyzed by enzymes of ectotherms, relative to the homologous reactions of birds and mammals. Moreover, the relative contributions of the enthalpies and entropies of activation to $\Delta G^{\ddagger}$ differ markedly and, we feel, adaptively, between ectothermic and avian–mammalian enzymic reactions.

METHODS

Thermodynamic activation parameters were determined for muscle-type (M_4) lactate dehydrogenase (L-lactate: NAD oxidoreductase, EC 1.1.1.27) enzymes from various ectothermic and endothermic organisms. The purified enzymes were generously provided by Drs. N. O. Kaplan and F. Stolzenbach.

Before measurements of lactate dehydrogenase activity, the purified enzymes were incubated in 0.20 M phosphate buffer (pH 7.5)–0.01 M 2-mercaptoethanol, for 24 hr. The activity of the enzymes was assayed spectrophotometrically by monitoring the decrease in absorbance at 340 nm as a function of time. The assay solution contained 0.10 M phosphate buffer (pH 7.5); 1.2 mM NADH; and various concentrations of pyruvate. The reaction was initiated by adding enough enzyme to yield an absorbance change of 1 A_{340} unit. Assays were performed at 5°, 15°, 25°, and 35°. At each temperature, dehydrogenase activity was measured at six concentrations of pyruvate; duplicate assays were used for each concentration. V_{max} values were obtained by the double-reciprocal method of Lineweaver and Burk.

Thermodynamic functions were calculated according to the following relationships (4):

$$\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\,\Delta S^{\ddagger},$$
$$\Delta H^{\ddagger} = Ea - RT,$$
$$\Delta S^{\ddagger} = 4.576\,(\log K - 10.753 - \log T + Ea/4.576\,T),$$

and K (in sec^{-1}) = V_{max}/mg of Enzyme × molecular weight × 10^{-3} mmol/μmol × 1 min/60 sec, where the molecular weight of the enzyme is expressed in mg/mmol. Activation energy (Ea) was calculated from the Arrhenius equation by the method of least squares. All Arrhenius plots were linear over the range of temperature used.

RESULTS

Thermodynamic activation parameters for several lactate dehydrogenase reactions are listed in Table 1, along with the

corresponding values for the reactions catalyzed by different variants of glyceraldehyde-3-phosphate dehydrogenase and glycogen phosphorylase-*b*. The values other than for lactate dehydrogenase have been computed by us using data published by others (5, 6). To the best of our knowledge, Table 1 presents all of the reliable information available concerning the $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$ characteristics of homologous enzymic reactions.

DISCUSSION

The use of $\Delta G^{\ddagger}$ and $\Delta H^{\ddagger}$ as indexes of catalytic efficiency

All previous studies that have attempted to compare the catalytic efficiencies of enzymes of differently thermally-adapted organisms have used the Arrhenius activation energy, $Ea = \Delta H^{\ddagger} + RT$, as the index of the capacity of an enzyme to reduce the energy barrier to a reaction (2). Even though it has been clear for some time that the true height of the energy barrier is the magnitude of $\Delta G^{\ddagger}$, biologists have continued to rely on *Ea* values since estimates of $\Delta S^{\ddagger}$ and, therefore, $\Delta G^{\ddagger}$ can be obtained only if the number of enzyme molecules present in the assay system is accurately known (7).

As is apparent from the equation, $\Delta G^{\ddagger} = Ea - RT - T\Delta S^{\ddagger}$, *Ea* is a valid index for comparison of the abilities of different enzymes to reduce the energy barrier to a given reaction only if the entropy of activation is the same in all cases. As the data of Table 1 indicate, this is certainly not the case. The large differences in $\Delta S^{\ddagger}$ between ectothermic and avian-mammalian reactions significantly reduce the differences in the observed $\Delta G^{\ddagger}$ that would be predicted on the basis of *Ea* differences. Thus, even though for the enzymes studied there is a positive correlation between the magnitude of *Ea* and the adaptation temperature of the species, *Ea* cannot be used as a quantitative index of catalytic efficiency. If one were to compute the differences in catalytic rate between the ectothermic and avian-mammalian variants of a particular reaction using *Ea* as a measure of the energy barrier to the reaction, he would predict that the ectothermic reactions would occur at rates several orders of magnitude greater than the rates of the avian-mammalian reactions (2). In fact, the observed V_{max} values differ only by a factor of 3-5, (Table 1). The observed differences in V_{max} activity, while much smaller than would be predicted on the basis of Ea differences, nonetheless seem to represent a significant temperature adaptation on the part of ectothermic enzyme, particularly at low temperatures.

The fact that ectothermic enzymes display higher catalytic efficiencies than the homologous enzymes of birds and mammals raises an interesting question concerning enzymic evolution. If we assume that modern avian-mammalian enzymes evolved from an ancestral enzyme that was similar to present-day ectothermic enzymes, how do we rationalize an apparent loss in catalytic activity during the evolution of warm-blooded forms? Speculations as to the basis of this seemingly paradoxical evolutionary change are presented elsewhere (3).

Contributions of $\Delta H^{\ddagger}$ and $T\,\Delta S^{\ddagger}$ to $\Delta G^{\ddagger}$

In addition to differing in their abilities to reduce the $\Delta G^{\ddagger}$ "barrier" to chemical reactions, ectothermic enzymes differ from the homologous enzymes of birds and mammals in terms of the relative contributions of the enthalpy ($\Delta H^{\ddagger}$) and entropy ($T\,\Delta S^{\ddagger}$) of activation to $\Delta G^{\ddagger}$. For all of the enzymes we have examined, the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values of the ectothermic reactions are lower than the corresponding values for the avian and mammalian enzymic reactions. Thus, the mean $\Delta S^{\ddagger}$ value of the ectothermic reactions listed in Table 1 is -6.3 entropy units (e.u.), whereas the mean $\Delta S^{\ddagger}$ value for the avian and mammalian reactions is $+4.5$ e.u. Correspondingly, the average $\Delta H^{\ddagger}$ value for the avian and mammalian reactions exceeds the average $\Delta H^{\ddagger}$ value for the ecto-

TABLE 1. *Thermodynamic activation parameters for lactate dehydrogenase reactions*

Enzyme	Animal	Assay temp. (°C)	V_{max}*	*Ea* (cal/mol)§	$\Delta H^{\ddagger}$ (cal/mol)	$\Delta S^{\ddagger}$ (e.u.)	$\Delta G^{\ddagger}$ (cal/mol)
Muscle-type lactate dehydrogenase	Rabbit	5	0.975×10^3	13,100	12,550	−2.3	13,200
		35	1.08×10^3	13,100	12,500	−2.5	13,250
	Chick	5	1.93×10^3	11,100	10,550	−8.4	12,850
		35	1.41×10^3	11,100	10,450	−8.7	13,150
	Tuna	5	8.6×10^2	9,350	8,800	−13.3	12,500
		35	4.5×10^3	9,350	8,750	−13.5	12,900
	Halibut	5	4.12×10^3	9,300	8,750	−13.5	12,500
		35	2.1×10^3	9,300	8,650	−13.7	12,900
D-Glyceraldehyde† -3-phosphate dehydrogenase	Rabbit	5	6.1	19,000	18,450	11.4	15,300
		35	180	19,000	18,400	11.3	14,900
	Lobster	5	22.7	14,500	13,950	−2.2	14,550
		35	220	14,500	13,900	−2.9	14,800
	Cod	5	18.5	14,500	13,950	−2.6	14,700
		35	225	14,500	13,900	−2.9	14,800
Muscle‡ glycogen phosphorylase-*b*	Rabbit	0	0.804	21,200	20,650	17.2	15,950
		30	60	21,200	20,600	17.8	15,200
	Lobster	0	4.5	15,900	15,350	1.1	15,050
		30	70.8	15,900	15,300	0.8	15,100

* μmol of substrate per min per mg of enzyme.
† Values computed on the basis of data given in Cowey (5).
‡ Values computed on the basis of data given in Assaf and Graves (6).
§ Standard deviations on Ea values were less than ±10%.

thermic reactions by about 14,200 joules/mol (3400 cal/mol). We feel that these differing contributions of enthalpic and entropic energies to $\Delta G^{\ddagger}$ may reflect important adaptations to the different thermal environments in which these two classes of enzymes function.

As seen from an evolutionary perspective, we hypothesize that several factors may have conferred selective advantage on the relative energetic dependencies of ectothermic and avian–mammalian enzymes. First, as has been suggested by numerous workers in the past (2), low enthalpies of activation render chemical reactions relatively temperature-independent. Thus, in ectotherms, which often experience 10–20° changes in body temperature diurnally and/or seasonally, rates of metabolism may be held relatively stable in the face of temperature changes if the enthalpies of activation of metabolic reactions are low.

While this argument may provide a partial explanation for the low $\Delta H^{\ddagger}$ values characteristic of ectothermic reactions, it cannot explain the evolutionary acquisition of a larger dependence on $\Delta H^{\ddagger}$ in avian and mammalian systems. Although the gaining of the homeothermic condition no doubt reduced, or even eliminated, the advantages of low $\Delta H^{\ddagger}$ values for purposes of rate-stabilization in the face of changing body temperature, we feel that there are other bases for the differing reliances on enthalpic and entropic activation energies between ectotherms and birds or mammals.

During the evolution of homeothermy, body temperatures became higher, as well as more stable. In contemporary birds and mammals, the temperature of the cell is near 40°, i.e., the heat content (enthalpy) and the entropy of the environment in which enzymes function are higher than in the case of ectothermic organisms. We propose that this change in enthalpy and entropy of the local environment of the enzymes had two selective influences on enzymic function. First, the higher entropy of the avian and mammalian cellular components relative to ectotherms may make it more difficult to form the enzyme–substrate activated complex from an entropy standpoint. Conversely, the higher heat content of the homeothermic cell should make enthalpic activation more likely. If we assume that $\Delta G^{\ddagger}$ values for a particular enzymic reaction are relatively fixed, as the data of Table 1 suggest, then avian and mammalian enzymes may have altered their enthalpic versus entropic contributions in order to render their catalytic function more consistent with the energy characteristics of their cellular environment.

In ectothermic organisms, metabolism often occurs at temperatures below 10°. Thus, relative to birds and mammals, the cellular constituents of ectotherms are low in heat content and entropy; in forming the activated complex, it may be relatively simple for ectothermic enzymes to keep entropy increases to a minimum (e.g., glycogen phosphorylase-*b*) or, in fact, to substantially reduce the entropy of the enzyme–substrate complex (e.g., glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase). By so exploiting the entropy characteristics of its cellular environment, the ectotherm appears to minimize the difficulties that might stem from the relatively low amounts of enthalpy present to activate the enzyme–substrate complex.

The structural basis of $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ differences

In this paper, we will only speculate briefly about the structural basis of the differences in the $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ values between ectothermic and avian–mammalian enzymes. Recent studies have shown that the substrate-binding sites of phylogenetically different variants of a particular enzyme are highly similar (8–12). Furthermore, reaction mechanisms for the conversion of substrate to product(s) appear to be the same for different variants of a particular enzyme (13). Therefore, it appears likely that the entropy and enthalpy differences during the activation event cannot be accounted for at the active site of the enzyme.

We also suggest that the basis for the lower $\Delta S^{\ddagger}$ characteristics of ectothermic enzymes is not the result of protein–solvent interactions. Any decrease in activation entropy due to the structuring of water molecules around exposed hydrophobic aminoacid residues during catalysis would lower $\Delta S^{\ddagger}$ values only at low temperatures (14). The entropy of activation values for enzymes listed in Table 1, however, do not change appreciably with temperature.

Thus, the most probable basis for the differing $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ values among variants of the same enzyme lies in the internal structure of the protein. Conformational changes during catalysis apparently generate a more rigid or ordered structure in the interior of ectothermic enzymes than in homologous enzymes from birds or mammals.

As we shall discuss in a forthcoming publication, slight differences in aminoacid composition can account for the observed thermodynamic differences among homologues of a given enzyme.

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