Supplementary Material Available: Full listings of fractional atomic coordinates and interatomic bond distances and angles of the **1b**-piperidine saltex (5 pages). Ordering information is given on any current masthead page.

Photoinduced Enzyme-Catalyzed Reduction of Nitrate (NO_3^-) and Nitrite (NO_2^-) to Ammonia (NH_3)

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The reduction of nitrate is of broad interest as a means of mimicking reduction processes of oxido-nitrogen substrates in nature and of developing novel nitrogen fixation systems.[1] Reduction of nitrate to nitrite (eq 1) is catalyzed in nature by the

$$NO_3^- + 2e^- + 2H^+ \rightarrow NO_2^- + H_2O \qquad (1)$$

$$NO_2^- + 6e^- + 8H^+ \rightarrow NH_4^+ + 2H_2O \qquad (2)$$

$$NO_3^- + 8e^- + 10H^+ \rightarrow NH_4^+ + 3H_2O \qquad (3)$$

enzyme nitrate reductase.[2] Reduction of nitrite to ammonia (as ammonium ions) (eq 2) is catalyzed in nature by the enzyme nitrite reductase.[2,3] Substantial efforts are directed toward the reduction of NO_3^- by electrochemical and photochemical means. Electrochemical reduction of NO_3^- has been accomplished by using catalytic material electrodes,[4] modified electrodes,[5] or in the presence of homogeneous catalysts[6,7] such as Co(III) or Ni(II) cyclams, Ru(II) bipyridine or Fe(III) porphyrin. Photosensitized reduction of NO_3^- to NO_2^- has been reported by using *N*-methylphenothiazine or *N,N'*-tetramethylbenzidine,[8] and reduction to ammonia was reported to occur at Pd-TiO_2 illuminated suspensions.[9] We have recently applied enzymes as biocatalysts for the photosensitized regeneration of NAD(P)H cofactors[10,11] and performed various biotransformations through photochemical means.[12] Here we wish to report on the photoinduced reduction of NO_3^- to ammonia using the two enzymes nitrate reductase and nitrite reductase as catalysts and photogenerated *N,N'*-dimethyl-4,4'-bipyridinium radical cation, viologen radical, $MV^{\bullet+}$, that act as an electron carrier and is recognized by the biocatalysts.[13]

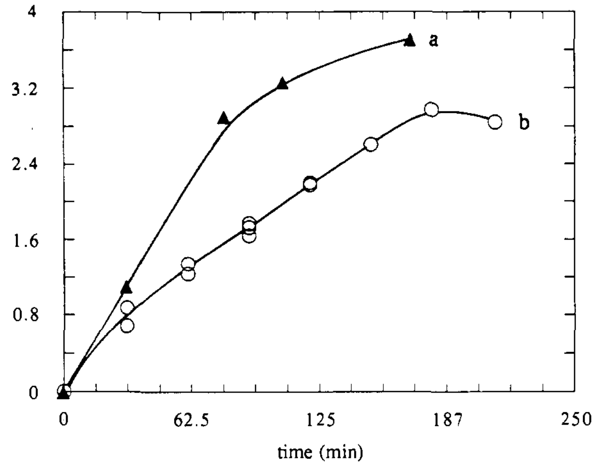

Figure 1. Rates of products formation as a function of illumination time. In all systems $[Ru(bpy)_3^{2+}] = 7.4 \times 10^{-5}$ M, $[Na_2EDTA] = 0.02$ M. (a) (▲) NO_2^- formation, pH 7.0, Tris buffer 0.1 M, $[MV^{2+}] = 3.2 \times 10^{-4}$ M, $[NO_3^-] = 9.9 \times 10^{-3}$ M, nitrate reductase 0.2 U. (b) (○) NH_4^+ formation, pH 8.0, Tris buffer 0.1 M, $[MV^{2+}] = 4.2 \times 10^{-4}$ M, $[NO_2^-] = 0.01$ M, nitrite reductase 0.06 U.

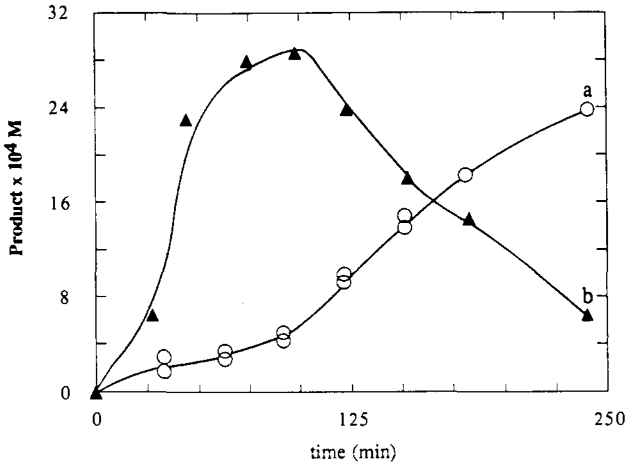

Figure 2. NO_2^- and NH_4^+ concentrations in the combined system, as a function of illumination time. (a) (○) NH_4^+. (b) (▲) NO_2^-. pH = 8.0, Tris buffer 0.1 M, $[Ru(bpy)_3^{2+}] = 7.4 \times 10^{-5}$ M, $[Na_2EDTA] = 0.02$ M, $[MV^{2+}] = 4.2 \times 10^{-4}$ M, $[NO_3^-] = 0.01$ M, nitrate reductase 1.0 U, nitrite reductase 0.35 U.

Illumination (λ > 420 nm) of an aqueous 0.05 M phosphate buffer solution, pH = 7.0, that includes Ru(II) tris-bipyridine, $Ru(bpy)_3^{2+}$, as photosensitizer, 7.4×10^{-5} M, *N,N'*-dimethyl-4,4'-bipyridinium, MV^{2+}, 3.2×10^{-4} M, as electron relay, EDTA, 0.02 M, as sacrificial electron donor, NO_3^-, 9.9×10^{-3} M, and the enzyme nitrate reductase (E.C. 1.9.6.1 from Escherichia coli), 0.2 units, results in the reduction of NO_3^- to nitrite (eq 1). The rate of NO_2^- formation[14] at time intervals of illumination is shown in Figure 1a. The quantum yield of NO_2^- formation corresponds to $\phi = 0.08$. After 310 min of illumination, ca. 60% of the original NO_3^- was converted to nitrite. The initial rate of NO_2^- formation is 0.07 μmol·min⁻¹. Illumination (λ > 420 nm) of an aqueous buffer solution, pH = 8.0, that includes $Ru(bpy)_3^{2+}$, 7.4×10^{-5} M, MV^{2+}, 4.2×10^{-4} M, as electron carrier, EDTA, 0.02 M, as

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Table I. Turnover Numbers for Components[a] Involved in the Photosensitized Reduction of NO_3^- and NO_2^- to Ammonia

	$Ru(bpy)_3^{2+}$	MV^{2+}	nitrate[e] reductase	nitrite[e] reductase
NO_3^- reduction[b]	80	18.5	6.2×10^4	
NO_2^- reduction[c]	38.5	7		2.4×10^4
combined system[d]	32	6	9×10^4	2.1×10^4

[a] Turnover number (TN) is defined as TN = mol of product formed/mol of component. [b] 60% conversion of NO_3^- to NO_2^-. [c] 28.5% conversion of NO_2^- to NH_4^+. [d] 23.8% conversion of NO_3^- to NH_4^+. [e] Molecular weight of nitrate reductase and nitrite reductase was estimated as 200 000 cf. ref 2b.

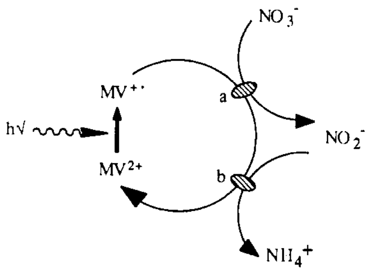

Figure 3. Scheme for biocatalyzed sequential reduction of NO_3^- to NH_4^+. a = nitrate reductase, b = nitrite reductase.

sacrificial electron donor, NO_2^-, 0.01 M, and the enzyme nitrite reductase (E.C. 1.6.6.4), 0.06 units, isolated from spinach leaves,[15] results in the formation of ammonia. The rate of ammonia formation[16] at time intervals of illumination is displayed in Figure 1b. The quantum yield of ammonia formation is $\phi = 0.06$. Control experiments reveal that all components in the two systems are required to effect the reduction of NO_3^- or NO_2^-, respectively. Also, no reduction of NO_3^- or NO_2^- takes place in the two systems in the dark. Exclusion of the enzymes nitrate reductase or nitrite reductase from the respective systems results in the formation of $MV^{\bullet+}$. Addition of the enzymes to the respective systems that include NO_3^- or NO_2^- and photogenerated $MV^{\bullet+}$ results in the depletion of $MV^{\bullet+}$ and reduction of NO_3^- to nitrite or of NO_2^- to ammonia, respectively.

Illumination ($\lambda > 420$ nm) of a photosystem that includes $Ru(bpy)_3^{2+}$, 7.4×10^{-5} M, as photosensitizer, $MV^{\bullet+}$, 4.2×10^{-4} M, as electron relay, EDTA, 0.02 M, the substrate nitrate, and the two enzymes nitrate reductase, 1.0 units, and nitrite reductase, 0.35 units, results in the reduction of NO_3^- to ammonia (eq 3) through the intermediate formation of nitrite. The rate of ammonia formation in this system is shown in Figure 2a, and curve 2b shows the amount of NO_2^- that is present in the system at time intervals of illumination. It is evident that only after a concentration of NO_2^- that corresponds to 3×10^{-3} M is formed, ammonia is effectively produced. During the illumination, no $MV^{\bullet+}$ is accumulated in the system. This suggests that the route of NO_2^- and ammonia formation is limited by the photochemical process that generates $MV^{\bullet+}$. The quantum yield of ammonia formation in the system that includes the two enzymes corresponds to $\phi = 0.08$. Table I summarizes the turnover numbers of the various components in the different systems. It is evident that the components are recycled during the reduction of NO_3^- and NO_2^- and that the enzymes exhibit stability in the artificial media. Figure 3 represents the schematic sequential cycle that leads to the reduction of NO_3^- to ammonia through nitrite as an intermediate. The photoinduced electron-transfer process generates $MV^{\bullet+}$ that acts as electron carrier for the two enzymes.

The primary step involves the reduction of NO_3^- to nitrite in the presence of nitrate reductase. The latter photoproduct acts as substrate for the enzyme nitrite reductase that mediates the reduction of NO_2^- to ammonia. The relatively high quantum yields for NO_2^- or ammonia formation are noteworthy. These originate from effective charge separation of $MV^{\bullet+}$ in the photosensitized electron-transfer process and the subsequent complementary dark reduction[17] of MV^{2+} by the oxidation product of the sacrificial electron donor, EDTA.

We thus describe the light driven reduction of NO_3^- to ammonia using an artificial photosystem and two biocatalysts. Further experiments to immobilize the enzymes and design of organized assemblies for this process are under way in our laboratory.

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Tailored Semiconductor–Receptor Colloids: Improved Photosensitized H_2 Evolution from Water with TiO_2-β-Cyclodextrin Colloids

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Redox reactions in microheterogeneous semiconductor systems are of much interest as a means of converting light to chemical energy.[1,2] Either direct excitation of a semiconductor or a photosensitizing dye, adsorbed on the particle surface, can activate electron (or hole) transfer to solution species at the semiconductor–liquid interface. Surface recombination and back electron (or hole) transfer from solution species to either the semiconductor or the adsorbed dye molecule can lower the overall yield of the desired product(s) and thus the conversion efficiency of light to chemical energy. Electrostatic interactions in colloidal semiconductor dispersions have been utilized to control interfacial electron transfer and to improve the quantum yield for H_2 evolution from water.[3] Surface adsorption of β-cyclodextrin (β-CD) to semiconductor colloids has been found[4] to improve the kinetics for charge transfer from the photoexcited semiconductor to electron acceptors retained in the β-CD cavity. β-Cyclodextrin also served to stabilize the colloids against aggregation.

Dye sensitization of a semiconductor affords the possibility of using sub-bandgap light and the electronic transport properties of the semiconductor to effect charge separation. Both organic dyes, such as Rhodamine B, and transition-metal complexes have been utilized as photosensitizers of wide bandgap semiconductor materials.[5,6] Diffusion-limited charge transfer from the pho-

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