slowly than 1c, 11 can be isolated as a reaction product.

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Registry No. 1a, 1111-97-3; **1b**, 999-79-1; **1c**, 3355-29-1; (*E*)-**2c**, 78739-45-4; (*Z*)-**2c**, 78739-46-5; **2d**, 78739-47-6; (*E*)-**2f**, 78739-48-7; (*Z*)-**2f**, 78739-49-8; **2g**, 78739-50-1; (*E*)-**2h**, 78739-51-2; (*Z*)-**2h**, 78739-52-3; **2i**, 78739-53-4; **8g**, 78739-54-5; **8i**, 78739-55-6; **9**, 78739-56-7; **11**, 78739-57-8; 1-chloro-3-methyl-1,2-butadiene, 27822-67-9; zinc chloride, 7646-85-7; 1,3-butadiene, 106-99-0; 4-methyl-1,3-pentadiene, 926-56-7; isoprene, 78-79-5; (*E*)-1,3-pentadiene, 2004-70-8; 2,3-dimethyl-1,3-butadiene, 513-81-5; 2,4-dimethyl-1,3-pentadiene, 1000-86-8.

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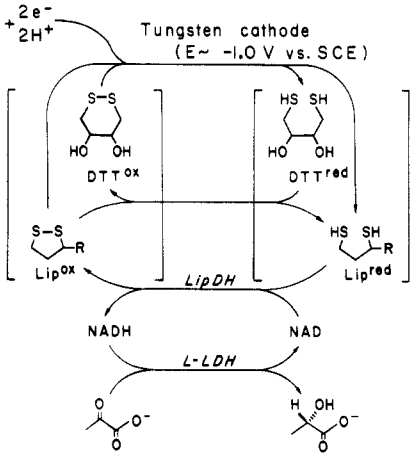
A Combined Electrochemical/Enzymatic Method for in Situ Regeneration of NADH Based on Cathodic Reduction of Cyclic Disulfides

Summary: NADH can be regenerated in situ from NAD by a process having as first step the electrochemical reduction of the disulfide groups of lipoamide and oxidized dithiothreitol to the corresponding dithiols; the dihydrolipoamide in this mixture reduces NAD to NADH in a reaction catalyzed by lipoamide dehydrogenase.

Sir: Here we describe a method for regeneration of NADH from NAD based on the ability of a tungsten cathode to reduce stable cyclic disulfides to the corresponding dithiols selectively in neutral aqueous solutions (Scheme I). Previous work in electrochemical methods for NAD(P)H regeneration have concentrated on direct cathodic reduction[1-4] and have not been sufficiently selective for reduction at the 4-position of the nicotinamide ring to permit high turnover numbers for those cofactors.[5] In the method reported here, the electrochemical step is reductive cleavage of a disulfide bond; the reduction of NAD to NADH occurs in a subsequent, regiospecific, enzymatic step. It is, of course, important that the rate of the cathodic reduction of the disulfide group(s) be much faster than the rate of any direct competing cathodic reduction of NAD, but in practice, this type of selectivity seems to be more easily achieved than that required in discriminating between positions in the nicotinamide ring.

Scheme I. NADH Regeneration Based on Electrochemical Reduction of Disulfides [Abbreviations: LipDH = Lipoamide Dehydrogenase, L-LDH = L-Lactate Dehydrogenase, DTT = Dithiothreitol, Lip = Lipoamide ($R = CH_2CH_2CH_2CH_2CONH_2$)]



Scheme I is based on the capability of a tungsten cathode to reduce even very stable cyclic disulfides in neutral aqueous solution without corrosion, excessive H_2 production, or unacceptable rates of reduction of NAD.[6] In particular, D,L-6,8-thioctic acid amide (D,L-lipoamide, Lip), D,L-6,8-thioctic acid (D,L-α-lipoic acid), and *trans*-4,5-dihydroxy-1,2-dithiane (oxidized dithiothreitol, DTT^{ox}) are reduced quantitatively[7] to the corresponding dithiols at potentials of −1.0 to −1.5 V (vs. SCE) with current efficiencies of >80%. Scheme I should not be interpreted as implying an electron-transfer mechanism for the reduction; in fact, we hypothesize that surface tungsten hydrides are the actual reducing species. The reaction does not, however, proceed by electrochemical production of H_2 followed by subsequent tungsten-catalyzed reduction of the disulfides. Tungsten in *not* a catalyst for this reduction: no reaction occurs when disulfides are exposed to H_2 in the presence of tungsten in the absence of an applied voltage.

Cyclic voltammetry shows a broad, apparently irreversible wave for reductions of these disulfides. In the presence of the concentrations of thiols and disulfides used

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(5) The reduction step must be >99.8% selective for a 4-position to achieve a turnover number of 1000.
(6) Several groups have reported the reduction (in H_2O) of lipoic acid at a DME but have not identified the products. Patriarche, G. J.; Vire, J. C.; Mairesse-Ducarmois, C. A.; Vandenblack, J. L.; Christian, G. D. *J. Electroanal. Chem.* **1979**, *104*, 147-153. B. Nygard, *Arkiv. Kemi* **1967**, 75-88, 89-97. Sawyer reports no reduction of the disulfide bond of lipoic acid derivatives at a gold electrode at −2.0 V (SCE) (Howie, J. K.; Houts, J. J.; Sawyer, D. T. *J. Am. Chem. Soc.* **1977**, *99*, 6323-6326), and we observe no reduction at platinum. We find that reduction of Lip^{ox} at a mercury pool or lead cathode occurs at −2.0 V (SCE), and that the product reduces Ellman's reagent, but that it is not a substrate for LipDH and that it does not match authentic Lip^{red} in HPLC retention time. Treatment of this product with DTT^{red} releases Lip^{red}. We suggest that it is a mercury(II) thiolate complex of Lip^{red} having high stability. Since chelating dithiols have very high association constants with Hg(II) (log K = 41 for 1,3-dithiopropanesulfonic acid: Perrin, D. D. "Stability Constants of Metal-Ion Complexes"; Pergamon Press: Oxford, 1979; Part B) such stability is not surprising.
(7) The conversion of disulfides to dithiols was quantitative using assays based on Ellman's reagent, HPLC, and activity as substrate for LipDH.

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here, the overvoltage for the production of hydrogen at the tungsten electrode is 0.85 V (H_2O, pH 7.8, 0.1 M Na_2SO_4), and dihydrogen evolution becomes rapid at −1.7 V (vs. SCE). We use a mixture of Lip^{ox} and DTT^{ox} in Scheme I to increase the overall rate of cathodic disulfide reduction. DTT^{ox} is reduced approximately twice as rapidly as Lip^{ox} at −1.0 V, and a major pathway in the reductions of a mixture of the two disulfides is one in which DTT^{ox} is converted to DTT^{red}, and this DTT^{red} in turn reduces Lip^{ox} to Lip^{red} by thiol–disulfide interchange.[8] DTT^{red} is not a substrate for LipDH.

We illustrate the operation of the regeneration sequence summarized in Scheme I with the synthesis of L-lactate from pyruvate. A 2-L, three-necked, round-bottomed flask containing a stirring bar and attached to an argon line was used as the reaction vessel. The working electrode was 40 ft of coiled tungsten wire[9] (0.030-in. diameter, surface area approximately 300 cm^2) and the reference electrode was an unexceptional SCE. The counter electrode was 3 ft of coiled platinum wire (0.040-in. diameter), separated from the working solution by a porous ceramic Soxhlet extraction thimble (VWR) inserted in the center neck of the flask. During electrolysis, the solution in the anode compartment was purged with a slow stream of Ar to remove any O_2 formed.[10] The flask was charged with 500 mL of imidazole (Im)–H_2SO_4 buffer (50 mM, pH 7.8). D,L-Lipoamide (2.05 g, 10 mmol),[11] DTT^{ox} (1.52 g, 10 mmol), sodium pyruvate (0.55 g, 5 mmol), and xanthine (0.3 g, 2 mmol)[12] were added, and the solution was readjusted to pH 7.8. LipDH (EC 1.6.4.3, from torula yeast) and L-LDH (EC 1.1.1.27) were coimmobilized in PAN gel:[13] 140 mL of swollen gel added to the reactor contained 340 U (μmol min^{-1}) of LipDH and 460 U of L-LDH. NAD (0.14 mmol) was added, the potential of the tungsten electrode was adjusted to −1.0 V (vs. SCE), and sodium pyruvate (14.3 g, 130 mmol, in 100 mL of Im·H_2SO_4 buffer) was added at 1.5 mmol h^{-1}. Reaction was complete in 3.5 days; under these conditions the cathodic reduction of disulfides was overall rate limiting, at least at the start of the reaction.[14] The gel was allowed to settle, the supernatant decanted, and the lactic acid produced isolated as its zinc salt as described previously[15] (13.8 g, 96% pure, 115 mmol, 85% yield based on pyruvate, 96% ee). The turnover numbers[16] (and recovered activities) of the components were as follows: LipDH, 2.6×10^7 (88%); L-LDH, 3.6×10^7 (90%); NAD, 920; Lip, 13. No effort was made to recover Lip or NAD. Only approximately 5% of the NAD(H) originally added to the reaction mixture remained active at the conclusion of the reaction; most of the Lip was still present in active form.

This synthesis demonstrates one practical procedure for the electrochemical regeneration of NAD. The efficient reduction of stable disulfides to strongly reducing dithiols represents a new electrochemical reaction and should be useful in other areas of enzymology, especially in protection of enzymes against autoxidation. The isolation of enantiomerically enriched product establishes that the ultimate reduction step—pyruvate to L-lactate—is enzymatic. The immobilization of the enzyme seems to be important to the success of the procedure: immobilization protects the enzymes against deactivation at the electrode surface and protects the electrode surface against poisoning by adsorbed proteins.

The present limitations of this regeneration procedure are that the system is specific for NAD,[17] that the turnover number achieved for NAD is lower by factors of 2–5 than those observed in comparable preparations of L- or D-lactic acid using purely enzymatic regeneration systems[15,18,19] (probably because of electrochemical reduction of NAD to a biologically-inactive product), and that, in common with many electrochemical syntheses, the reaction is complicated by the equipment required.

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Registry No. NADH, 58-68-4; NAD, 53-84-9.

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(10) The anodic products of reaction were not identified.

(11) The presence of Lip^{ox} in large excess (5–10 times the solubility of Lip^{ox} in the buffer employed) resulted in greater selectivity of the tungsten cathode against NAD and pyruvate reduction.

(12) Inclusion of nitrogen heterocycles (nicotinamide, nicotinic acid, AMP, adenine, xanthine) in the reaction mixture substantially reduced the rate of cathodic reduction of NAD, probably by competition for sites active for the reduction of heterocycles on the electrode surface (see Bresmahan et al.[1]).

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A Stereoselective Approach to Acyclic Systems via Condensations of α-Lithiosulfinyl Carbanions and Aldehydes

Summary: The stereochemical outcome of condensations of α-lithiosulfinyl carbanions with aldehydes is presented, and demonstrates useful methodology for generating 1,2-asymmetry, as well as construction of 1,3-asymmetric relationships in acyclic systems.

Sir: Development of synthetic methodology for relative asymmetric induction has been recognized as a challenging problem in the chemistry of complex acyclic molecules. In part, as an outgrowth of interest in the synthesis of ionophore antibiotics, new methods have recently demonstrated relative asymmetric induction in acyclic systems.[1] Although a number of procedures establish 1,2-asymmetry,[2] construction of 1,3-asymmetric relationships presents

(1) For an excellent review on acyclic stereocontrol, see: Bartlett, P. A. *Tetrahedron* **1980**, *36*, 2.

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