Molecular Dynamic Simulation of Malate Dehydrogenase

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Abstract

The activity of cytoplasmic malate dehydrogenase (cMDH)is perturbed by solution crowding. Since specific movements of a surface loop in the region of the enzyme's active site have been implicated in the activity of this enzyme, it seems likely that flexibility of this and other portions of cMDH are important for kinetic activity. Solution crowding might limit that flexibility. We have examined the flexibility and conformational mobility of cMDH using molecular dynamic simulations. The enzyme, in the absence of cofactor and substrate, in the presence of cofactor only, and in the presence of cofactor and substrate, was examined in 100 ps simulations using the AMBER force field. In all simulations the protein was placed in a box of solvent water. The structure of cMDH,

crystallized at high ammonium sulfate concentrations in the presence of NAD,was used as the starting structure. The cofactor was removed from the pdb file, the apo enzyme energy_minimized, and the dynamic simulation performed. Cofactor (NADH) was added to the structure that evolved during the molecular dynamics simulation of the apo enzyme. The complex was energy_minimized, and the simulation of the enzyme_cofactor complex performed. A similar process was followed for an enzyme_NADH_oxaloacetate complex. Results show the structure of cMDH changes relative to the crystalline enzyme during simulations. Active site ligand additions cause further perturbations. Flexible areas include the active site and the subunit interface. We gratefully acknowledge the support of the Ohio Supercomputer Center.

Introduction:

Cytoplasmic Malate Dehydrogenase is a critical enzyme for coordinating the metab olism of the cytoplasm and the mitochondrion. It catalyzes the interconversion of oxaloacetate and malate using the cofactors NADH and NAD. Malate dehydrogenase is a dimeric enzyme with 35 kD subunits. Eadie-Hofstee analysis indicates the enzyme is negatively cooperative with respect to its use of substrate and cofactor (**Figure 1**). The magnitude of cooperativity depends on pH and on ionic strength suggesting an electrostatic component to interactions leading to cooperativity [1]. We have also shown that cofactor binding to cMDH is negatively cooperative (**Figure 2**). It is interesting that the enzyme's kinetic properties change in solutions made as concentrated as the cytoplasm with proteins or the hydrophilic polymer, Ficoll (compare the red and the green points in **Figure 1**). In the case of NADH utilization, the kinetics change from apparently cooperative to apparently Mi chaelis Menton (straight versus curved line) [2].

It is reasonable that conformations most pertinent to cooperativity differ from those found in structures deduced from X-ray crystallography [3]. Reported crystal structures of cMDH were determined on crystals formed at high ammonium sulfate concentrations and substantial concentrations of macromolecules [4]. Both of these conditions mitigate against cooperativity in substrate/cofactor utilization.   However, during molecular dynamic (MD) simulations a relevant
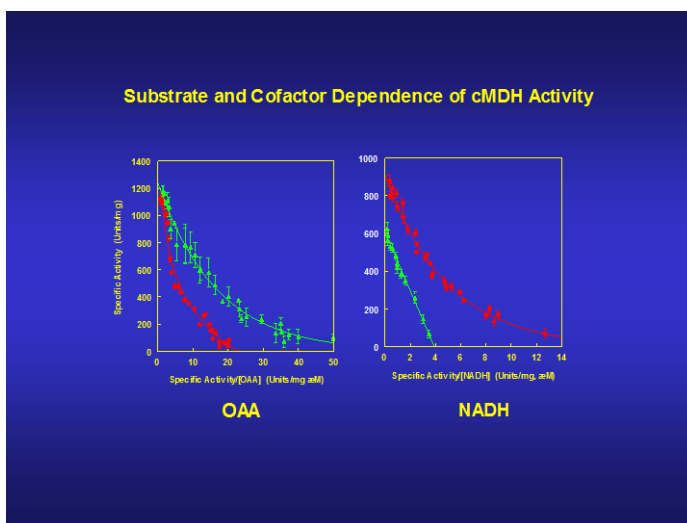
Figure 1: Substrate (oxaloacetate) and cofactor (NADH) dependence of cMDH activity at pH 8.0 (50mM HEPES) 25°C. Red symbols correspond to assays performed without additives. Green symbols correspond to assays performed in the presence of 300 mg/ml Ficoll to simulate *in vivo* conditions. Lines represent the best fit of experimental data
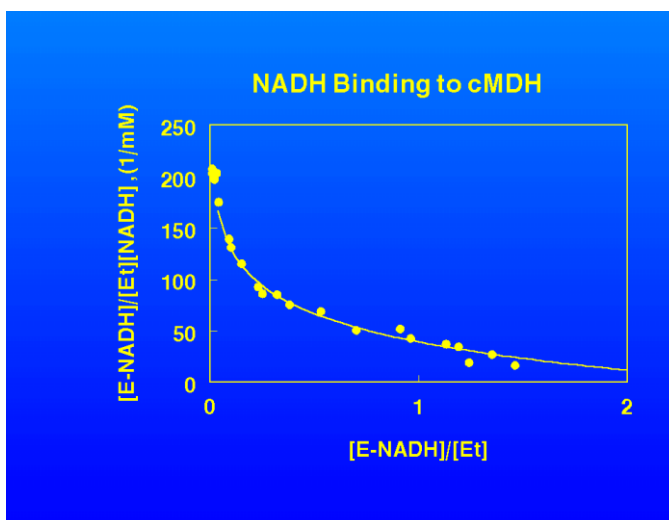

Figure 2: Scatchard plot describing NADH binding to cMDH at pH 8.0 (50 mM HEPES) 25°C. Points represent experimental data and the line represents the best fit of that data. The observed curvature is expected for a negatively cooperative binding interaction.

structure may evolve.

As part of a general effort to understand both the molecular basis for cooperativity in cMDH, and to

determine the reason for the enzyme=s sensitivity to solution crowding, we have studied apo MDH
and the cMDH-NADH complex using molecular dynamic simulations.

MD was carried out as outlined in **Figure 3**. For apo cMDH the starting structure was that reported
from which NAD and sulfate was removed [5]. The structure was energy minimized, placed in a
solvation box, and warmed in successive 50° K steps to 300 degrees. The starting structure for the
simulation of cMDH with bound NADH was the final structure in the apo simulation. Initial
placement of cofactor was done by superimposing the structure of E. coli MDH, which has NAD on
the cMDH subunits and placing the NADH the same way in cMDH subunits [6]. As with apo
cMDH, a program of minimization, hydration, warming, and equilibration was followed (**Figure 3**).
Extended simulations representing a time span of 100 ps were produced .

Results of simulations are shown in part in **Figure 4**. **Figure 4A** represents a backbone
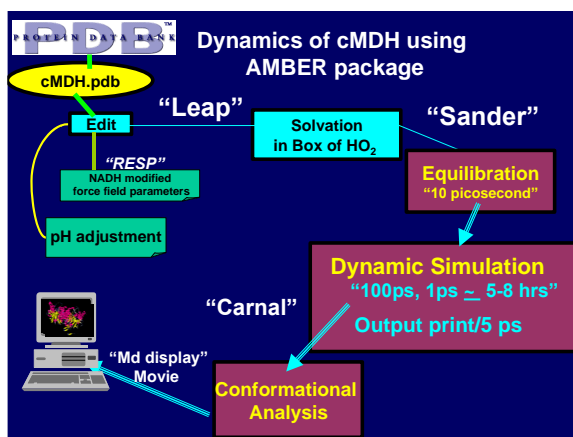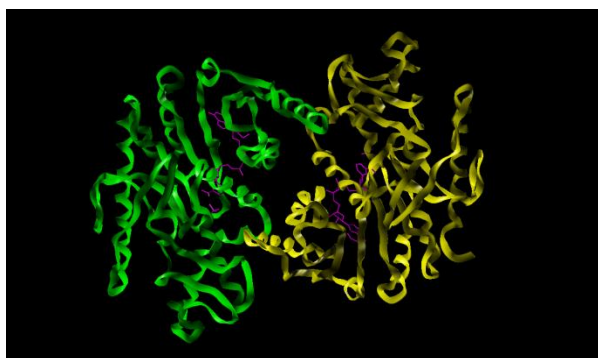

Figure 3: sketch of the followed steps of dynamic simulation process.
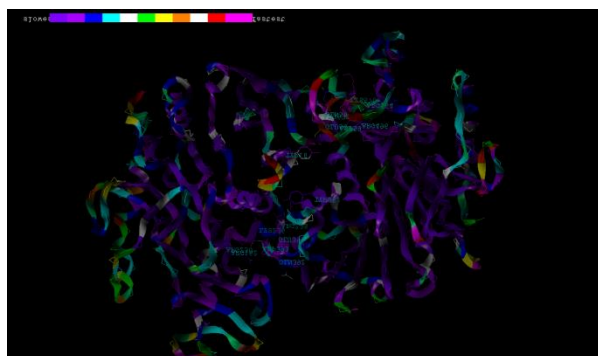
trace of subunit "A" of cMDH in yellow and subunit "B" in green. They show how the subunit
interfaces mesh, with contacts between three helical regions. For subunit B they are, from top to
bottom, the ∀-2G..∀-3G helices, the ∀-B helix, and the ∀-C helix.

Flexibility and conformational changes are coded in cMDH structures in **Figure 4 B and D** for apo-
cMDH , and Figure **4C and E** for cMDH-NADH   These structures are colored according to the
size of standard deviation in the position of the ∀-carbon of each amino acid. Structure B and C
summarize the whole simulation while D and E summarize the last 10% of it. Upon comparing B

and C, its clear that motions + changes are greater in MDH-NADH than the apo enzyme. Comparisons of the last 10% of MD for each structure were completely purple indicating that the difference in coloring in B and D reflects differences in conformational changes rather than flexibility. Readjusting the coloring scale, to look at the detail of the last 10% of each structures' MD, Figure D an E were obtained. They indicate flexibility in both simulated structures is very similar, with the cMDH-NADH complex being somewhat more flexible. However, the interface region at the lower right is an area of flexibility.
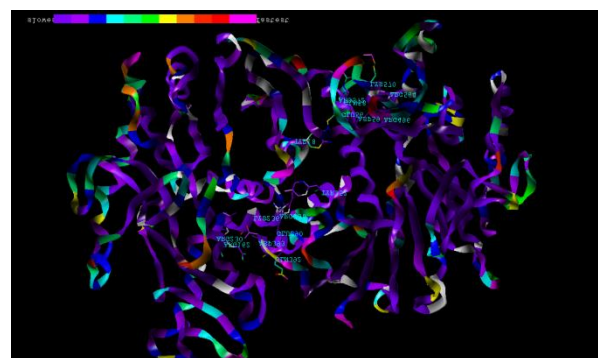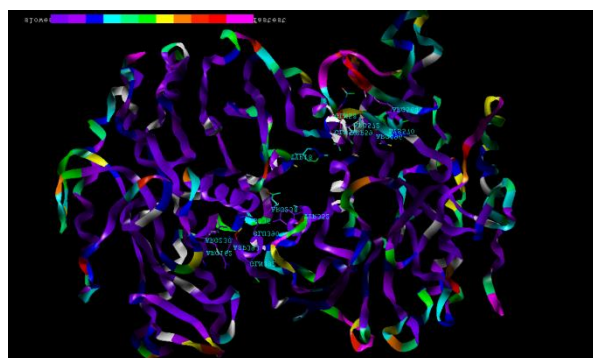

4A

4B
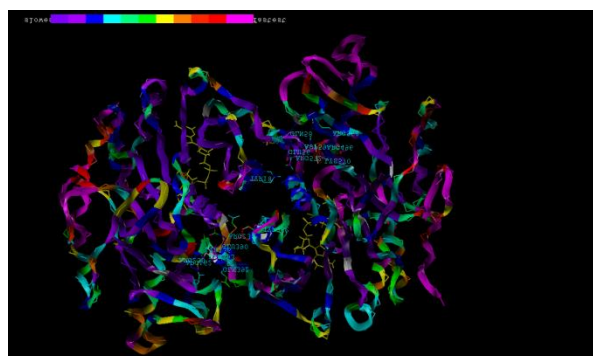
4C

4D

4E

Figure 4. **Figure 4A** represents a backbone trace of subunit "A" of cMDH in yellow and subunit "B" in green. They show how the subunit interfaces mesh, with contacts between three helical regions. For subunit B they are, from top to bottom, the ∀-2G..∀-3G helices, the ∀-B helix, and the ∀-C helix. Flexibility and conformational changes are coded in cMDH structures in **Figure 4 B and D** for apo-cMDH , and Figure **4C and E** for cMDH-NADH

Throughout the MD simulation, little change occurred in the structure of cMDH, including a loop of amino acids near the enzyme's active site. In contrast, during MD simulations clear changes occurred in the cMDH-NADH complex. The loop changed its position substantially (**Figure 5**), though it is not as closed as reported ternary complexes. Changes in backbone structures comprising the cofactor binding site as well as the loop are shown during initial and intermediate stages of MD simulations of the cMDH-NADH complex. **Figure 6A** shows results for the "A" subunit indicating only modest changes in backbone conformation occur. In contrast, changes in the "B" subunit are substantial (**Figure 6B**). All elements of the "B" subunit cofactor site change. It is reasonable to expect that affinity of cofactor binding also changes.
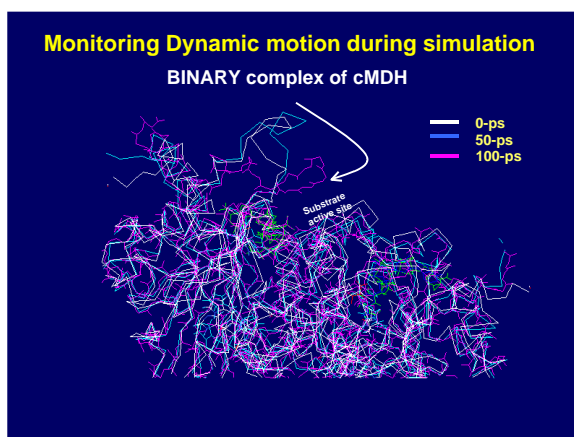

Figure 5: The loop changed its position substantially during the dynamic simulation 0-100 ps

Among regions whose positions change during the simulation is the ∀2G helix (564-572, Figure 6B). This is one of the interface helices mentioned with respect to Figure 4. To estimate the relative magnitude of movement in each subunit at the subunit interface, subunits were superimposed on the basis of helices that form the interface. It is clear that at the end of cMDH
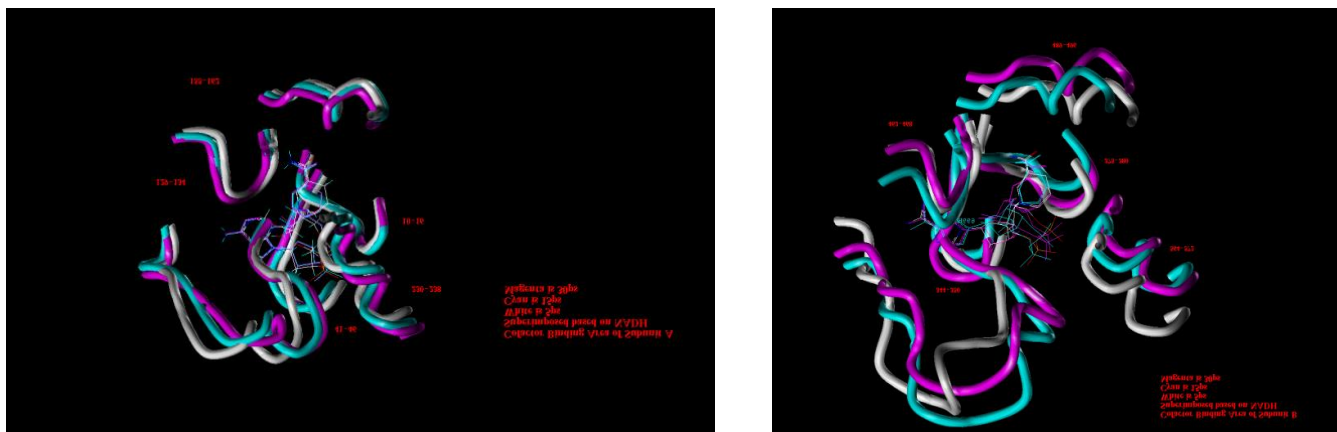
Figure 6: A indicating only modest changes in backbone conformation occur. **B**. All elements of subunit cofactor site change

simulations, the backbone structures of apo-cMDH subunits are very similar in the interface region (**Figure 7a**). In contrast they diverge, particularly in the ∀2G helix (left of **Figure 7b**), in the cMDH-NADH complex. This is primarily a result of movement in subunit B. For reference, subunits of the crystallographically determined structure are shown in **Figure 7c**. The close superposition of the subunit interfaces suggests that under conditions of crystallization, the subunit
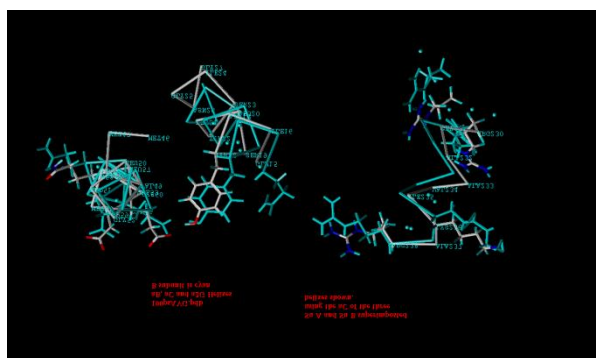

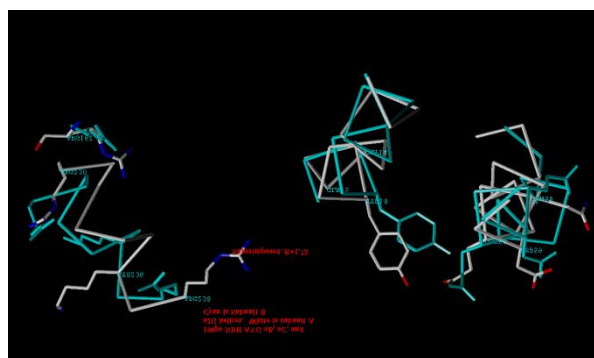interface does not reflect cooperativity while it does at the conclusion of MD simulations.

Figure 7A   Figure 7B

Specific subunit-subunit interactions were monitored during MD simulations of the cMDH-NADH complex. An example of results is shown in **Figures 8 a and b** for apo and NADH-cMDH.
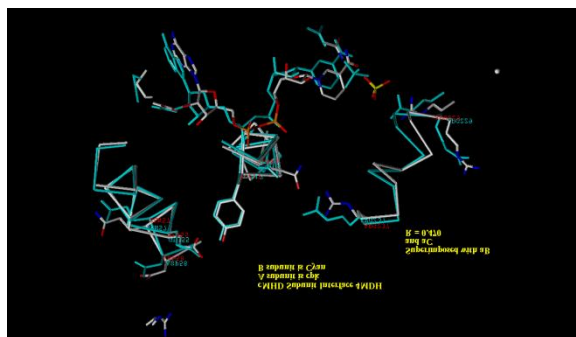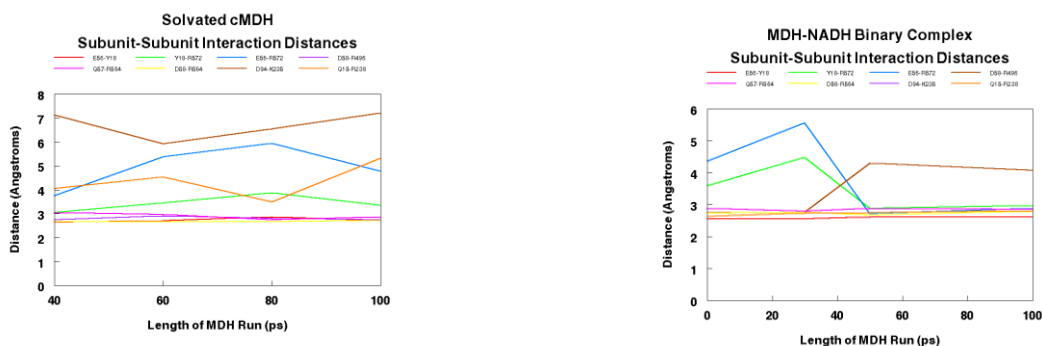
Figure 7C

Figure 7A: the backbone structures of apo-cMDH subunits are very similar in the interface region
In contrast they diverge, particularly in the ∀2G helix. **Figure 7b**: in the cMDH-NADH complex.
Figure 7c This is primarily a result of movement in subunit B. For reference, subunits of the crystallographically determined structure are shown in.

In general, the bonding pattern does not change during MD simulation of apo cMDH (**Figure 8a**).

In contrast interface bonding does change in the NADH complex (**Figure 8b**), consistent with

cooperativity. Much of the change involves the ∀2G helix.

Based on simulation results, it is tempting to speculate on a cooperative pathway for cMDH-NADH

to account for cMDH's binding activity. If NADH binding perturbs the ∀2G helix and so disrupts

interactions involving D58 and E 55, this will perturb the active site residue, R161,which otherwise

binds D58 itself [Table 1& 2].

Figures 8 a and b for apo and NADH-cMDH. .

Conclusion

Molecular Dynamic Simulations indicate substantial conformational changes occur in the structure of cMDH as a result of NADH binding. Results suggest a cooperative pathway by which cofactor binding at one subunit effects the binding affinity of the other subunit.

References

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Table-1: RMS Deviation between Superpositioned Subunit B and Subunit A During MD Simulation

Simulation	Apo	NADH adduct
Initial	1.13	0.521
30 ps	1.085	1.338
100 ps	1.30	1.235
MDH/NAD (crystal)		0.470
MDH/tetrahydroNAD/α-ketomalonate		0.394

Table-2: Subunit interactions

Interaction	Apo MDH		hold MDH	
	Subunit A	Subunit B	Subunit A	Subunit B
E55-Y17	2.7	2.59	2.62	5.53
E55-R238*	4.78	2.78	2.72	6.48
D58-R161*	2.75	2.82	4.07	2.77
D58-R230*	2.73	2.76	2.79	2.88

Authors Column

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