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NADH Oxidase Activity of Gold Nanoparticles in Aqueous Solution

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Abstract—Nanoparticles of Au(0) stabilized by Triton X-100 in water catalyze NADH oxidation. Oxygen and potassium ferricyanide can serve as electron acceptors from NADH. The NADH oxidase activity of Au(0) (normalized to gold concentration) is 0.08 turnover/min in air and 0.32 turnover/min in argon in the presence of K_3FeCN_6 (2×10^{-4} mol/l). The catalytic activity decreases with increasing gold concentration in the solution used for particle preparation and, accordingly, with increasing size of gold particles.

INTRODUCTION

The catalytic activity of gold nanoparticles in liquid-phase oxidation has attracted researchers' interest both as a source of fundamental knowledge and as a basis for new technologies. The most research has been devoted to reactions catalyzed by Au nanoparticles stabilized on metal oxide supports, including CO oxidation and olefin epoxidation [1]. Carbon-supported gold nanoparticles are catalytically active in the liquid-phase oxidation of glycols and aldehydes [2, 3].

At the same time, the catalytic properties of a broad class of gold nanoparticles stabilized by organic monolayers in solutions remain to be studied [4, 5]. Therefore, it is of interest to investigate the oxidation of NADH[1] with dioxygen involving Au(0) nanoparticles stabilized with Triton X-100 in aqueous solutions.

EXPERIMENTAL

Nanoparticles were prepared by an earlier described procedure [5] using twice-distilled water, $HAuCl_4 \cdot nH_2O$ (reagent grade, Aurat), and Triton X-100 (Aldrich). The standard preparation procedure consisted in the irradiation of a freshly prepared aqueous solution of $HAuCl_4$ (4×10^{-5} mol/l) and Triton X-100 (10^{-2} mol/l) in a quartz cell ($1 \times 1 \times 4$ cm) with a Foton bactericide lamp ($W = 12$ W) for 25 min in air at room temperature. The cell was placed at a distance of 1.5–2 cm from the lamp. Before each irradiation run, the cell was washed with aqua regia to remove the Au(0) traces.

NADH oxidation was conducted in air at room temperature (19 ± 2°C) in a quartz cell ($0.5 \times 1 \times 4$ cm). The reaction mixture ($V = 1$ ml) contained an aqueous solution (pH ~5.5) of nanoparticles ([Au] = 4×10^{-5} mol/l, [Triton X-100] = 10^{-2} mol/l) and NADH ($[NADH]_0 = 2 \times 10^{-4}$ mol/l). The NADH concentration was monitored spectrophotometrically at $\lambda = 340$ nm ($\varepsilon = 6220$ l mol^{-1} cm^{-1}). All spectroscopic measurements were carried out on a Specord M-40 spectrophotometer.

For measurements in argon, the water to be used in the preparation of $HAuCl_4$, Triton X-100, NADH, and $K_3[Fe(CN)_6]$ solutions was pumped and saturated with argon. Particle preparation and NADH oxidation were carried out in argon-filled cells with a sealing lock. A solution of $K_3[Fe(CN)_6]$ (0.2 μmol in 20 μl of water per milliliter of the reaction mixture) was syringed into the cell through a rubber septum. The reaction rate was determined for an initial period of 5 min. Turnover number was calculated based on $[HAuCl_4]$ added.

The initial molar concentration of NADH was equal to the molar concentration of dissolved oxygen. The initial rate of NADH oxidation was shown to be independent of NADH concentration for $[NADH]_0 >$ 200 μmol/l.

RESULTS AND DISCUSSION

The formation of nanoparticles upon irradiation of the solution of $HAuCl_4$ and Triton X-100 was indicated by the reaction mixture changing its color from almost colorless to pink. The spectrum of the solution also changed: the absorption band at 320 nm due to Au^{3+} disappeared, and a band at 528 nm due to gold nanoparticles appeared (Fig. 1).

In the method used in this work, Triton X-100 reduced Au^{3+} and stabilized the resulting nanoparticles. According to an earlier report [5], the size of these nanoparticles depends on the [Au]/[Triton] ratio, being ~6 nm under the standard conditions chosen. Since 1 nm^3 of gold contains 59 Au atoms [6, 7], a nanoparticle of this diameter contains ~6500 gold atoms.

Nanoparticles thus prepared showed NADH oxidase activity. A kinetic curve typical of NADH oxidation

[1] NADH is the reduced form of nicotinamide adenine dinucleotide, which is used in biochemistry as a two-electron donor, and its oxidized form is NAD^+.

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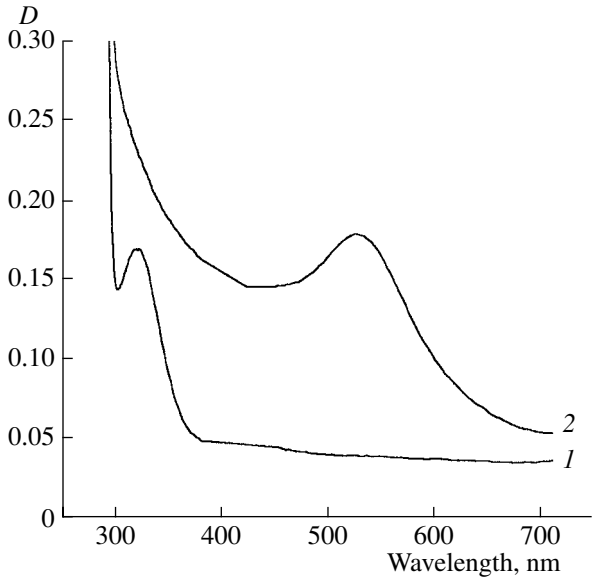

Fig. 1. Optical spectra of an aqueous solution of $HAuCl_4$ (4×10^{-5} mol/l) and Triton X-100 (10^{-2} mol/l) (*1*) before and (*2*) after irradiation with a bactericide lamp.

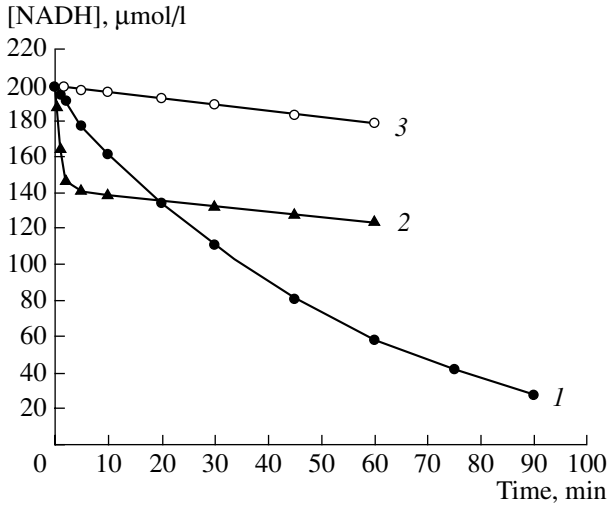

Fig. 2. Kinetic traces of NADH oxidation with dioxygen (*1*) in a solution of gold nanoparticles ([Au] = 4×10^{-5} mol/l), (*2*) in a solution of an unirradiated $HAuCl_4$ (4×10^{-5} mol/l) + Triton X-100 (10^{-2} mol/l) mixture, and (*3*) in a solution of Triton X-100 (10^{-2} mol/l) irradiated in the presence of gold.

with dioxygen catalyzed by Au nanoparticles is presented in Fig. 2 (curve *1*).

For the given reaction and particle preparation conditions, the initial rate of NADH oxidation was, on the average, 3 ± 1 μmol l^{-1} min^{-1}, one order of magnitude higher than the rate of NADH autooxidation (Fig. 2, curve *3*). Under the same conditions, Au^{3+} ions were only reduced by NADH, most likely to Au^0, as was evident from the observed reaction stoichiometry (3NADH : 2Au; curve *2*).

As can be seen from the data presented in Fig. 2, the reaction rate decreases with time. Vigorous stirring of the solution in order to facilitate oxygen diffusion exerted no considerable effect on the oxidation kinetics. If, after the reaction had been conducted for 30–40 min, NADH (in a microquantity of water) was added to the reaction mixture in order to restore the initial NADH concentration, the oxidation rate somewhat increased but did not reach the initial value. Apparently, the retardation of the reaction is due to not only NADH consumption but also catalyst deactivation. The total turnover number for the particles prepared by the standard procedure was 4–5 (based on total gold added).

The catalytic properties of the nanoparticles—activity and total turnover number–depend considerably on their quality. In turn, the quality of the particles is determined by preparation conditions, specifically, the purity of the reactants, the cleanness of the glassware, and irradiation parameters.

Some characteristics of the NADH oxidase activity of the gold nanoparticles are listed in the table.

The data presented in the table (rows 1 and 2) indicate that the initial rate of NADH oxidation normalized to the initial $HAuCl_4$ concentration decreases with an

Characteristics of the NADH oxidase activity of gold nanoparticles

Run no.	Nanoparticle preparation and NADH oxidation conditions	Initial rate of NADH oxidation, turnover/min
1	Standard conditions (see Experimental)	0.08 ± 0.02
2	Particles are prepared at	
	(a) $[HAuCl_4] = 1 \times 10^{-5}$ mol/l	0.15
	(b) $[HAuCl_4] = 50 \times 10^{-5}$ mol/l	0.03
	Other conditions are standard	
3	Particles are prepared and NADH is oxidized in argon	0
4	See run 3; difference: 0.0002 mol/l $K_3Fe(CN)_6$ was added to the reaction mixture (the initial $K_3Fe(CN)_6$ concentration was equal to the concentration of dissolved oxygen at an air pressure of 1 atm)	0.32

increase in this concentration. The initial gold concentration is known to determine the size of the resulting particles and, accordingly, the total surface area of the catalyst [5]. According to data presented in [5], the particle diameter in runs 1 and 2b is equal to 6 and 23 nm, respectively. If we express the initial NADH oxidation rate in these runs in terms of the number of NADH molecules oxidized on 1 nm^2 of the nanoparticle surface in 1 min (N), we will obtain nearly equal conversion rates, namely, $N_1 \sim 5$ nm^2/min and $N_{2b} \sim 7$ nm^2/min. Note that these data coincide, within one order of magnitude, with data reported for other catalytic reactions involving gold nanoparticles. For instance, for the low-temperature (350 K) CO oxidation catalyzed by TiO_2-supported gold nanoparticles of diameter 3.5 nm, the reaction rate is 4 turnover/s per particle, which corresponds to ~8 turnover/min per square nanometer of the particle surface [1].

It was found that electrons from NADH can be accepted by either dioxygen or potassium ferricyanide. For the reaction in argon (row 3 of the table), the rate of NADH oxidation is zero, while this rate is 12–13 μmol/min or 0.32 turnover/min (row 4) after potassium ferricyanide (2×10^{-4} mol/l) is added to the reaction mixture. This result clearly demonstrates that the role of gold nanoparticles as a catalyst is coupling of the two-electron oxidation of NADH and the one-electron reduction of the acceptor.

Note that gold nanoparticles show a rather high specificity towards NADH. Attempts to use such two-electron donors as catechol and ascorbic acid as oxidation substrates have failed. Since NADH undergoes oxidation in the presence of gold nanoparticles and catechol (which has a very similar oxidation potential) does not, it is believed that the species accepted by a gold nanoparticle is the hydride ion, which can be donated by NADH and not by catechol. This property of gold nanoparticles is probably due to surface Au^+ ions.

It is noteworthy that the reaction that we discovered models the NADH–ferricyanide oxidoreductase activity of the Au protein from *Micrococcus luteus*. This protein is of interest because its active center contains a gold complex with a flavonoid (rutin) and can catalyze the NADH-dependent oxidation of methane to methanol [6, 7].

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