10, where the eclipsing of the tertiary methyl group and the alkyl chain offsets the thermodynamic advantages of chelate formation, resulting in a partitioning of the rearrangement through conformations B and C (Figure 4). The observed sensitivity of the reaction to the nature of the cationic species provides evidence that supports the role of multidentate chelation in defining the transition state. Rearrangement of the magnesium enolate of **10a** is considerably more selective than that of the lithium species, affording only **11a**, although yields are significantly diminished. Also consistent with the proposed chelation model is the deterioration of selectivity observed in the presence of a cation-coordinating additive; for example, in 20% HMPA–THF, rearrangement of **8b** gives an mixture of **9b** and a *Z* olefinic product, while the rearrangement of **10b** becomes essentially nonselective.

The [2,3] Wittig rearrangement of **1** and related tertiary substrates represents a powerful entry to highly functionalized acyclic intermediates, which incorporate structural features not readily accessible by other linear protocols. In particular, the ability to develop a remote stereochemical relationship across a trisubstituted olefin of defined geometry should prove a valuable addition to existing methodology.[18] The availability of both the homoallylic hydroxyl and allylic alkoxy substituent as control elements should facilitate further stereoselective functionalization of the trisubstituted olefin, for example, by directed hydrogenation[19] or osmylation,[20] and we envision tertiary [2,3] Wittig products such as **3** as advanced precursors to a variety of functional arrays present in polyketide-derived natural products. Application to the synthesis of biologically significant targets is under way and will be the subject of future reports.

Acknowledgment. We thank the National Institutes of Health (Grant AI-19632) for partial support of this work. We wish to thank Professor Clarence Pfluger for obtaining the X-ray crystal structure of **3**.

Supplementary Material Available: Experimental Procedures and analytical data for all new compounds (7 pages). Ordering information is given on any current masthead page.

(18) Review of trisubstituted olefin synthesis: (a) Faulkner, D. J. *Synthesis* **1971**, 175. (b) Reucroft, J., Sammes, P. G. *Q. Rev. Chem. Soc.* **1971**, *25*, 135. See also ref 17b and references therein.

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Changing Coenzymes Improves Oxidations Catalyzed by Alcohol Dehydrogenase[1]

Summary: Oxidation of alcohols to aldehydes catalyzed by HLADH proceeds more smoothly if the oxidant, NAD^+, is replaced by a stronger oxidant, e.g., thionicotinamide or 3-acetylpyridine adenine dinucleotide.

(1) Presented in part at the American Chemical Society Meeting, New Orleans, LA, September 1987. Abstract ORG 193.

Sir: One goal of current efforts in site-directed mutagenesis is to improve existing enzymes for use in synthesis by modification of the amino acid chain of an enzyme.[2] A complementary approach, described below, is to optimize the coenzymes to be used in such syntheses.

As an example reaction, we have examined the oxidation of ethanol with NAD^+ catalyzed by alcohol dehydrogenase from horse liver, EC 1.1.1.1 (eq 1). This oxidation, like

$$CH_3CH_2OH + NAD^+ \rightleftharpoons CH_3CHO + NADH + H^+ \quad (1)$$

$$K_{eq} = 0.0004 \text{ at pH } 7.0^3$$

other oxidations involving NAD^+, proceeds poorly due to inhibition by the product acetaldehyde (K_i(acetaldehyde) = 0.6 mM, noncompetitive).[4,5] Examination of 13 different analogues of NAD^+ identified thionicotinamide adenine dinucleotide ($SNAD^+$) and acetylpyridine adenine dinucleotide ($APAD^+$), both of which are stronger oxidants than NAD^+, as useful substitutes for NAD^+ because they show increased rates and yields of acetaldehyde. Similar substitutions in other oxidoreductases will allow the reevaluation of previously impractical reactions.[5]

Kinetic parameters of eq 1 were measured for two classes of NAD^+ analogues by steady-state kinetics (see Table I) under identical conditions. These values allow direct comparison of the different analogues and are consistent with values measured under similar conditions.[6-9]

The first class of NAD^+ analogues contain modifications only in the adenine portion, thus substitution of these analogues does not alter the equilibrium constant of eq 1. No significant changes in the inhibition constants for acetaldehyde were observed. The second class of analogues contain modifications in the carboxamide portion of the nicotinamide ring. The largest changes in the inhibition constant for acetaldehyde were observed with $SNAD^+$, $APAD^+$, and formylpyridine adenine dinucleotide which showed increases by factors of 10, 60, and 100, respectively. These three analogues are stronger oxidants than NAD^+ and shift the equilibrium constant of eq 1 toward aldehyde formation by factors of 15, 130, and 92, respectively. Several analogues that are weaker oxidants than NAD^+ were ineffective coenzymes.

The reactivity of two analogues that showed less product inhibition, $SNAD^+$ and $APAD^+$, was further investigated to determine whether they would be suitable for enzyme-catalyzed syntheses. Both analogues showed a V_{max} approximately twice that for NAD^+. Two procedures for regeneration of NAD^+—NH_4^+/2-oxoglutarate[10] and methylene blue/O_2[5]—could also be used to regenerate $SNAD^+$ and $APAD^+$. Oxidation of the reduced coenzymes (0.12 mM) by 2-oxoglutarate (14 mM) and ammonia (220 mM), catalyzed by glutamate dehydrogenase (bovine liver)

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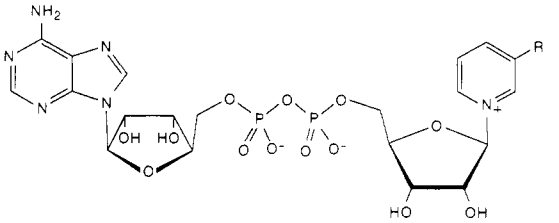
Table I. Kinetic and Thermodynamic Parameters for Several Analogues of NAD$^+$

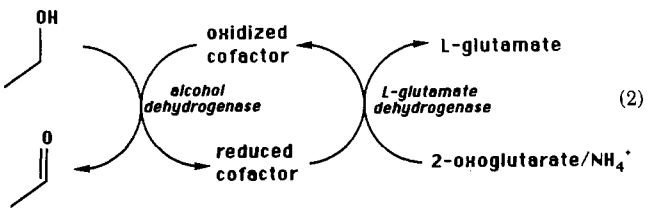


coenzyme	R	$E^{o\prime}$, mV	apparent K_i (CH_3CHO),[a] mM	V_{max}, %
nicotinamide adenine dinucleotide (NAD^+)	$C(O)NH_2$	−320	0.6	100[b]
nicotinamide hypoxanthine dinucleotide	$C(O)NH_2$	−320	0.5	99[c]
nicotinamide guanine dinucleotide	$C(O)NH_2$	−320	0.3	91[c]
nicotinamide 1,N^6-ethenoadenine dinucleotide	$C(O)NH_2$	−320	1.0	19[c]
nicotinamide riboside 4-(adenine-9-yl)-*n*-butyldiphosphate	$C(O)NH_2$	−320	0.7[d]	80[d]
thionicotinamide adenine dinucleotide ($SNAD^+$)	$C(S)NH_2$	−285[e,f]	6	224
3-acetylpyridine adenine dinucleotide ($APAD^+$)	$C(O)CH_3$	−258[f]	35	214
3-formylpyridine adenine dinucleotide	C(O)H	−262[f]	60	29
3-benzoylpyridine adenine dinucleotide	$C(O)C_6H_5$	−250[g]	20[h]	32[g]
3-cyanopyridine adenine dinucleotide[i]	CN	≈−200[j]		<0.2[k]
3-aminopyridine adenine dinucleotide	NH_2	<−400[j]		<1[l]
3-aldoximepyridine adenine dinucleotide	CH(=NOH)	≈−400[j]		<5[m]
3-iodopyridine adenine dinucleotide[n]	I	−354[n]		<0.1[o]
nicotinic acid adenine dinucleotide	COO^-	≈−400[j]		<0.1[p]

[a] The inhibition by acetaldehyde of the oxidation of ethanol (30 mM in 0.1 M borate buffer, pH 9, 25 °C, 2 mM coenzyme) was measured in solutions containing added acetaldehyde by monitoring the rate of formation of reduced coenzyme by UV spectroscopy (340 nm, ϵ 6220 M^{-1} cm^{-1} for coenzymes with an intact nicotinamide ring; formation of SNADH was monitored at 395 nm, ϵ 11300 M^{-1} cm^{-1}; formation of APADH or FPADH was monitored at 365 nm, ϵ 7800 M^{-1} cm^{-1}). The apparent inhibition constants were determined from a Dixon plot. The rate observed with no added acetaldehyde is taken to be V_{max}. Mixture of EE and ES isozymes was used unless otherwise noted. [b] Specific activity under these conditions is 0.57 ± 0.02 units/mg of protein for Sigma catalog no. A6128. [c] Pure EE isozyme, prepared as described in item *f* (below). [d] Reference 9. [e] Reference 7. [f] Scharschmidt, M.; Fisher, M. A.; Cleland, W. W. *Biochemistry* **1984**, *23*, 5471–78. [g] Samama, J.-P.; Hirsch, D.; Goulas, P.; Biellmann, J.-F. *Eur. J. Biochem.* **1986**, *159*, 375–80. [h] K_m value at pH 8 from item *g* (above). [i] Prepared as described by Biellmann, J.-F.; Jung, M. J. *FEBS Lett.* **1970**, *7*, 199–200, and purified by paired ion chromatography on a C-18 column. [j] Estimated from a plot of σ_p vs $E^{o\prime}$. [k] No activity was detected under these conditions as shown by monitoring the formation of reduced coenzyme either by UV at 324 nm or by HPLC (reverse phase column eluted with a linear gradient of 0–50% B over 8 min, 1 mL/min. A = H_2O, 5 mM tetrabutylammonium phosphate pH 6; B = $MeOH/H_2O$, 60/40, 5 mM tetrabutylammonium phosphate, pH 6). [l] Anderson, B. M.; Kaplan, N. O. *J. Biol. Chem.* **1959**, *234*, 1226–32. [m] Biellmann, J.-F.; Samama, J.-P.; Wrixon, A. D. *Biochimie* **1973**, *55*, 1469–72. [n] Prepared as described by Abdallah, M. A.; Biellmann, J.-F.; Samama, J.-P.; Wrixon, A. M. *Eur. J. Biochem.* **1976**, *64*, 351–60, and was pure by both anion exchange and paired ion chromatography. [o] Formation of reduced coenzyme could not be detected by HPLC as described in item *k* (above). An assay where the formation of reduced coenzyme is coupled to the formation of a colored dye (Abdallah, M. A.; Biellman, J.-F. *Eur. J. Biochem.* **1980**, *112*, 331–33) also showed no activity above background under these conditions. [p] Formation of reduced coenzyme could not be detected by HPLC as described in item *k* (above).

proceeded effectively: V_{max}(NADH) = 100, V_{max}(SNADH) = 37, V_{max}(APADH) = 52 (imidazole buffer, pH 7.3). Similarly, oxidation of the reduced coenzyme with methylene blue and O_2 also proceeded effectively without a catalyst. (Rate constants for reaction of reduced coenzyme with methylene blue: 21 ± 6 M^{-1} s^{-1} for NADH, 6 ± 1 M^{-1} s^{-1} for SNADH, 5 ± 0.8 M^{-1} s^{-1} for APADH; pH 9, borate buffer.) Since the alcohol dehydrogenase catalyzed step is usually rate-limiting, the slightly slower rates of oxidation of the reduced coenzymes should not slow the overall rate of reaction. The analogues show higher values of K_m than NAD^+ (K_m(NAD^+) = 4–17 μM,[6,9] K_m($SNAD^+$) = 72 μM,[7] K_m($APAD^+$) = 40–90 μM[8,9]). In all cases the enzyme should be near saturation at 1 mM coenzyme.

The analogues $SNAD^+$ and $APAD^+$ were compared to NAD^+ in a small-scale oxidation of ethanol to acetaldehyde with regeneration of the coenzyme using 2-oxoglutarate/glutamic dehydrogenase (eq 2). Ethanol (100 mM, 1 mmol



(2)

in 10 mL) was oxidized to acetaldehyde in TES buffer (50 mM, pH 8) containing coenzyme (1 mM), bis(ammonium) 2-oxoglutarate (200 mM), alcohol dehydrogenase (10 units), glutamate dehydrogenase (50 units). After 25 min, enzymatic assay for acetaldehyde[11] showed 3.3 mM acetaldehyde with NAD^+ as coenzyme, 6.2 mM with $SNAD^+$, and 10 mM with $APAD^+$. The maximum value was reached after 65 min when assay showed 3.3 mM with NAD^+, 16 mM with $SNAD^+$, and 31 mM with $APAD^+$.

Since the overall equilibrium constant for eq 2 does not change with different coenzymes, the same amount of acetaldehyde should eventually form in each case. For practical purposes, however, more acetaldehyde (up to 9 times more) is formed with the analogues.

Oxidation of other alcohols with these analogues (Table II, Figure 1) showed a similar lessening of product inhibition by aldehyde, increase in V_{max} and higher yields in small-scale syntheses.

Although the overall equilibrium constant does not change upon substitution of analogues in eq 2, the free energy profile (Scheme I) does change in a manner that lowers the free energy of the intermediate electron carrier. This lowering of free energy rationalizes the more effective oxidations observed. Consistent with this explanation, analogues of NAD^+ with the same redox potential as

(11) An aliquot of the reaction mixture was passed through an ultrafiltration membrane to remove enzymes and assayed for acetaldehyde with NADH and yeast alcohol dehydrogenase.

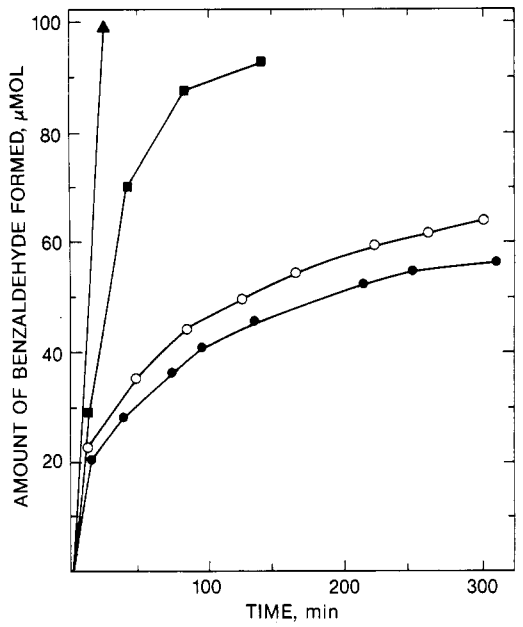
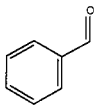
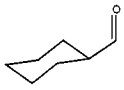


Figure 1. Comparison of coenzymes for the oxidation of benzyl alcohol to benzaldehyde catalyzed by HLADH. Oxidations using $APAD^+$ (▲) or $SNAD^+$ (■) are faster than those with NAD^+ (●). Conditions: benzyl alcohol (100 μmol, 10 mM) in TES buffer (50 mM, pH 8), coenzyme (1 mM), bis(ammonium) 2-oxoglutarate (200 mM), alcohol dehydrogenase (10 units, based on ethanol/NAD^+), glutamate dehydrogenase (50 units). Dilution of the NAD^+-containing reaction by a factor of two (○) also shows faster formation of benzaldehyde consistent with the notion that inhibition by benzaldehyde limits the rate of reaction.

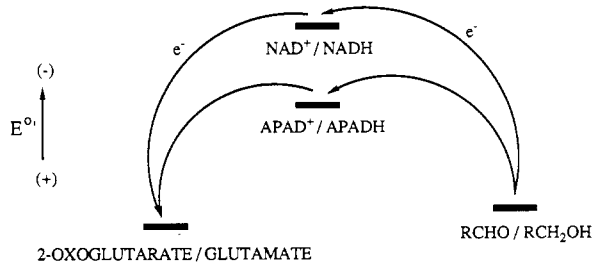
Table II. Oxidation of Other Alcohols Catalyzed by HLADH

product		coenzyme NAD⁺	SNAD⁺	APAD⁺
	K_i,[a] mM	0.10	1.4	3.1
	V_{max},[a] %	90	20	500
	synthesis,[b] % conversion	45	93	100
	K_i,[c] mM	3.7	150	600
	V_{max},[c] %	120	210	930
	synthesis,[d] % conversion	25	55	62
	K_i,[e] mM	0.40	2.5	16
	V_{max},[e] %	20	70	300
	synthesis,[e,f] % conversion	5	nd	22
	K_i,[g] mM	190	250	400
	V_{max},[g] %	90	140	120

[a] 2 mM benzyl alcohol, 50 mM TES buffer, pH 8, 25 °C (TES = *N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid). K_i is the inhibition constant for the product aldehyde. V_{max} is the relative rate of oxidation as compared to ethanol at pH 9, 25 °C. [b] Data in cation of Figure 1. [c] 30 mM 2-methylprop-2-en-1-ol, 50 mM TES buffer pH 8, 25 °C. [d] 100 mM 2-methylprop-2-en-1-ol, cofactor was regenerated as in caption of Figure 1. [e] 20 mM cyclohexanemethanol, 0.1 M borate buffer, pH 9, 25 °C. [f] Cofactor was regenerated with methylene blue (1 mM), catalase (0.1 mg/mL), 400-μmol scale, HLADH (1.2 units), 0.4 mM coenzyme. [g] 0.2 M cyclohexanol, 0.1 M borate buffer, pH 9.0, 25 °C.

NAD^+ (analogues where the adenine portion was modified) showed no improvement. Further, oxidation of cyclohexanol, which is thermodynamically easier that the oxidation of a primary alcohol,[5] showed (1) little product inhibition by cyclohexanone and (2) little change in product inhibition upon substitution of NAD^+ with $SNAD^+$ or $APAD^+$ (Table II).

Scheme I[a]



[a] The free energy of the intermediate electron carrier is lowered upon substitution of $APAD^+$ for NAD^+ in an oxidation of an aldehyde by ammonium 2-oxoglutarate.

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Synthesis and Chemiluminescence of an α-Hydroperoxyacyl Cyanide

Summary: An isolable enol (**3**) reacts with triplet dioxygen to afford an α-hydroperoxyacyl cyanide (**4**); treatment of **4** with base provides evidence for the intermediacy of a 1,2-dioxetan-3-one (**5**).

Sir: Since α-peroxy lactones (1,2-dioxetanones) were first suggested as key intermediates in bioluminescence,[1] they have been synthesized[2] and the chemienergization processes underlying their luminescence have become fairly well understood.[3] In this paper we report a new light-producing reaction, presumably involving α-peroxy lactones.

Treatment of isobutyryl cyanide[4] (2-oxo-3-methylbutanenitrile, **1**) with triethylamine and a large excess of trifluoroacetic anhydride led to formation of the enol

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