Supplementary Material: Structure of a Highly NADP⁺-Specific Isocitrate Dehydrogenase

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Structure Analysis

GenBank/NCBI database accession codes and organism names for “representative sequences” of (putative) monomeric IDHs that were used in sequence alignments were as follows (if more than one relevant sequence for a particular organism was available, the first in the default order at the NCBI website was typically used): Acinetobacter calcoaceticus, ZP_06056020; Azotobacter vinelandii, BAA11169; Campylobacter jejuni, YP_002343962; Chlorobium limicola, BAC00856; Colwellia maris, BAA03134; Colwellia psychrerythraea, BAE92863; Corynebacterium diphtheriae, CAE49148; Corynebacterium glutamicum, YP_224955; Francisella tularensis, YP_667588; Mycobacterium bovis, NP_853736; Mycobacterium leprae, CAA17935; Mycobacterium tuberculosis H37Rv, NP_214580; Neisseria meningitidis alpha14, YP_003083023; Pseudomonas aeruginosa, NP_251314; Pseudomonas fluorescens, YP_002873363; Pseudomonas psychrophila, BAH80317; Ralstonia eutropha, YP_841443; Rhodomicrobium vannielii, YP_004012323; Streptomyces lividans TK24, ZP_05522130; Vibrio cholerae, NP_230786; and Vibrio parahaemolyticus, NP_797390. Accession codes for three other sequences used for the putative monomeric IDH from Ralstonia eutropha were CAJ96713, YP_298398 and AAZ63554.
Comparison of CgIDH and AvIDH Structures

Figure 1: Structural comparison of the CgIDH and AvIDH holoenzyme. Domains I of CgIDH-Holo (maroon) and AvIDH-Holo (blue) are superimposed, with Domains II showing a 36° hinge-bending movement relative to each other. Also shown are Mg$^{2+}$ (orange ball) and NADP$^+$ (stick model) bound to CgIDH-Holo. AvIDH-Holo coordinates are from PDB ID 1J1W (Yasutake et al., 2003).
NADP$^+$ Binding

Figure 2: Overlay of CgIDH-Holo and Apo1 forms in the coenzyme binding region. CgIDH-Holo (maroon), -Apo1 (blue), with residues directly or indirectly interacting with NADP$^+$ (stick models). Also shown is the final $2mFo - DFc$ map (blue) contoured at 1.0 $\sigma$ level around NADP$^+$ atoms alongwith the final NADP$^+$ model (stick model). CgIDH-Apo1 coordinates are from PDB ID 2B0T (Imabayashi et al., 2006).
Figure 3: Stereo view of interference with NADP$^+$ binding in the apoenzyme. Domains I of CgIDH-Holo (green) & -Apo2 (red) molecules in the asymmetric unit in the current structure are superimposed; their symmetry-related neighbors (Neighbor-Holo, greenish yellow and Neighbor-Apo, orange, respectively) are also shown. Some of the hydrogen bonds formed (a double one between the side chains of Apo2 Arg596 and Neighbor-Apo Asp506, and one between the 2′-phosphate of NADP$^+$ and the Holo Arg645) are also shown (black dashes). The sidechain of Glu508 of Neighbor-Apo is disordered but well within interaction distance of the Arg645 side chain of the apo form. Neighbor-Holo lies farther from the coenzyme binding site than Neighbor-Apo.