1·2 is stabilized by two additional hydrogen bonds between the counteranion and the two hydroxyl groups of 1.

Recrystallization of 1·2 from acetone–hexane gave yellow prisms as 1·2·(acetone)$_2$. X-ray analysis of this crystal confirmed the presence of three hydrogen-bonding interactions (Figure 2).[8,9]

^{1}H NMR titration[10] of 2 with 1 gave a salt-formation constant (K) of (7.1 ± 0.2) × 10^2 M^{-1}. For compound 4, having only two hydroxyl groups, a K value for titration with 2 was found to be (2.1 ± 0.1) × 10^2 M^{-1}. The 3.4-fold enhancement of salt formation for 1 over 4 is attributable to preorganization in 1 for three hydrogen-bonding interactions.[11]

Supplementary Material Available: Listings of experimental details for the syntheses of 1, 3, and 4 and details of the X-ray diffraction analysis, plots of atom labels, and tables of atomic coordinates, equivalent isotropic thermal parameters, bond lengths, and bond angles for 1·2·(acetone)$_2$ (20 pages); a table of observed and calculated structure factors (34 pages). Ordering information is given on any current masthead page.

(8) The position of methyl group connected to phosphonate is disordered between two positions in the ratio of 1:1. Only one of the two positions is shown in Figure 2.

(9) Similar hydrogen-bonding arrangements are seen in the X-ray structure of ([$HOC_6H_4O(Ph)PO_2$][C_5H_5NH]·catechol): Poutasse, C. A.; Day, R. O.; Holmes, R. R. *J. Am. Chem. Soc.* **1984**, *106*, 3814–3820.

(10) In $CDCl_3$ at 30 °C, [1] or [4] = 0.32–5.0 mM and [2] = 1.0 mM, calculated by a nonlinear least-squares fitting.

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Studies of Complex Enzymatic Pyridine Nucleotide-Dependent Transformations: Structure and Cofactor Binding Correlation in CDP-D-glucose Oxidoreductase[1]

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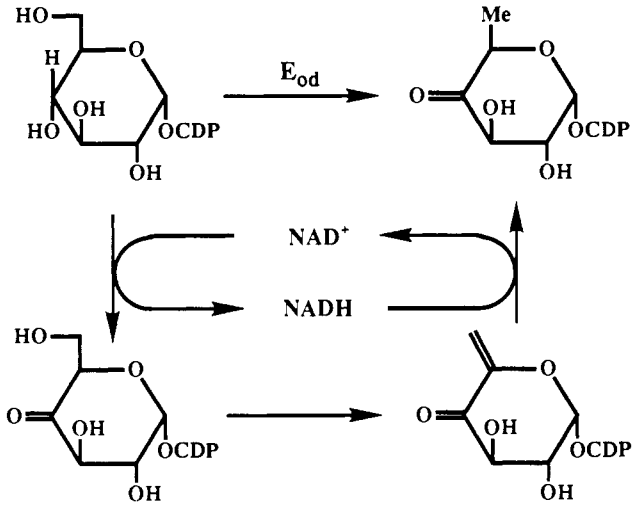
Many cellular processes are catalyzed by dinucleotide-binding proteins. Although in most nicotinamide dinucleotide-dependent reactions, NAD^+ or $NADP^+$ is in essence a cosubstrate, there exists a class of enzymatic reactions in which the coenzyme is tightly bound and acts as a catalytic prosthetic group.[2] This unique class of catalysts embodies a number of vital cellular enzymes which include the following: *myo*-inositol-1-phosphate synthase, central to signal transmission; UDP-galactose epimerase, essential for cellular metabolism; and dehydroquinate (DHQ) synthase, an indispensable step in the biosynthesis of aromatic amino acids. In studying the biosynthesis of 3,6-dideoxyhexoses,[3] we have recently purified a CDP-D-glucose oxidoreductase (E_{od}) from a strain of *Yersinia pseudotuberculosis*.[3h] The intramolecular oxidation–reduction catalyzed by this enzyme is illustrated in Scheme I. This catalyst and other nucleotidyl diphosphohexose-4,6-dehydratases represent another distinguished member of this overall redox-neutral class of enzymes and have been shown

Scheme I



to be the integral branching point from which all 6-deoxy sugars arise.[4] Distinct from most NAD^+ tight binding enzymes of this class, the purified E_{od} from *Yersinia* exhibits an absolute requirement for NAD^+.[3h] Clearly, such abysmal NAD^+ binding imposes an interesting challenge to our understanding of the mechanism of its catalysis. To further our exploration concerning the primary structure and catalytic mechanism of this enzyme, we have now cloned, sequenced, and expressed its gene in *Escherichia coli*.[5] Reported are our studies of the binding affinity for NAD^+ of this dehydratase and the sequence comparison of its cofactor binding motif with another well characterized member of its class, DHQ synthase. The insights gained from this study have helped in postulating primary structure-NAD^+ binding relationships of this prominent class of enzyme.

The *asc*B (E_{od}) open reading frame encodes a protein of 357 amino acids which was expressed in *E. coli* at 5% of the total soluble protein.[5] Interestingly, the purified recombinant enzyme was only 40% active prior to NAD^+ reconstitution. As defined in eq 1, the E_{od} catalyzed reaction clearly involves two distinct

$$E + NAD^+ \overset{K_{NAD}}{\rightleftharpoons} E\text{-}NAD^+ + S \overset{K_S}{\rightleftharpoons} E\text{-}NAD^+\text{-}S \xrightarrow{k_{cat}} E + P \quad (1)$$

binding events, with the dissociation constants for substrate and nicotinamide cofactor designated K_s and K_{NAD}, respectively. However, it follows a single substrate catalysis when all of the enzyme is converted to the NAD^+-bound form. Thus, in the presence of excess cofactor, the purified enzyme exhibits a K_m of 1.05 mM which is equivalent to K_s under saturation kinetics conditions. Since the equation defining the apparent K_m is equivalent to $K_s \cdot K_{NAD}/(K_s + [S])$), the dissociation constant for NAD^+ can be readily deduced by measuring the enzyme activity under limiting NAD^+ concentrations. Therefore, on the basis of the results shown in Figure 1A, a K_{NAD} of 117 nM can be calculated from the apparent K_m of 79.5 nM.

Surprisingly, the affinity for NAD^+ has only been reported for a small number of proteins. Within the class of enzymes that utilize NAD^+ as a catalytic prosthetic group, the best characterized example is the DHQ synthase from *E. coli*, which has a K_{NAD} of 80 nM under turnover conditions.[6] In comparison, the determined K_{NAD} of 117 nM for the E_{od} of *Yersinia*, also under saturating levels of substrate, suggests a 1.5-fold decrease in affinity for NAD^+. However, cofactor binding differentiation of these two enzymes is much more profound in the absence of substrate. For DHQ synthase, a K_{NAD} of 2 nM was determined, reflecting very tight NAD^+ binding.[6] In contrast a K_{NAD} of 5.4

(1) This work was supported in part by the National Institutes of Health Grant GM 35906. H.-w. L. is the recipient of a National Institutes of Health Research Career Development Award (GM 00559).

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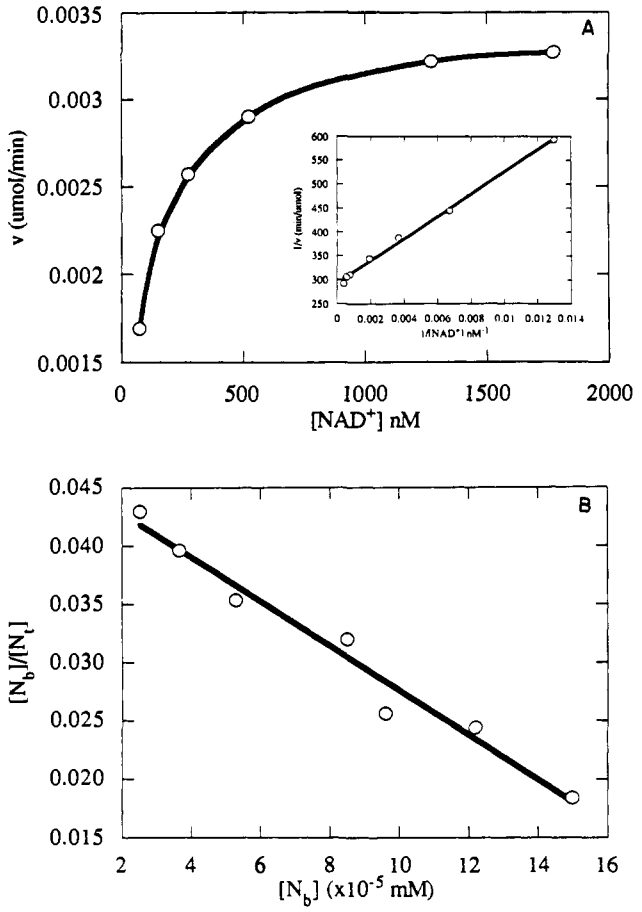

Figure 1. (A) Determination of K_{NAD} under turnover conditions. (B) Determination of K_{NAD} in the absence of substrate. The experiments were carried out by forced dialysis using [^{3}H]NAD$^+$ ([N_t]: 0-500 nM) with E_{od} (250 nM). The extent of bound ([N_b]) and free cofactor was assessed by scintillation counting. The K_{NAD} was deduced from the Scatchard plot (Segal, I. E. *Biochemical Calculations*, 2nd ed.; Wiley: New York, 1976; p 242).

μM, deduced from Figure 1B, was found for the free 4,6-dehydratase.

Since the active-site conformation must play an important role in controlling the binding affinity of coenzyme, the prodigious 2700-fold difference in NAD$^+$ affinity found for the *Yersinia* enzyme may simply reflect a distortion of the nicotinamide binding domain at, or near, the active-site. Comparison of the amino acid sequences and the tertiary structures of many NAD(P)$^+$ or FAD dependent enzymes has led to the identification of a compact ADP-binding domain preserved in most dinucleotide-binding proteins. Homology within this conserved binding region includes a glycine-rich phosphate binding loop, **GXGXXG**, near the N-terminus of the protein, and six predominantly hydrophobic residues forming a hydrophobic core of the dinucleotide binding $\beta\alpha\beta$ fold.[7,8] Contrary to the typical $\beta\alpha\beta$ fold found in most NAD$^+$ binding enzymes, including DHQ synthase,[9] an examination of the sequence of the *Yersinia* enzyme reveals the presence of an extended fold with the sequence of GHTGFKG.[10] The preferred alignment with the typical **GXGXXG** consensus, **GHTGFKG**, results in the placement of histidine near the N-terminal end of the α-helix which has been recently shown to interfere with the α-helix dipole[11] often thought to be important in ligand (or cofactor) binding.[12] The alternative alignment, **GHTGFKG**, provides a relatively bulky substitution, threonine, for the universally conserved second glycine which is believed to be important to minimize steric interaction with the nicotinamide cofactor.[8] Altering the third conserved glycine to lysine may disrupt the close interaction between the β-strand and α-helix.[8] These primary distinctions from the normal NAD$^+$ binding motif suggest the presence of a second possible cofactor binding consensus for this class of enzyme, which is severely perturbed electronically and/or sterically, resulting in diminished cofactor affinity.[13] These fundamental comparisons may assist in the prediction or tailor-design of desired protein-cofactor interactions of this unique class of nicotinamide binding enzymes as well as possibly many other essential ADP-binding proteins. In addition, assembling a cofactor binding motif(s) for this class of enzyme will help define the common residues important for their overall redox neutral reactions.

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(7) Where G refers to glycine, X refers variable amino acids, α designates an α-helix, and β defines a β-sheet.

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(10) All the known sequences of UDP-galactose-4-epimerase, CDP-tyvelose epimerase (Verma, N.; Reeves, P. R. *J. Bacteriol.* **1989**, *171*, 5694), TDP-D-glucose 4,6-dehydratase from *Saccharopolyspora erythrea* (Pissowotzki, K.; Mansouri, K.; Piepersberg, W. *Mol. Gen. Genet.* **1991**, *231*, 123), and *Salmonella typhimurium* (Jiang, X.-M.; Neal, B.; Santiago, F.; Lee, S. J.; Romano, L. K.; Reeves, P. R. *Mol. Microbiol.* **1991**, *5*, 695) contain a GGXGXXG fold which still follows the normal consensus.

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(13) The primary GXXGXXG sequence has also been found in the CDP-D-glucose 4,6-dehydratase from *Salmonella typhimurium* (Jiang, X.-M.; Neal, B.; Santiago, F.; Lee, S. J.; Romano, L. K.; Reeves, P. R. *Mol. Microbiol.* **1991**, *5*, 695) and TDP-D-glucose 4,6-dehydratase from *Streptomyces griseus* (Pissowotzki, K.; Mansouri, K.; Piepersberg, W. *Mol. Gen. Genet.* **1991**, *231*, 123); however, no information on cofactor binding in these particular systems is known.

1,2,3,4-Tetramethyl-5-(trifluoromethyl)cyclopentadienide: A Unique Ligand with the Steric Properties of Pentamethylcyclopentadienide and the Electronic Properties of Cyclopentadienide

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Cyclopentadienide (Cp) and pentamethylcyclopentadienide (Cp*) are two of the most widely used ligands in organometallic chemistry. Since the first synthesis and use of Cp* as a ligand a quarter of a century ago,[1] it has become well established that replacement of the five hydrogens on Cp by five methyl groups results in major changes in both the physical and chemical properties of transition metal complexes which differ only in the substitution of Cp* for Cp.[2] Unfortunately, dissection of the steric

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