Mechanistic Studies of Allosteric Regulation in the Type II Citrate Synthases

N.T. Nguyen[1], R. Maurus[1], L.J. Donald[2], H.W. Duckworth[2] and G.D. Brayer[1]

[1]Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, B.C. and [2]Department of Chemistry, University of Manitoba, Winnipeg, Manitoba

1.Abstract

The hexameric Type II citrate synthases are uniquely found in Gram-negative bacteria and are strongly and specifically inhibited by NADH through an allosteric mechanism. We have completed the first structure of a Type II citrate synthase and examined the binding mode of the allosteric inhibitor NADH. Variant kinetic studies have identified Arg109 as a particularly interesting residue in the NADH binding site. Arg109 seems to play at least two key roles in allostery, including screening against NAD+ binding and setting the proper binding affinity for NADH so that citrate synthase activity is properly tuned to the metabolic level of NADH. Surprisingly, an examination of the structure of the R109L variant enzyme (in the presence and absence of NADH) revealed a dramatic refolding of a flexible region of polypeptide chain in the distant active site region. These results suggest that Arg109 is also directly involved in the series of structural events that occur in switching between the R (active) and T (inactive) states of the enzyme. Subsequent analyses have determined the probable route of the allosteric signal from the NADH binding site to the active site region.

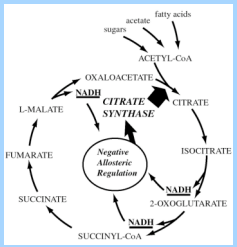
2. Overall Objectives

1. Characterize the inhibitory allosteric NADH binding site of hexameric Type II CS.

2. Determine the mechanism by which the allosteric signal is transmitted to the active site.

3. Introduction

1. Citrate synthase (CS) catalyzes the entry point of carbon into the TCA cycle.



2. Type II CS are unique in that:

- **these are found only in Gram-negative bacteria.**
- **these are active only in the hexameric form.**
- **these have two allosterically regulated conformational states:**
  - **the R-state is catalytically active.**
  - **the T-state is inactive - has NADH bound.**

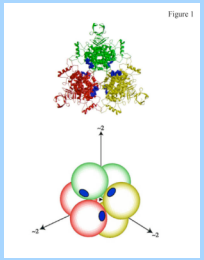
4. Features of the First Type II CS Structure

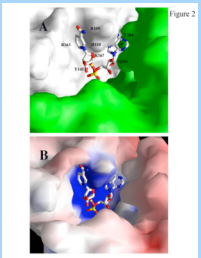
Highlights of Figure 1

1. Hexameric complex formation (from 3 dimers) depends on a specialized loop.

2. This complex exhibits only 3-fold symmetry/ dimer subunits have different conformations.

3. The allosteric NADH binding sites (in blue) are localized to dimer-dimer interfaces.





Highlights of Figure 2

1. Structural elements from two dimer units are needed to form the NADH binding sites.

2. NADH is bound in a highly unusual horseshoe configuration.

5. Kinetic Effects of NADH Binding Site Replacements

NADH binding and inhibition

	Kd, μM	Ki, μM	maximum inhibition, %
Wild type	1.6 ± 0.1	5.0 ± 0.6	99
R109L	1.16 ± 0.04	1.6 ± 0.3	94
H110A	5.2 ± 0.2	104 ± 14	71
T111A	6.6 ± 0.2	34 ± 8	50
Y145A	—	—	none
R163L	5.81 ± 0.04	—	none
K167A	4.1 ± 0.2	—	none
Q182A	6.1 ± 0.5	17 ± 3	97
N189A	6.9 ± 0.8	127 ± 25	50
T204A	10.2 ± 0.4	95 ± 27	53

Kinetic Analysis Highlights

1. Three variants show complete loss of NADH inhibition.

2. Only the R109L variant shows increased binding affinity for NADH.

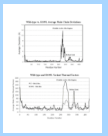
6. Special Structural Features of the R109L Variant

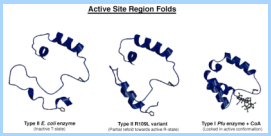
Highlights:

1. Little change at the site of substitution (without or with NADH).

2. Dramatic positional and mobility changes to two distant regions of polypeptide chain.

i) a helical linker region connecting the NADH site to the active site.
ii) the active site region which partial refolds towards the active R-state.

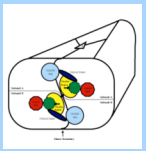




3. The schematic drawing above and to the right shows a comparison of the active site folds of the inactive T-state enzyme, the partially refolded R-state of the R109L variant, and the polypeptide chain conformation of this region in a non-allosteric Type I enzyme.

7. Allosteric Mechanism Proposal

Based on the available kinetic and structural data, it is possible to propose the following general outline for how the transition between the allosteric T and R-states occurs, and the role of NADH in this process.



8. Conclusions

1. Arginine 109 would appear to play three roles:

i) Screening at the entrance to the NADH binding site to prevent NAD^+ binding.

ii) Modulation of the level of NADH binding affinity to reflect metabolic needs.

iii) Providing the steric stimulus to propagate the allosteric signal.

2. A helical region provides the structural link between an NADH binding site on one dimer and the active site region on an adjacent dimer.

3. The active site unfolding/ refolding response depends on the status of the helical linker.

4. A mechanistic proposal outlining the general characteristics of the allosteric process in hexameric Type II *E. coli* CS has been developed.

9. Research Project Support

This work is funded by an operating grant from NSERC.