rate constants (k(inst)) were determined for heating and cooling. The Arrhenius plot of these rate constants is also shown in Figure 1.

Activation enthalpies determined in the nematic phases and in the smectic phase are the same (≈15 kcal mol^{-1}) within experimental error and also are similar to that obtained in benzene.[16,19] However, because of the small temperature range, these values are inherently inaccurate. The rate constants in the liquid crystalline phases are approximately 10 times as high as those in benzene, a solvent with a considerably lower viscosity. Ganapathy et al.[15] have found that, in a cholesteric mixture, DAI has an activation parameter similar to that in benzene and the isomerization reaction is not affected by the viscosity of the solvent. The mixed cyano-substituted oligophenyl would be expected to have a much greater dielectric constant than benzene, and this factor might be responsible for the higher reactivity observed. As for the difference in reactivity between the smectic and nematic phases, the smectic phase is clearly a more ordered microenvironment, adopting a layer-like structure as a consequence of the strong dipole–dipole interactions among cyano groups.[10] Formation of the transition state for the isomerization may be less favorable in this rigid microenvironment than in the corresponding nematic phases.

Work is continuing in an attempt to explore the generality of anti-Arrhenius behavior associated with reentrance.

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Chemically Induced Modification of Cofactor Specificity of Glucose-6-phosphate Dehydrogenase†

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Changing recognition of enzymes and other molecules in a desired manner has been accomplished using enzyme modifications,[1a-c] temperatures,[1d-f] solvent,[1g] substrate modifications,[1h,i] and chemically designed materials.[1j-m] In this paper we describe a general chemical approach to modifying cofactor specificity of NADP dependent enzymes. A vanadate ester derivative of NAD has the potential of being a structural and functional analog of 2′-NADP. Vanadate ($H_2VO_4^-$ or HVO_4^{2-}) is a good analog for phosphate ($H_2PO_4^-$ or HPO_4^{2-}), suggesting that organic vanadates will be good analogs of organic phosphates.[2,3] In aqueous solutions, vanadate will react on a millisecond time scale with hydroxyl groups in organic ligands and spontaneously form organic vanadate esters (eqs 1 and 2 in Figure 1), which are analogous to organic phosphates.[2,3] Primary, secondary, and tertiary alcohols will all react with vanadate to form vanadate esters, and the formation constant ranges from 0.1 to 10.[3] When organic vanadate esters form as in a solution of glycerol and vanadate, enzymes such as glycerol-3-phosphate dehydrogenase[4,5] are able to recognize and

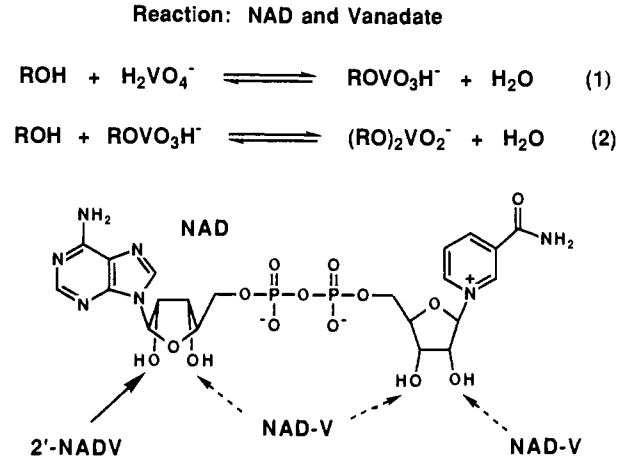

Figure 1. The vanadylation sites of NAD as well as the general reactions to form vanadate esters (eq 1) and vanadate diesters (eq 2).

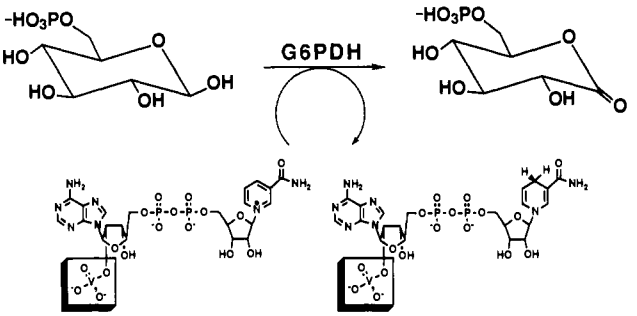

Figure 2. The reaction of glucose 6-phosphate with NADV catalyzed by glucose-6-phosphate dehydrogenase to form 6-phosphogluconate and NADVH.

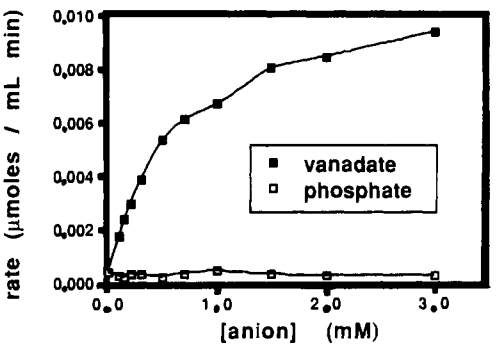

Figure 3. Rates of G6PDH reaction by addition of vanadate and phosphate to assay solutions containing NAD. Assay solutions contained 50 mM TAPS, 80 mM KCl, 2.0 mM NAD, 5.0 mM G6P, approximately 5.5 mg/mL G6PDH, and from 0.0 to 3.0 mM vanadate or from 0.0 to 3.0 mM phosphate at pH 9.0.

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Table I. Michaelis–Menten Constants Determined for Bakers' Yeast G6PDH[a,b]

cofactor	K_m (mM)[c]	V_{max} (mM min^{-1})[c]	k_{cat} (min^{-1})	k_{cat}/K_m (min^{-1} mM^{-1})
2′-NADP	0.025 (±0.001)	1.0 (±0.04)[b]	3.8×10^6	1.5×10^8
2′-NADV	0.003 (±0.002)	2.3×10^{-4} ($\pm 1.4 \times 10^{-4}$)	6.1	1.8×10^3
NAD	2.9 (±0.1)	7.7×10^{-5} ($\pm 0.3 \times 10^{-5}$)	2.0	0.69
c-2′,3′-NADP	1.2 (±0.2)	1.5×10^{-2} ($\pm 0.3 \times 10^{-2}$)	5.8×10^3	4.8×10^3
3′-NADP	13 (±5)	5.0×10^{-4} ($\pm 1.9 \times 10^{-4}$)	13	1.0

[a] Assay solutions contained 50 mM TAPS, 80 mM KCl, and 5.0 mM G6P at pH 9.0. With the exception of the NADV experiments detailed in the caption of Figure 3, the cofactors were varied from approximately $^1/_3K_m$ to $3K_m$. [b] Using the assay conditions described in footnote *a*, the G6PDH activity was 15× lower than that in Sigma's assay. G6PDH used in these experiments had a specific activity of about 14 units/mg. [c] The uncertainties were determined using the enzyme kinetics program written by Jacek Stanislawski and marketed by Trinity Software.

convert the organic vanadate substrate analog to product. A solution of vanadate and NAD is likely to form several organic vanadates since NAD contains four free secondary hydroxyl groups. One of these derivatives is 2′-NADV (Figure 1), which may function as a cofactor corresponding to 2′-NADP.

The possibility that 2′-NADV mimics 2′-NADP in biological systems was explored using bakers' yeast glucose-6-phosphate dehydrogenase (G6PDH), an enzyme which strongly prefers 2′-NADP to NAD (see Table I)[6] and is not inhibited by vanadate monomer.[7] Glucose-6-phosphate (G6P) is oxidized by G6PDH to 6-phosphogluconolactone, which spontaneously hydrolyzes to form 6-phosphogluconate (6PG) in the presence of the appropriate cofactor (Figure 2). The addition of 0.0030 mg of bakers' yeast G6PDH to an assay solution containing 2 mM NAD and varying concentrations of vanadate converts G6P to 6PG as shown in Figures 2 and 3. Twenty-fold rate enhancements were obtained in the presence of vanadate whereas negligible rates were observed in the absence of G6PDH. Similar experiments were carried out in the presence of phosphate or arsenate, but no significant rate increases were observed (Figure 3). These results are consistent with the formation of a covalent complex between vanadate and NAD, which is accepted by G6PDH as a cofactor.

Solutions containing NAD (from 0 to 15 mM) and vanadate (7.5 mM) were analyzed using ^{51}V NMR spectroscopy in order to determine the formation of NAD–V complexes. Vanadate monomer (V_1, −541 ppm), dimer (V_2, −562 ppm), and tetramer (V_4, −577 ppm) are observed in the spectra, and the equilibrium constants determined their respective concentrations under the assay conditions (see spectra and analysis in supplementary material). Previous ^{51}V NMR studies of solutions containing vanadate and nucleosides showed that analogous complexes form with stoichiometries of 1:1 and 2:2.[8-15] Analysis of a series of spectra showed that the resonance at −541 ppm is actually the result of overlapping signals for two different species; the 1:1 monovanadate ester complex (NAD–V) and V_1. Since NAD has four hydroxyl groups capable of formation of a monoester, the formation constant of 2′-NADV ($K_{eq,NADV}$ = 12 M^{-1}) is calculated to be $^1/_4$ of the total formation constant for the NAD–V monoester complexes (49 M^{-1}). Analogous approaches showed that the additional complex at −523 ppm was a 2:2 complex with a formation constant of 5.2×10^6 M^{-3}.

Assay solutions contained NAD and 2′-NADV, which both bind to G6PDH and are converted. Using steady-state approximations, the 2′-NADV equilibrium constant from ^{51}V NMR studies, Michaelis–Menten constants for NAD and NADV, the concentrations of NAD and V_1, and the fact that $V_{max,NADV}$ can be described in terms of $V_{max,NAD}$ ($V_{max,NADV} = \beta V_{max,NAD}$), a kinetic expression including two alternative substrates (eq I) can be derived. Detailed derivations are included in the supplementary material.

$$\frac{1}{v} = \frac{K_{m,NAD}K_{m,NADV}/[NAD] + K_{m,NAD}K_{eq,NADV}[V_1] + K_{m,NADV}}{V_{max,NAD}K_{m,NADV} + \beta V_{max,NAD}K_{m,NAD}K_{eq,NADV}[V_1]} \quad (I)$$

The kinetic data were obtained using constant [V_1] from 0.0 to 0.05 mM. The complexity and practicality of the kinetics dictated that the experiments be performed at modest concentration ranges. The calculated constants are shown in Table I. $K_{m,NADV}$ is 10 times lower than $K_{m,NADP}$, but 2′-NADV overall has a significantly lower k_{cat} than 2′-NADP. If no change in enzyme mechanism takes place, these results suggest that the 2′-NADV binds more tightly to the enzyme than 2′-NADP, but the additional stabilization results in a lower k_{cat}. The k_{cat}/K_m ratio suggests, however, that 2′-NADV overall is a reasonable cofactor for bakers' yeast G6PDH.

Despite many recently reported biological activities of vanadium in microorganisms such as cofactor activity in nitrogenases[18] and bromoperoxidases,[19] no analogous function for vanadium in mammals has emerged. The activity of 2′-NADV as a coenzyme for 2′-NADP dependent dehydrogenases is likely to affect many biosynthetic pathways. Switching the metabolic pathways from the energy-producing NAD dependent enzymes to the biosynthetic NADP dependent enzymes may be a factor in the insulin mimetic activity of vanadium compounds in the treatment of streptozotocin-induced diabetes in the rat.[2] Since vanadate (V(V)) reduces to vanadium(IV) in most cellular environments, the discovery would be of biological interest if a vanadium(IV)–NAD complex also had activity as a cofactor.[16,17] Rate enhancements are observed when vanadyl cation is added to an assay solution containing NAD and bakers' yeast G6PDH. Analogous detailed analyses have not yet been completed with other NADP dependent dehydrogenases; however, 6-phosphogluconate dehydrogenase from sheep liver and alcohol dehydrogenase from *Thermoanaerobium brockii* also show rate enhancements upon addition of vanadate to NAD-containing assay solutions.[20] The substrate specificity of alcohol dehydrogenase from *Thermoanaerobium* is such that this enzyme is valuable as a catalyst in organic synthesis.

Changes in pyridine nucleotide specificity have previously been engineered in both *Escherichia coli* glutathione reductase[21] and *Bacillus stearothermophilus* glyceraldehyde-3-phosphate dehydrogenase[22] using site-directed mutagenesis. In comparison,

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the use of vanadium to alter the cofactor specificity of 2'-NADP dependent dehydrogenases is simple, rapid, and convenient and demonstrates the power of simple chemical reactions in modifying enzyme specificity.

Supplementary Material Available: Description of aqueous vanadate reactions, kinetic derivations, and experimental data (12 pages). Ordering information is given on any current masthead page.

On the Second-Order Polarizability of Conjugated π-Electron Molecules with Octupolar Symmetry: The Case of Triaminotrinitrobenzene

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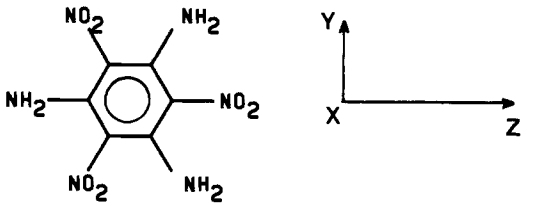
Interest in the quadratic nonlinear optical (NLO) properties of organic molecules and materials keeps steadily growing. Experimental data are now starting to provide a better understanding of the relationship between molecular structure and NLO properties,[1-4] which is of major importance to guide synthesis and materials processing toward novel systems with enhanced characteristics. Compounds with a quadratic NLO response have traditionally been developed according to the general recipe of having an electron-donating group and an electron-accepting group linked by a conjugated segment. *p*-Nitroaniline (pNA) and its derivatives constitute prototypical examples of such an approach. In this case, the second-order polarizability β is often described within the dipolar approximation on the basis of the so-called two-state model:[5]

$$\beta_\mu(0) = \frac{3}{2}\frac{(\mu_{ge})^2\Delta\mu}{(\hbar\omega_{ge})^2}$$

where $\Delta\mu = \mu_e - \mu_g$ is the difference between the dipole moments in the main excited state and the ground state; $\hbar\omega_{ge}$, the transition energy; and μ_{ge}, the transition dipole moment.

One of the implications of this model is that it is most favorable to have as large as possible a change in dipole moment upon excitation in order to maximize charge separation, and therefore β. However, the expansion of the β tensor in irreducible components can be shown to contain, in addition to a dipolar contribution, an *octupolar contribution*. It has recently been proposed[6] to address specifically the optimization of the octupolar contribution at the microscopic and macroscopic levels. Initial second-harmonic-generation (SHG) powder measurements[7] have helped in identifying 1,3,5-triamino-2,4,6-trinitrobenzene (TATB),



as a prototype candidate molecule and crystal exhibiting a significant β value despite the fact that by symmetry the dipole moment is 0 in all electronic states (in the absence of structural relaxations). Systematic utilization of the two-state model in the dominant context of dipolar systems may have obscured possible significant contributions[6] to β coming from more than a single excited state.

In TATB, the donor and acceptor groups are located at alternate positions on the benzene ring. X-ray diffraction studies indicate that the molecule is planar in the solid state because of strong intra- and intermolecular hydrogen bonding.[8] TATB thus adopts D_{3h} symmetry and is noncentrosymmetric. The crossed character of the intramolecular charge transfers between the amino and nitro groups (for each of them, there are one para and two ortho interactions) leads to a complete cancellation of the vector (dipolar) part of the β tensor. Therefore, β cannot be measured by use of the EFISHG (electric field induced second harmonic generation) technique,[5,9] which is based on the alignment of the molecules along their dipole moment axis.

The components of the β tensor have been evaluated for TATB and pNA, using three different and complementary theoretical methods.[10] The results are given in Table I. It is remarkable to note that the three independent theoretical approaches consistently lead to similar conclusions. Our results thus demonstrate that the intrinsic quadratic nonlinear response of TATB (modulus of the β tensor) is about 1.6-1.8 times larger than that of pNA. This establishes that *octupolar contributions to β are significant.*

The main difference between the pNA and TATB β tensors lies in the strongly different "off-diagonal" β_{zyy} values, while the "diagonal" β_{zzz} values are comparable. In TATB, D_{3h} symmetry imposes $\beta_{zyy} = \beta_{zzz}$,[6] thus making β_{zyy} substantially higher than for C_{2v} molecules such as pNA. It is the additional contribution from the β_{zyy} component which strongly increases the modulus of β, by almost a factor of 2, from pNA to TATB.

The sum-over-states approach allows us to identify the π-electron excited states that contribute most significantly to the off-resonant quadratic nonlinear response. We find, *in marked contrast to the two-state model*, that in addition to the ground state S_0, a set of three doubly degenerate π-electron excited states

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