Biophysical Journal, Volume 96

Supporting Material

A link between hinge-bending domain motions and the temperature dependence of catalysis in IPMDH

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SUPPLEMENTARY RESULTS AND DISCUSSION

H/D exchange of ligand-bound states.

Hydrogen/deuterium exchange relaxation spectra were measured at room temperature for the unliganded IPMDH and the IPMDH+NAD, IPMDH+IPM complexes as well as for the IPMDH+IPM+NADH non-productive ternary complex (Figure S3). The overall flexibility of the IPMDH+IPM complex was found to be almost indistinguishable from that of the ligand-free enzyme while the IPMDH+NAD and the IPMDH+IPM+NADH complexes exhibit increased conformational rigidity. The relaxation spectra are not perfectly parallel; larger differences are seen at the larger ln k_0t values, corresponding to amide protons exchanging on longer timescales. The direct protection of amide protons by the bound coenzyme does not explain this effect; it seems likely that the binding of NAD globally rigidifies the entire Domain 1. The substrate IPM, however, has a negligible effect on conformational flexibility; even though it induces partial closing of the domains, this is apparently insufficient to protect a significant number of amide protons.

Differential scanning calorimetry of ligand-bound states.

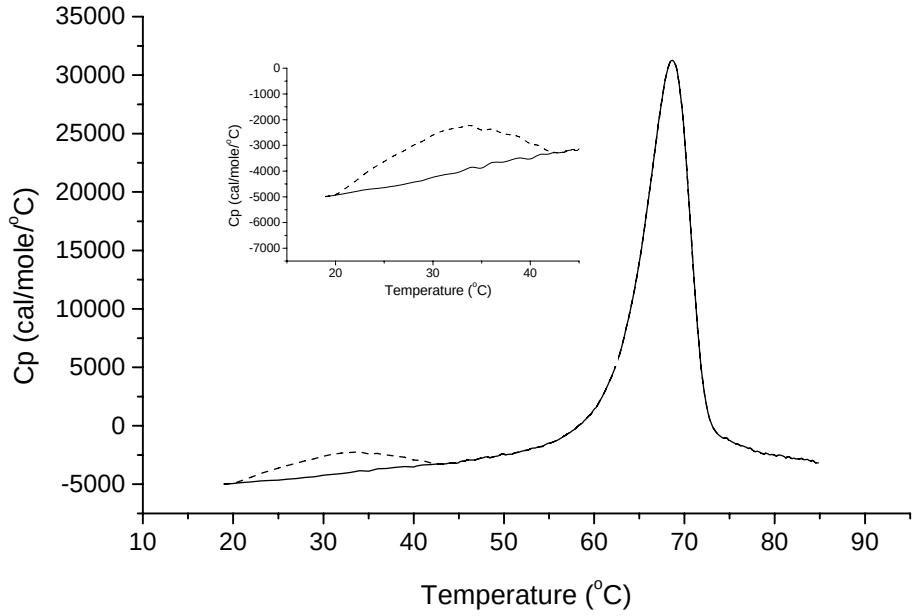
Table S1 shows the denaturation temperatures and the half-widths of the peaks observed in the heat capacity curves of various ligand-bound forms of IPMDH, as measured by DSC. The half-widths of the peaks are inversely proportional to the van't Hoff enthalpy changes of the corresponding transitions. The transition temperatures vary between 68.4 and 72.5 ^{o}C. The difference between these temperatures is small compared to that reported in similar studies (1). In contrast to the results obtained from H/D exchange, here IPM causes the larger effect. NAD(H) only induces a small change, comparable to the standard error of the measurement. Bound IPM, however, increases the transition temperature by ~4°C, and also noticeably decreases the half-width of the peak, which indicates a higher enthalpy of unfolding, and suggests a more compact structure (2). The minor difference between the data for the IPMDH+IPM and the ternary complexes suggests that the IPM-bound form is more closed-like, while the IPMDH+NAD complex resembles more an open state. A more detailed, quantitative evaluation of these data such as a comparison of the enthalpy changes is not possible because the thermal denaturation of IPMDH is irreversible, and therefore no reliable enthalpy values can be extracted.

Flexibility changes during the catalytic cycle.

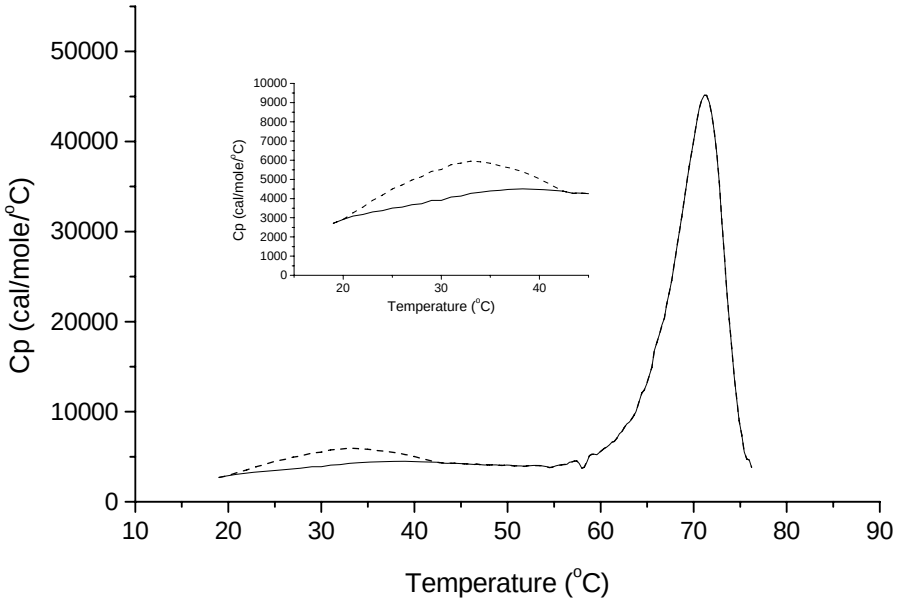
Putting together all the pieces of information gained from our experiments performed with various ligand-bound forms of IPMDH, a putative model emerges that describes how the conformation and the flexibility of IPMDH vary during its catalytic cycle. In solution, an equilibrium exists between the open and closed states as well as a large number of intermediate substates between these two extremes. Bound ligands influence this equilibrium and shift it towards more closed forms while also modulating the overall conformational flexibility of the protein. At the intracellular concentration of the coenzyme ([NAD^+]=0,8 mM, [NADH]=1μM(3)), the equilibrium state of IPMDH is the NAD^+ saturated form, which is a fairly rigid molecule with an open-like conformation. When the substrate IPM binds to this form, it induces domain closure, and the catalytic reaction is carried out. The ligand-free state after the dissociation of the products is quite open and highly flexible, and can bind both NAD^+ and IPM as also testified by the Random Bi Bi mechanism of IPMDH (4). Thus, both the more open-like but less flexible NAD^+-IPMDH complex and the more closed-like but flexible IPM-IPMDH complex can serve as an intermediate on the pathway to the reactive ternary complex.

Supplementary Figure 1

A

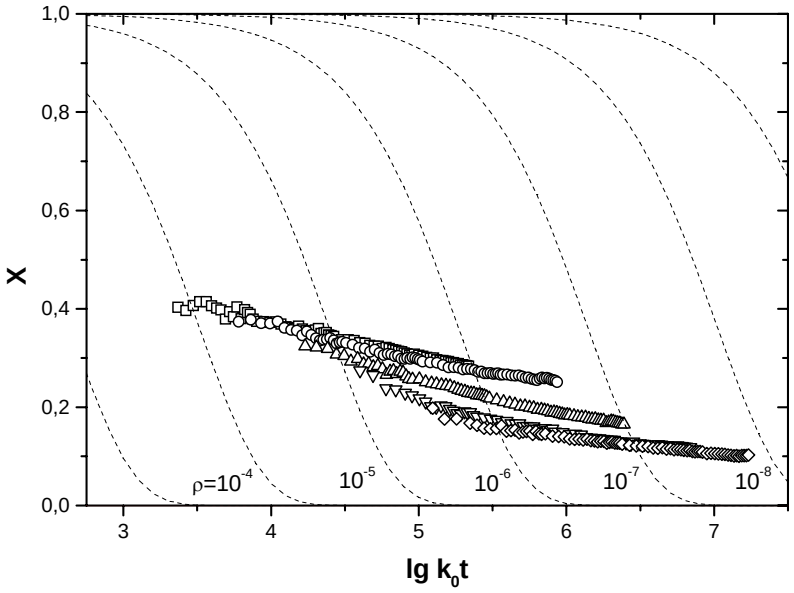


B



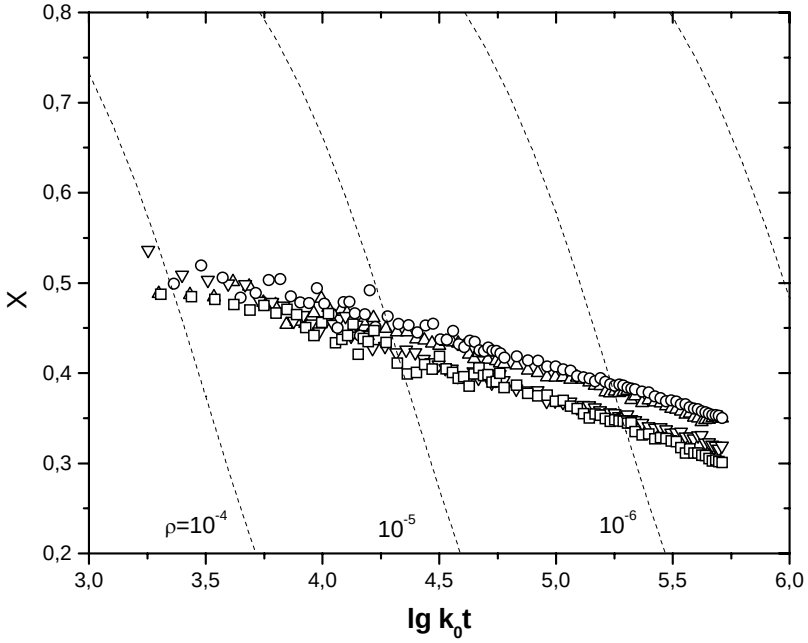
The measured heat capacity curves of apo-IPMDH (*A*) and IPM-bound IPMDH (*B*) (*solid lines*). The calculated curves (*dashed line*) were derived from the van't Hoff plot for IPM (Figure 3B), assuming a temperature-induced conformational transition between two static conformational states of IPMDH with different affinities towards IPM, with an enthalpy of 26 kcal/mol and a midpoint of 31 ^{o}C. The insets are magnified versions of the temperature range of the assumed transition.

Supplementary Figure 2



Hydrogen/deuterium exchange data displayed as relaxation spectra for *E. coli* IPMDH at pD 8.15 at various temperatures (20(□), 32(○), 41(△), 50(▽) and 59(◊) °C). X, the fraction of unexchanged peptide hydrogens is plotted vs. lg k_0t, where t is the time and k_0 is the chemical exchange rate constant. The dashed lines represent the exchange rate curves for hypothetical polypeptides characterized by a given probability (the ρ values) of solvent exposure of the peptide groups.

Supplementary Figure 3



Hydrogen/deuterium exchange relaxation spectra for different ligand-bound states of *E. coli* IPMDH at pD 8.15 at 30°C. The apoenzyme (□) and IPMDH+IPM binary complex (▽) show similar flexibility distributions. The NAD(H)-bound states, i.e. the IPMDH+NAD binary (△) and the IPMDH+IPM+NADH non-productive ternary (○), complexes are more rigid than the other forms.

Supplementary Table 1 The effect of different ligands on the thermostability of IPMDH

Unfolding temperatures for the various liganded forms of IPMDH obtained by differential scanning calorimetry, and the respective half-widths of the associated peaks.

	T_m (^oC)	$\Delta T_{½}$ (^oC)
IPMDH	68.4	4.8
IPMDH+NAD	68.8	4.1
IPMDH+NADH	69.1	4.6
IPMDH+IPM	72.1	3.4
IPMDH+NADH+IPM	72.5	3.0

References

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