Protein hydration changes during catalysis: A new mechanism of enzymatic rate-enhancement and ion activation/inhibition of catalysis

(Conformational change/free energy of activation/activation volume/catalytic rate-enhancement)

PHILIP S. LOW AND GEORGE N. SOMERO
Scripps Institution of Oceanography, University of California, San Diego, Box 1529, La Jolla, Calif. 92037

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ABSTRACT There exists a linear correlation between the effect of a salt on the rate of an enzymatic reaction and its effect on the activation volume ($\Delta V^+$) of the reaction. Salts that increase $\Delta V^+$ invariably decrease the rate of the reaction, and vice versa. The salt effects on reaction rate are, however, much larger than would be predicted solely on the basis of pressure-volume work changes derived from the observed alterations in $\Delta V^+$. Different inorganic salts affect reaction rates and activation volumes in a manner that reflects the salts' positions in the Hofmeister series.

These observations, taken in conjunction with data on the effects of salts on protein functional group (aminoacid side-chains and peptide linkages) hydration, lead us to propose the following hypothesis to account for salt activation and inhibition of catalysis. Aminoacid side-chains and peptide linkages located on or near the protein surface change their exposure to water during conformational events in catalysis. These protein group transfers are accompanied by large volume and energy changes that are due largely to changes in the organization of water around these groups. When these transfer processes occur during the rate-limiting step in catalysis, these energy and volume changes can contribute to the free energy of activation ($\Delta G^+$) and the activation volume of the reaction. By influencing the degree to which water can organize around transferred protein groups, salts can modify both the $\Delta G^+$ (rate) and the $\Delta V^+$ of a reaction.

Many workers have considered the energy and volume changes that accompany the large-scale conformational changes during protein denaturation (1–5). These energy and volume changes have been attributed mainly to the transfer of protein groups (aminoacid side-chains and peptide linkages) from the relatively nonpolar interior of a protein to the surrounding water (1–5).

Since conformational changes may also occur during catalysis (6), the transfer of protein groups at the water–protein interface may also contribute to the energy ($\Delta G^+$) and volume ($\Delta V^+$) changes of catalysis (7). To investigate this hypothesis, we have examined the effects of certain neutral salts on the activation volume ($\Delta V^+$) and rate of several enzymic reactions since neutral salts are known to modify the energy and volume changes that accompany transfer of protein group analogues between nonpolar solvents and water (8–10).

MATERIALS AND METHODS

The enzymes and assay procedures used in these studies are described in the previous paper in this series (7).

In most cases the effects of salts on $\Delta V^+$ and $V_{max}$ were determined simultaneously. However, in a few cases in which the effects of a given enzyme took place over two or more days, salt effects on $V_{max}$ were re-examined separately to eliminate the possibility of a time-dependent denaturation effect. Variations in the $\Delta V^+$ for a given enzyme and set of assay conditions were extremely small; activation volumes agreed to within approximately ±3 cm$^3$/mol for measurements performed over a several month time period, using the same enzyme preparation.

RESULTS

The effects of different salts on the activation volumes and maximal velocities of the lactate dehydrogenase (EC 1.1.1.27; L-lactate:NAD$^+$ oxidoreductase), malate dehydrogenase (EC 1.1.1.37; L-malate:NAD$^+$ oxidoreductase), and isocitrate dehydrogenase (EC 1.1.1.41; threo-D,isoctirate: NAD$^+$ oxidoreductase (decarboxylating]) reactions, all of which display no specific ion requirements, and on the pyruvate kinase (EC 2.7.1.40; ATP:pyruvate 2-O-phosphotransferase) reaction, which requires a monovalent cation (K$^+$), NH$_4^+$ are shown in Figs. 1–4. When $\Delta V^+$ is plotted as a function of $V_{max}$ for each salt-enzyme combination, a striking linear correlation is observed. Regardless of the direction (activation or inhibition) and the magnitude of the salt effect, the linear correlation between $V_{max}$ and $\Delta V^+$ is maintained. For most of the reactions examined, the slope of a plot of $\Delta V^+$ against percent activation or inhibition is relatively similar (Table 1).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Dialyzed</th>
<th>Salt</th>
<th>$\Delta V^+$/$V_{max}$</th>
<th>$\Delta V^+$/$V_{max}$/$V_{max}$/$2$ (cm$^3$/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PK</td>
<td>Yes</td>
<td>CsCl</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>PK (in 7 mM KCl)</td>
<td>Yes</td>
<td>LiCl</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>PK</td>
<td>No</td>
<td>KCl</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>PK</td>
<td>Yes</td>
<td>KF</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>PK (in 40 mM KCl)</td>
<td>No</td>
<td>NaI</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>LDH</td>
<td>Yes</td>
<td>KSCN</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>LDH</td>
<td>Yes</td>
<td>LiCl</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>LDH</td>
<td>Yes</td>
<td>KCl</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>IDH (in glycerol)</td>
<td>No</td>
<td>n-Propanol</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>MDH</td>
<td>No</td>
<td>KCl</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Average for all enzymes</td>
<td></td>
<td></td>
<td>19.2</td>
<td></td>
</tr>
</tbody>
</table>

Values were calculated by subtracting the $\Delta V^+$ value at half of the maximal velocity at optimal salt concentration from $\Delta V^+$ at optimal salt concentration. The values for both $\Delta V^+$/$V_{max}$ and $\Delta V^+$/$V_{max}$/$V_{max}$ were based on the least square fit of the plots of $\Delta V^+$ against rate. PK, pyruvate kinase; LDH, lactate dehydrogenase; IDH, isocitrate dehydrogenase; MDH, malate dehydrogenase.
DISCUSSION

It is evident from these results that the mechanism by which salts activate or inhibit enzymic reactions must involve a process that has the following characteristics. First, the process must occur with a volume change. Second, different salts and varying concentrations of salts must modify this volume change differently. The effects of the different salts on the volume and rate changes generally follow the Hofmeister series (7, 11). Third, the volume change that accompanies the process must be intimately linked with the energetics of the process such that, as the volume change decreases, the process becomes more exergonic, and vice-versa (see Figs. 1–4).

With regard to this association between $\Delta V^\ddagger$ and reaction rate, it is vitally important to emphasize that the effect of salts on enzymic reaction rates is not simply a consequence of salt-induced changes in $\Delta V^\ddagger$. The effect of $\Delta V^\ddagger$ on the absolute rate of a reaction ($k$) at constant temperature and pressure is indicated by the equation (12),

$$k = (K T / h) e^{-E^*/RT} e^{-P\Delta V^*/RT} e^{\Delta S^*/R}$$

where all other symbols have their usual thermodynamic meanings. A change in $\Delta V^\ddagger$ of 20 cm$^3$/mol at 278°K and 1 atmosphere pressure would cause a change in rate ($k$) of less than 1%, i.e., a change in rate due to a change in $\Delta V^\ddagger$ of 20 cm$^3$/mol would be negligible. However, we observed that a salt-induced change in $\Delta V^\ddagger$ of 20 cm$^3$/mol was associated with an approximately 50% change in rate (Table 1). Thus, changes in the $P\Delta V^\ddagger$ term in the above equation cannot be
FIG. 4. The relationship between reaction rate and activation volume at different solute concentrations for several enzyme-solute combinations. (A) Malate dehydrogenase (MDH)-KCl. (B) M₄ lactate dehydrogenase (LDH)-LiCl. (C) Pyruvate kinase (PK)-NaI; 40 mM KCl was added to the reaction mixture to insure a measurable rate of activity. (D) M₄ LDH-KSCN. (E) Isocitrate dehydrogenase (IDH), n-propanol. (F) M₄ LDH-KCl.
the source of the rate changes we observed. Furthermore, if 
\( \Delta V^1 \) were responsible for the change in rate, then the 
reaction velocity would be logarithmically related to \( \Delta V^2 \). We 
observed a linear correlation between rate and \( \Delta V^2 \) in 
all cases. Thus, as stated above, the change in \( \Delta V^2 \) is a correlate 
of, but is not the cause of, salt-induced changes in reaction velocity.

The inability of salt-induced changes in \( \Delta V^1 \) to account 
sufficiently for the salt-induced rate changes indicates that the 
process responsible for the change in \( \Delta V^2 \) is characterized 
by another energy change of much greater magnitude than that due to \( P\Delta V^2 \). To determine the source of this larger 
energy change, let us examine the process by which salts 
affect \( \Delta V^2 \). In a previous paper (7) we presented evidence 
that aminoacid side-chains and peptide linkages located near the 
protein–water interface may change their exposure to water 
during catalytic conformational changes. Because these 
groups alter the density of the nearby water, their 
movement results in volume changes. Salts modify these volume 
changes by affecting the degree to which water organizes 
around these groups (7).

The energy changes that accompany protein group transfers 
to relatively nonpolar regions on or within the protein and water 
can be estimated by examining the free energies of transfer of protein group analogues between 
nonpolar solvents and water. Table 2 lists the energetic costs 
of several model transfer processes which we suggest may 
simulate actual events on an enzyme’s surface during a 
conformational change, e.g., during the activation event in catalysis. Transfer processes such as these, which occur during 
the rate-determining step of an enzymic reaction, could contribute to the \( \Delta G^1 \) and \( \Delta V^1 \) of the reaction. The “energy barrier” \( \Delta G^2 \) to 
enzymic reaction will be raised or lowered by an amount equal to the net free energy of transfer 
from all such processes occurring during the rate-determining step. This adjustment of \( \Delta G^1 \) by transfer processes 
can be most easily understood by making a hypothetical comparison 
of two transition states that differ only in the exposure of a single carboxylate moiety. The existence of an 
exposed carboxylate group in the transition state will decrease its energy by approximately 4.5 kcal/mol (Table 2). This 
reduction in the free energy of the transition state will increase the probability of its formation and thereby accelerate the 
velocity of the reaction. It is clear that the exposed carboxyl-
ate group need not be at the catalytic site of the enzyme. The 
only requirement is that the exposure of the group be 
a obligatory part of the activation event. Further, it is clear 
that complete exposure or withdrawal of a protein group is 
not necessary. For example, a reduction in \( \Delta G^1 \) of 1000 
calories/mol could be effected by exposing a peptide linkage 
completely or by increasing the exposure of a carboxylate group 
only very slightly (Table 2).

If the effects of salts on both \( \Delta V^1 \) and \( \Delta G^2 \) derive from 
salt-induced changes in the hydration of protein groups which 
change their exposure to water during catalysis, one would predict a correlation between salt effects on \( \Delta V^1 \) and 
\( \Delta G^2 \). Our results (Figs. 1–4) are fully consistent with this 
prediction. We propose, therefore, that salts, by modifying the 
hydration spheres around the transferred protein groups, 
will affect not only the volume changes but also the energetics 
of the transfer processes. This explanation of the observed 
salt effects allows an interpretation of both ion activation 
and ion inhibition of catalysis. The difference between ion 
activation and inhibition is due to the direction of the net 
transfer processes. The following examples will illustrate our 
point.

In the case of the malate dehydrogenase reaction (Fig. 
4A), for example, we suggest that one or more water-constricting 
groups on the protein surface increase their exposure 
to water during the formation of the activated enzyme–substrate complex. These transfer processes lead to reductions 
in both \( \Delta V^1 \) and \( \Delta G^1 \) (Table 2). When salts are present 
in the medium, hydration of the exposed water-constricting 
groups will generally be hindered, and the normal reduction 
in \( \Delta V^1 \) and \( \Delta G^1 \) will be smaller. The salts thus appear to 
increase \( \Delta V^1 \) and \( \Delta G^1 \), leading to the observed rate inhibition.

For an ion-activated enzyme like pyruvate kinase, the 
direction of group transfer would be the opposite, as will 
the salt effects on \( \Delta V^1 \) and rate. Groups on the protein surface 
that constrict water will be withdrawn fully or partially into 
the protein during the activation event. Available data on 
pyruvate kinase support this hypothesis. Each of the four 
subunits of pyruvate kinase contains a carboxylate group 
which binds a potassium ion (14). Neutralization of four 
carboxylate groups by K+ would be expected to lead to a volume 
change of approximately 45 cm3/mol. We observed 
that potassium ion reduces the activation volume of the pyruvate 
kine reaction by approximately 40 cm3/mol. The effect 
of potassium ion on the free energy change of this transfer 
process, on the basis of model transfer studies (Table 1), 
would be to reduce \( \Delta G^1 \) by approximately 4.5 kcal/subunit 
or by approximately 18 kcal/mol of enzyme. The observation 
that no detectable pyruvate kinase activity occurs in the 
absence of potassium ion is consistent with the fact that an 
increase in \( \Delta G^1 \) of 18 kcal/mol would make the reaction 
kinetically prohibitive at biological temperatures.

It is apparent that the magnitudes of the free energy 
changes accompanying group transfer reactions (Table 2) 
are sufficiently large to provide adequate “raw material” for 
adjusting \( \Delta G^1 \) values to whatever levels are optimal on 
biological grounds. For example, by fully exposing one additional 
peptide linkage or carboxylate group during a step in catalysis, 
the rate of that step could be enhanced 7-fold or 
2000-fold, respectively. When one considers the vast numbers 
of charged and polar groups that are situated at or near 
the protein-water interface for enzymes (15), it becomes 
clear that protein group transfer reactions provide a resource 
for catalytic rate enhancement for all types of enzymes. Thus, difficulties in accounting for rate enhancement of en-

### Table 2. Free energies of transfer (\( \Delta G^1 \)) of protein functional groups from ethanol to water

<table>
<thead>
<tr>
<th>Protein functional group</th>
<th>( \Delta G^1 ) (calories/mol)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH2OH</td>
<td>668</td>
<td>a</td>
</tr>
<tr>
<td>CH3</td>
<td>1908</td>
<td>a</td>
</tr>
<tr>
<td>Methionyl sulfur</td>
<td>-736</td>
<td>a</td>
</tr>
<tr>
<td>-OH</td>
<td>-900</td>
<td>a</td>
</tr>
<tr>
<td>CH3=N—(peptide backbone)</td>
<td>-1145</td>
<td>a</td>
</tr>
<tr>
<td>Dipolar ion</td>
<td>-3722</td>
<td>a</td>
</tr>
<tr>
<td>-COO—</td>
<td>-4500</td>
<td>b</td>
</tr>
</tbody>
</table>

* E. J. Cohn and J. T. Edsall (2).

* Calculated according to the method of Cohn and Edsall, from data given by A. Vigues (13) and W. Kauzmann (3) for transfers from hydrocarbon to water.
zyme-catalyzed reactions in comparison with nonenzymic reactions (6, 16, 17) might be partially resolved on the basis of our "group-transfer-hydration" hypothesis. The observation that large numbers of enzymes are activated or inhibited by salts (11, 18–21) suggests that changes in protein group hydration are a common occurrence during catalysis.

Our mechanism differs from other mechanisms of enzymic rate-enhancement, e.g., orientation effects, semi-conduction, strain, oriented energy transfer, orbital steering, acid-base catalysis, and covalent catalysis (6, 17, 22–27), in that the transfer processes are not restricted to the catalytic site. Our mechanism emphasizes that a major portion of the enzyme molecule may play an important role in the energetics of catalysis. Thus, an important mechanism for reducing the free energy of the activated complex may involve transfer processes on the enzyme's surface at regions remote from the catalytic site. The requirement for biological catalysts (enzymes) to be macromolecular may become clearer as this "group-transfer-hydration" mechanism becomes better understood in structural terms.

Finally, whereas we have discussed this mechanism only in the single context of catalytic activation processes, free energy changes stemming from hydration changes may also contribute significantly to the energetics of such biological processes as muscle contraction (28, 29), nerve impulse conduction (30), ATP hydrolysis (31), hemoglobin-ligand binding (32), and protein subunit assembly (33, 34) where, in all cases, changes in hydration have been observed.

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