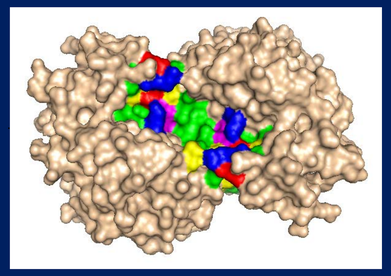
The Bell Laboratories: Systems Biology, Structural Biology, Biophysics & Bioinformatics

Understanding the Interface: Exploring Malate Dehydrogenase using Computational and Experimental Approaches

Ellis Bell[1], James Burnett[2], Michael Scwabe[1] & Jessica Bell[1]
[1]University of San Diego, [2]University of Pittsburgh



Abstract

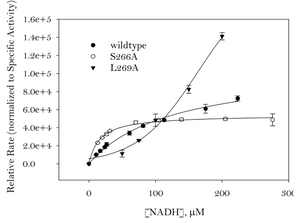
Dimeric Malate Dehydrogenase exhibits properties attributed to subunit interactions. The dimer interface comprises 47 residues, clustered in four groupings in the sequence, 15 residues are conserved in eukaryata, with 7 more functionally conserved. Structures of watermelon glyoxysomal MDH, with or without the allosteric ligand, Citrate, bound to one subunit, were examined to explore the nature of subunit contacts (using the program HINT). In addition, to examine second sphere residues with potential roles in catalysis, and to establish differences in conserved crystallographic water molecules we used POOL and DRoP respectively. Intra- and inter-molecular HINT analysis with no ligands bound versus the dimer with Citrate bound to one subunit indicates that D87 forms multiple hydrogen bonds within the interfacial 266-270 loop region, some having increased intensity with Citrate bound, (mobile loop closed) as compared to no ligands bound, (mobile loop open). Further analysis suggests R196 and T268 lose favorable interactions with D87 on the opposite subunit, while E256 loses unfavorable interactions with D90 upon citrate binding which draws S266 further into the active site causing T268 to shift away from D87 and closer to Q58. This affects the L269-Q58 interaction across the interface. S266A and L269A mutants show loss of citrate inhibition and binding, and diminished substrate inhibition. Mutants T268D and I88A show little impact on cofactor binding although I88A becomes monomeric as shown by SEC and cross-linking. Coupled with changes in S266, T268 and L269 interactions across the interface, it appears that subunit interactions are triggered by cofactor induced changes in L269-Q58 interactions between subunits.

Experimental Results

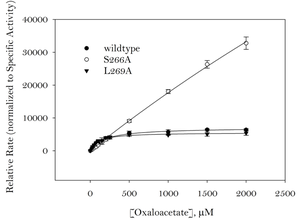
Site Directed Mutagenesis shows critical roles for Serine 266 and Leucine 269 in ligand induced subunit interactions

Mutant	Specific Activity	Km(NADH),μM	Km(OAA),μM
wildtype	100%	126 (15)	186 (11)
I88A	0.11%	Could Not Determine	Could Not Determine
T255E	2.6%	258 (81)	41 (23)
S266A	15.5%	16.4 (1.2)	Linear
L269A	1.6%	Linear	98 (9)

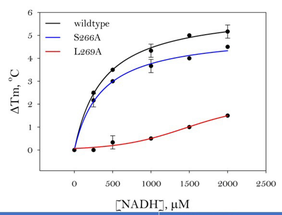
Effects of Mutations on NADH Saturation
Michaelis Menten Kinetics,
0.05M Phosphate, pH 8.0



Effects of Mutations on Oxaloacetate Saturation
Michaelis Menten Kinetics
0.05M Phosphate, pH 8.0



NADH Binding Determined by Fluorescence Based Thermal Shift Effects of Mutations



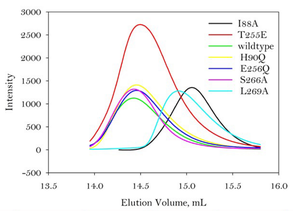
Mutant	K_D (St Dev), μM
Wildtype	373 (26)
S266A	354 (42)
L269A	"linear"

Leucine 269 Involved in Normal Saturation by NADH but not Oxaloacetate Saturation

Serine 266 Governs Oxaloacetate Saturation but not NADH Binding

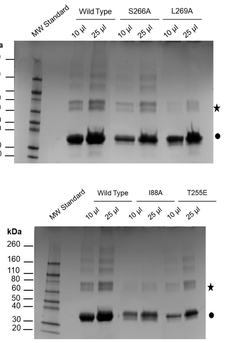
As judged by SEC and Cross-Linking Data, mutants retain dimeric structure, although L269A & I88A appear to have distorted dimer structures

Size Exclusion Chromatography
MDH wildtype & Mutants



- Calculated by assuming a linear relationship between adjacent molecular weight standards and a dimer molecular weight for wildtype of 68,800
- Caveat: Since the molecular weights must be either 68,000 (dimer) or 34,400 (monomer), the apparent molecular weights reflect shape factors.

Mutant	wildtype	T255E	I88A	S266A	L269A
Elution Volume, mL	14.43	14.53	15.08	14.49	14.66
Apparent Molecular Weight*	68,800	67,090	36,400	68,000	64,500

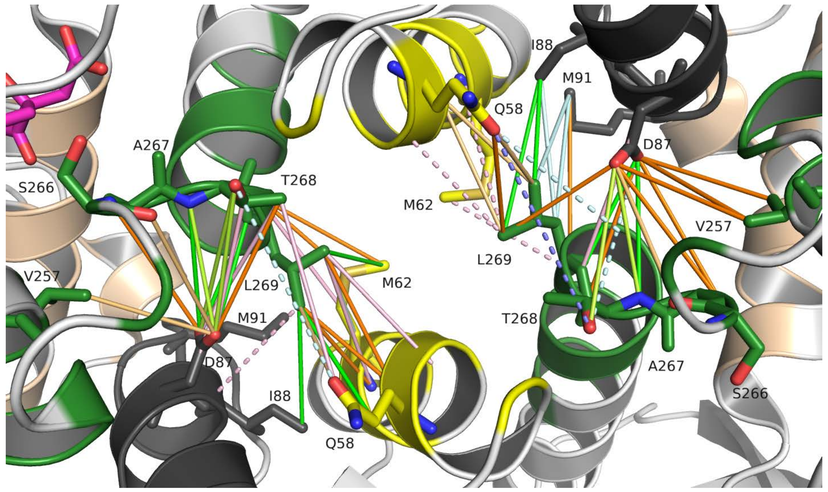


Major Conclusions:

The Interface is a dynamic region of the molecule with many attractive and repulsive interactions

Ligand Binding induces changes in the dynamics and interactions at the interface

S266 and L269 play a key role in connecting the active site to the interface, with L269 transmitting ligand induced information to the opposite subunit in the dimer.



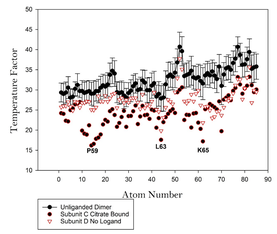
The Mechanism of Subunit Interactions in MDH

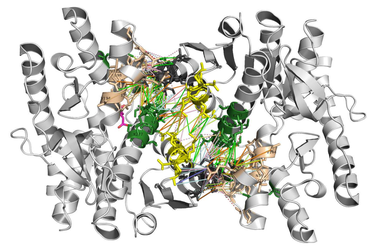
Citrate binding to the MDH subunit causes a conformational change in the 266-269 loop that is relayed across the subunit interface by rearrangement of non-polar interactions. The hydroxyl group of T268 moves, placing it further away from D87. In response to this, Q58 from the unbound subunit is drawn closer to the citrate-bound subunit and L269 compensates by shifting closer to region II in the unbound subunit. Moreover, in the unbound subunit , D87 is further removed from Q58, which increases its electronegativity. As a result, this increases the repulsive forces with the 266-269 backbone in the unbound subunit, causing the loop to shift to a position similar to the 266-268 loop in the citrate-bound subunit.

Computational Analysis

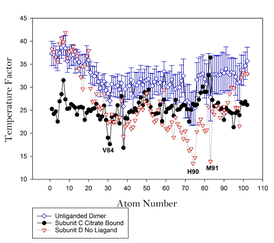
The Subunit Interface comprises 4 regions of sequence. Temperature Factors are taken from the 1SMK.pdb structure of watermelon Glyoxysomal MDH. HINT analysis to examine the nature of the interface contacts was conducted on the AB Dimer (no ligand bound) and the CD Dimer (with Citrate bound to the C subunit). Analysis of the temperature factors show that Citrate binding has distinct positional effects at the interface and induces changes in the unliganded subunit.

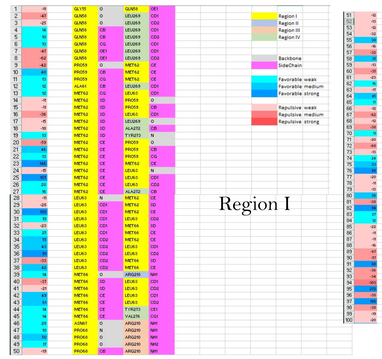
Effect of Citrate on Interface Flexibility
Region I Q58 to P68





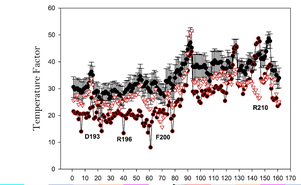
Effects of Citrate on Interface Flexibility
Region II: N80 to G94





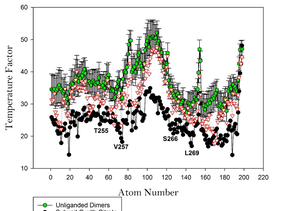
Region II

Effect of Citrate on Interface Flexibility
Region III: L192 to V212



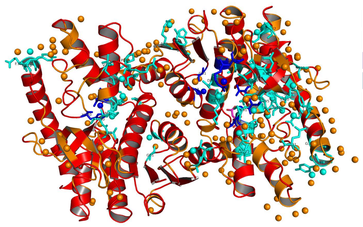
Region III

Impact of Citrate Binding on Interface Flexibility
Region IV: R249 to K277



Region IV

Blue/red coloring for atomic level forces unique to AB or CD. The forces in AB that disappear in CD are indicated by dashed lines, while newly evolved forces in CD are solid lines. The forces that are common to both are shown in solid lines as well, but are colored differently. Forces that become more negative are colored orange, while forces that become more positive are green. Forces that become less negative are colored light orange, while forces that become less positive are colored limon



100-91%
90-81%
80-71%
≤70%
No Associated Water Molecules

Water molecules and the interacting protein residues are colored by DRoP based upon the percent conservation within the set ⌈100-91% (blue), 90-81% (cyan), 80-71% (green), ≤70% (orange)⌋; protein residues not in contact with water molecules are colored red.

This Project is supported by NSF-1726932 EHR-IUSE
Principal Investigator: Ellis Bell
Co-Principal Investigators:
Joseph Provost & Jessica Bell
& NSF -MCB-0448905*: Principal Investigator: Ellis Bell*

For Details of Experimental Approaches Scan Code