Time-resolved Crystallographic Studies of Isocitrate Dehydrogenase Using Intermediate Trapping, Photolytic Triggering, and Synchrotron Radiation*

Barry L. Stoddard, Jill Bolduc, and Dave Dyer
The Fred Hutchinson Cancer Research Center, Seattle, Washington, USA

Andrew Mesecar, Bruce Cohen, Mike Brubaker, and Daniel E. Koshland, Jr.
University of California at Berkeley, Emerville, California, USA

Time-resolved crystallography uses a group of related techniques for reaction trapping and data collection. The experiments are designed to accumulate a specific catalytic intermediate throughout the crystal for a short period of time, during which diffraction data is collected. In this context, three parameters can be defined: the method used to isolate the intermediate, the longest exposure time allowed for data collection (which is limited either by the lifetime of the intermediate or by the lifetime of the crystal during the experiment), and the method of data collection. At one end of the spectrum is a single-turnover experiment, triggered by a photolytic event, in which the lifetime of the rate-limited species is short, necessitating rapid data collection using a synchrotron light source and Laue diffraction. At the other extreme is a reaction intermediate trapped chemical and physical techniques to produce an extended lifetime in the crystal, usually visualized by the use of a slower method of data collection. The enzyme isocitrate dehydrogenase catalyzes the decarboxylation of isocitrate to a-ketoglutarate and exhibits a catalytic mechanism with several rapid steps prior to the rate-limiting dissociation of products. In order to accumulate the initial ES complex and the subsequent enol intermediate, site-directed mutants were used to impose specific kinetic barriers, and steady-state accumulation of the rate-limited species was used in conjunction with Laue data collection. Molecular dynamics simulations provided significant additional details of the structure of the ES complex and of the factors that contribute to an efficient hydride transfer reaction. A subsequent development, demonstrating the importance of comparative studies using multiple strategies of intermediate trapping, is the use of small structural perturbations in the active site ES complex to evaluate the contribution of precise substrate alignment to catalytic rate enhancement during hydride transfer. Steady-state, freeze-trapping methods were used to visualize and compare the resulting complexes. Most recently, the structure of the rate-limited product complex formed during a single synchronized round of turnover has been determined using photolytic liberation of caged substrate and Laue X-ray data collection. The experiment was conducted with three different caged compounds, each possessing a unique mechanism leading to the formation of the ES complex. Photoreaction efficiency and subsequent substrate affinities and binding rates in the crystal are critical parameters for these experiments.

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