Receptor for a Phosphonic Acid Monoester: Salt Formation Stabilized by Three Hydrogen-Bonding Interactions

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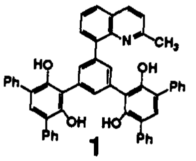
Hydrogen bonding to a counteranion within a salt plays an important role in the chemistry of artificial receptors for anionic substrates such as phosphates[2] and carboxylates.[3] We became interested in the hydrogen-bonding effect on the salt formation in equilibrium with an acid and a base. In an apolar solvent such as chloroform, an acid and a base are in equilibrium with a contact ion pair.[4] If a base has functional groups capable of hydrogen bonding to a counteranion, the equilibrium will shift to the salt stabilized by additional hydrogen bonding. On the basis of this concept, we have developed the basic receptor for oxo acids. Here we report ^{1}H NMR spectroscopic and X-ray crystallographic evidence that the salt between compound **1** and methyl phenylphosphonate (**2**) is stabilized by three hydrogen-bonding interactions.



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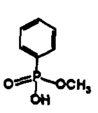

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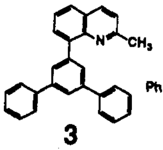

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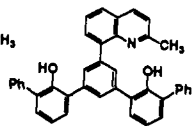

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We performed salt-formation experiments by ^{1}H NMR spectroscopy[5] using **1** and a reference compound **3**. In a DMSO-d_6 solution of a 1:1:1 mixture of **1**, **3**, and *p*-toluenesulfonic acid (TsOH),[6] the CH_3 signals of **1** and **3** underwent downfield shifts of 0.05 and 0.06 ppm, respectively, due to partial N-protonation. The nearly identical shift values suggest that the proton affinity (basicity) of the nitrogen atom of **1** is nearly equal to that of **3**.[7] Actually, ^{1}H NMR titration of **1** or **3** with TsOH at 30 °C gave almost identical salt-formation constants, which mean association constants with H^+ of $(9.4 \pm 0.5) \times 10^1$ M^{-1} for **1** and $(9.5 \pm 0.9) \times 10^1$ M^{-1} for **3**.

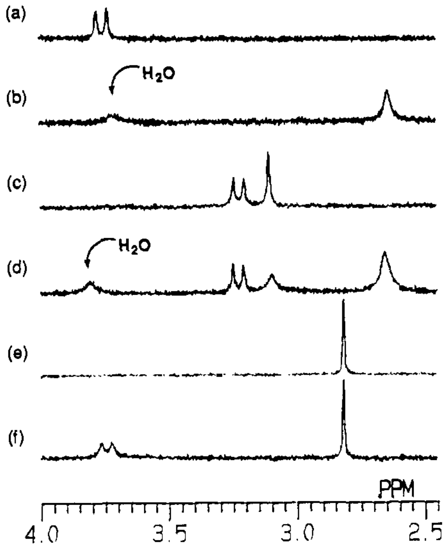

Figure 1. ^{1}H NMR spectra in $CDCl_3$ at −60 °C (270 MHz): (a) [**2**] = 0.75 mM; (b) [**1**] = 0.75 mM; (c) [**1**] = [**2**] = 0.75 mM; (d) [**1**] = 3.0 mM, [**2**] = 0.75 mM; (e) [**3**] = 0.75 mM; (f) [**3**] = [**2**] = 0.75 mM.

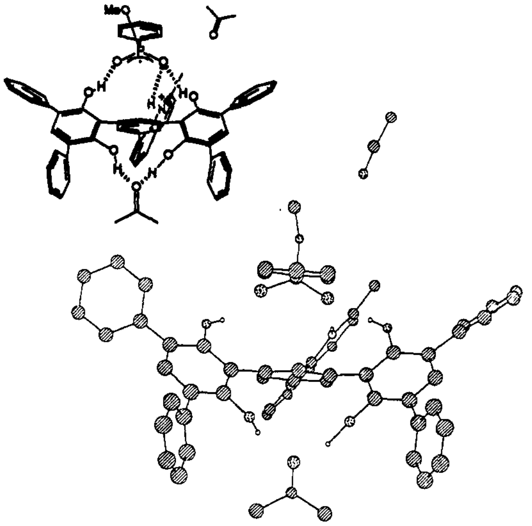

Figure 2. X-ray structure of **1·2·**(acetone)$_2$.

In chloroform, however, the selectivity between **1** and **3** was observed. In a $CDCl_3$ solution of a 1:1:1 mixture of **1**, **3**, and **2**,[6] the CH_3 signal of **1** moved downfield by 0.18 ppm, whereas that of **3** showed no shift. This result indicates that the salt **1·2** is more stable than the salt **3·2**.

For a mixture of **2** and more than 1 equiv of **1**, only one CH_3 signal of **1** was observed at 30 °C due to the fast exchange between free **1** and salt-formed **1**. At −60 °C (Figure 1), however, this exchange became slower on the NMR time scale. For example, in the case of a **1**:**2** ratio of 4 (Figure 1d), two CH_3 signals were observed in a 3:1 intensity ratio; they correspond to the signal of free **1** and that of salt-formed **1** (Figure 1b,c). The absence of the signals of free **1** in the case of a **1**:**2** ratio of 1 (Figure 1c) shows complete salt formation, whereas the salt formation between **3** and **2** is not observed even at −60 °C (Figure 1e,f).

The OH signal of **1** (δ 5.86 at 30 °C, 6.3 (broad) at −60 °C in the absence of **2**) became too broad to be observed upon addition of **2** at 30 °C. When the sample was cooled to −60 °C, the OH signals appeared at δ 5.80 (2 H) and 10.78 (2 H). The lower field protons are assignable to the OH protons which form hydrogen bonds with the counteranion. This result indicates that the salt

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(5) Tetramethylsilane (TMS) was used as an internal standard.

(6) [**1**] = [**3**] = [TsOH] or [**2**] = 0.75 mM. For a $CDCl_3$ solution of a 1:1:1 mixture of **1**, **3**, and TsOH, the downfield shift of the CH_3 signal of **1** was 0.34 ppm and the CH_3 group of **3** showed no shift.

(7) In DMSO-d_6 solutions of TsOH, tetraethylammonium *p*-toluenesulfonate, **1** + TsOH, or **3** + TsOH (each 0.75 mM), the chemical shift values of the sulfonate moieties were identical within 0.005 ppm. This result suggests that TsOH is almost completely dissociated in DMSO and that the sulfonate anions form solvent-separated ion pairs or free ions.

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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.

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(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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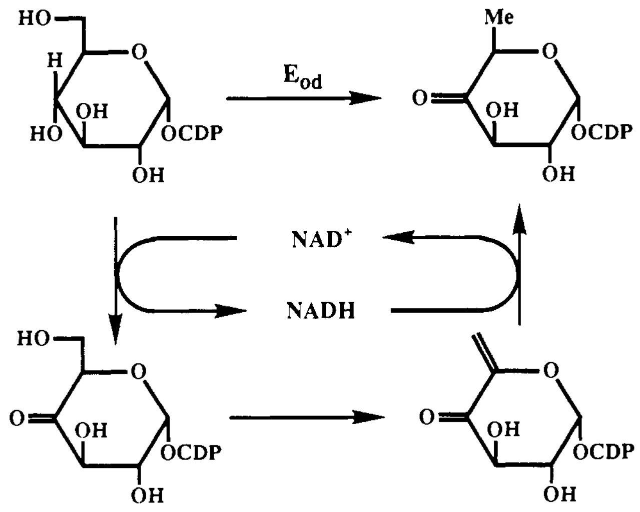
Many cellular processes are catalyzed by dinucleotide-binding proteins. Although in most nicotinamide dinucleotide-dependent reactions, NAD$^+$ or NADP$^+$ is in essence a cosubstrate, there exists a class of enzymatic reactions in which the coenzyme is tightly bound and acts as a catalytic prosthetic 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 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.

Scheme I



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

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

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

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