

Specific Intermolecular Association between NAD^+ and $\text{Rh}(\text{terpy})_2^{3+}$

Keisuke UMEDA, Hiroshi IKEDA, Asao NAKAMURA, and Fujio TODA*

Department of Bioengineering, Faculty of Bioscience and Biotechnology,
Tokyo Institute of Technology, Nagatsuta-cho, Midori-ku, Yokohama 227

The intermolecular association of NAD^+ and $\text{Rh}(\text{terpy})_2^{3+}$ was confirmed with NMR spectroscopy. The protons of adenine in NAD^+ was shifted to upfield by the addition of $\text{Rh}(\text{terpy})_2^{3+}$. ROESY spectrum showed intermolecular NOE between aromatic protons of those compounds. From these observations, the structures of complex of $\text{Rh}(\text{terpy})_2^{3+}$ and NAD^+ were proposed.

Many works for the photochemical reduction of NAD^+ to give 1,4-NADH have been reported in this decade,¹⁾ and recent studies have been targeted to the development of the enzyme-free system for the NAD^+ -reduction.²⁾ The importance in the reduction of NAD^+ is the regioselectivity in which 4-position of nicotinamide is hydrated into 1,4-dihydro-form.

Recently, we have proposed bis(2,2'; 6,2"-terpyridine)rhodium(III) ($\text{Rh}(\text{terpy})_2^{3+}$) as a catalyst, and it was clarified that $\text{Rh}(\text{terpy})_2^{3+}$ catalyzed the reduction of NAD^+ to give 1,4-NADH regioselectively and have the great stability in the photoreduction cycle.³⁾ The mechanism for the regioselective reduction catalyzed by $\text{Rh}(\text{terpy})_2^{3+}$ remains obscure and is now of interest. Considering the fact that NaBH_4 , which is a small size of reductant, reduces NAD^+ into the mixture of 1,2-, 1,4-, and 1,6-NADH,^{3,4)} the regioselective catalysis is presumably due to the stereospecific interaction of NAD^+ with the reduced form of $\text{Rh}(\text{terpy})_2^{3+}$. In order to

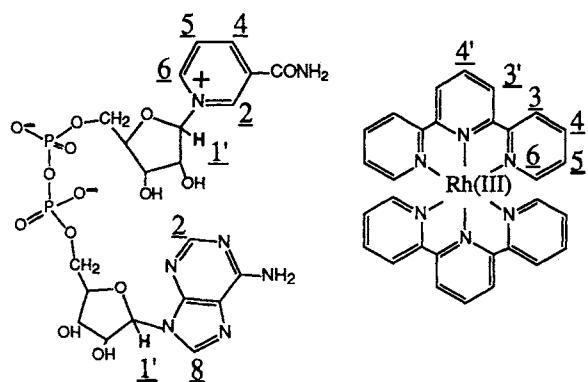


Fig. 1. Structures of NAD^+ and $\text{Rh}(\text{terpy})_2^{3+}$. The numbering refers to assignments of chemical shifts.

of $\text{Rh}(\text{terpy})_2^{3+}$ was used instead, and the observation on the interaction of NAD^+ with the original form of $\text{Rh}(\text{terpy})_2^{3+}$ seems to be sufficient enough to estimate that of NAD^+ with the reduced form of $\text{Rh}(\text{terpy})_2^{3+}$. In this report, we describe the intermolecular interaction between NAD^+ and $\text{Rh}(\text{terpy})_2^{3+}$.

confirm the specific interaction, proton nuclear magnetic resonance (NMR) was used in this study. NMR is one of the greatest tactics providing the information about inter- or intramolecular interaction.⁵⁾ Although the best way is that the interaction of NAD^+ with the reduced form of $\text{Rh}(\text{terpy})_2^{3+}$ (perhaps $\text{Rh}(\text{H}^-)(\text{terpy})_2^{3+}$)⁶⁾ should be studied directly, it is difficult to measure this interaction because NAD^+ reacts with catalytic active form of $\text{Rh}(\text{terpy})_2^{3+}$ within the period of experiment. Therefore, the original form

500 MHz ^1H NMR was measured with Varian VXR-500S spectrometer at 25 °C. NAD^+ and $\text{Rh}(\text{terpy})_2^{3+}$ were dissolved in D_2O containing 0.1 mol dm^{-3} NH_4HCO_3 .

Table 1. Assignments of ^1H NMR for NAD^+ and $\text{Rh}(\text{terpy})_2^{3+}$ in D_2O containing 0.1 mol dm^{-3} NH_4HCO_3 at 25 °C

NAD ⁺								terpy ^{a)}											
Nicotinamide ^{a)}				Adenine ^{a)}				T-C ₃ H		T-C ₄ H		T-C ₅ H		T-C ₆ H		T-C ₃ 'H		T-C ₄ 'H	
N-C ₂ H	N-C ₄ H	N-C ₅ H	N-C ₆ H	N-C ₁ 'H	A-C ₂ H	A-C ₈ H	A-C ₁ 'H												
9.32	8.82	8.18	9.13	6.07	8.16	8.41	6.02	8.72	8.30	7.52	7.72	9.00	8.95						

a) Chemical shifts are described in ppm downfield from TSP. N-, A-, and T- denote nicotinamide, adenine, and terpyridine.

The chemical shifts for protons of NAD^+ and $\text{Rh}(\text{terpy})_2^{3+}$ were summarized in Table 1. Figure 2 shows changes in chemical shifts for NAD^+ -protons shifted from originals with various concentration of $\text{Rh}(\text{terpy})_2^{3+}$.

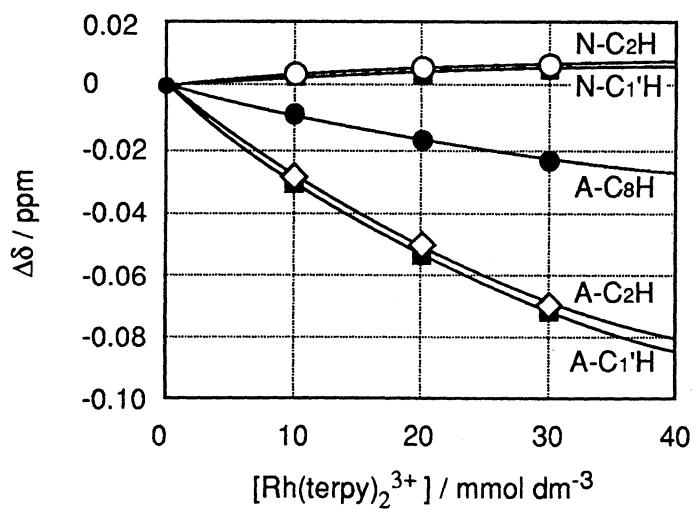


Fig. 2. Changes in resonances in ppm for NAD^+ protons shifted as the variation of $\text{Rh}(\text{terpy})_2^{3+}$ concentration. The concentration of NAD^+ is 2 mmol dm^{-3} . $\Delta\delta$ (ppm) were determined with external TSP. $\Delta\delta$ of another protons of nicotinamide ring which were not shown hardly shifted.

middle domain between shielding field and deshielding field. In order to evaluate the association constant (Kasso), $\Delta\delta$ for A-C₂H, A-C₈H, and A-C₁'H were fitted with Eq.1⁷⁾ which is derived from Benesi-Hildebrand equation. It is clear from Fig. 2 that Eq.1 is good agreement with results for $\Delta\delta$, suggesting 1:1 complex of NAD^+ and $\text{Rh}(\text{terpy})_2^{3+}$, and Kasso obtained for each proton are summarized in Table 2.

$$\frac{1}{\text{Kasso } Q} + \frac{[\text{Rh}(\text{terpy})_2^{3+}]_0 + [\text{NAD}^+]_0}{Q} = \frac{[\text{Rh}(\text{terpy})_2^{3+}]_0}{\Delta\delta} \quad (1)$$

$Q = \delta_{\text{complex}} - \delta_{\text{free}}$ $([\text{Rh}(\text{terpy})_2^{3+}]_0 \text{ or } [\text{NAD}^+]_0 \text{ is the primary concentration})$

It is clear that the resonances of adenine protons, especially A-C₂H and A-C₁'H, shifted to upfield considerably. On the other hand, the resonances for protons of nicotinamide are almost maintained in original chemical shifts. The upfield shift observed in the resonances for adenine protons is presumably caused by the effect of the ring-current shielding of *terpy* as the ligand, and it is unlikely that rhodium as the centered metal affects this upfield shift because rhodium in $\text{Rh}(\text{terpy})_2^{3+}$ is not paramagnetic. Therefore A-C₂H and A-C₁'H surely associate with the inside of *terpy* ring, whereas protons of nicotinamide are hardly affected by the ring-current shielding, indicating that those protons are out of the shielding field or lie in the

These results demonstrate that NAD^+ is associated with $\text{Rh}(\text{terpy})_2^{3+}$ but are not sufficient evidence to define the stereospecific interaction between both of compounds. In order to confirm whether the protons of both

Table 2. Association constants for adenine-protons of NAD^+ to the ligand of $\text{Rh}(\text{terpy})_2^{3+}$

K _{asso} / mol ⁻¹ dm ³		
A-C ₂ H	A-C ₈ H	A-C _{1'} H
13	10	15

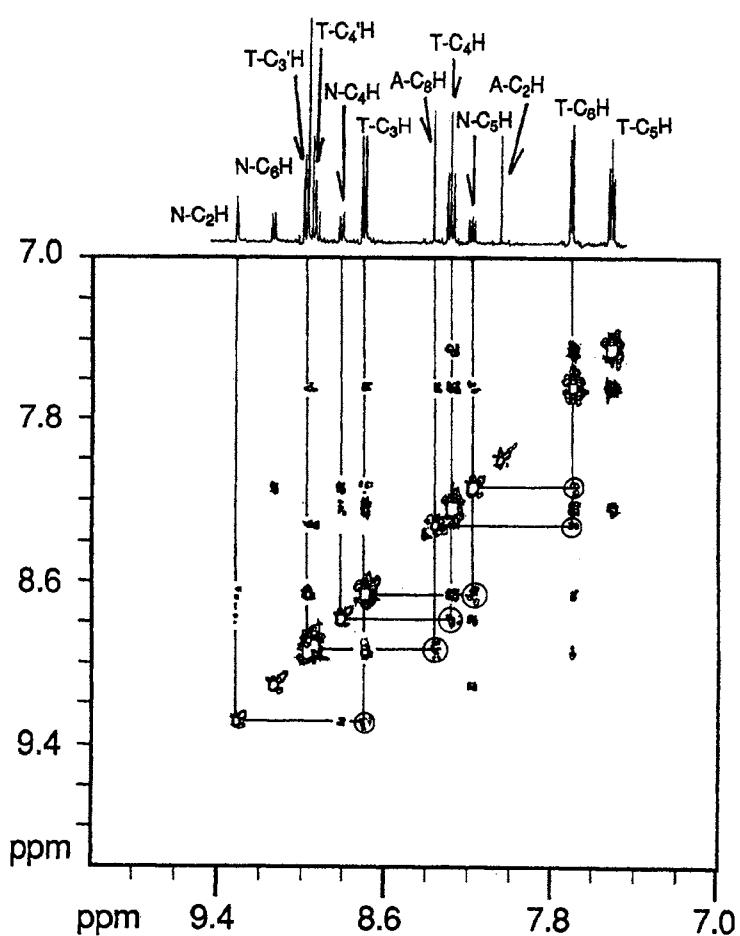
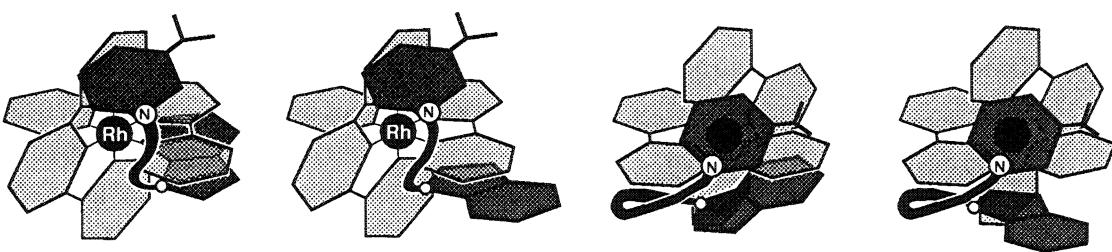


Fig. 3. ROESY spectrum and 1D ^1H NMR spectrum of the mixture composed of NAD^+ and $\text{Rh}(\text{terpy})_2^{3+}$ measured at 25 °C. NOE signals for intermolecular protons are linked by lines.

to rhodium rather than 2- or 6-position. Moreover *rhodium-hydride* complex ($\text{Rh}(\text{H}^-)(\text{terpy})_2^{3+}$),⁶⁾ which is produced by photochemical reduction of $\text{Rh}(\text{terpy})_2^{3+}$, presumably catalyze the reductive formation of 1,4-NADH. These estimations lead to a conclusion that 4-position of nicotinamide is the most probable to be reduced because the position is close to rhodium.

compounds are close each other enough to give NOE or not, ROESY spectrum of the mixture of NAD^+ and $\text{Rh}(\text{terpy})_2^{3+}$ was measured at 25 °C. Figure 3 presents the ROESY spectrum and 1D ^1H NMR spectrum of aromatic groups. In Fig. 3, some weak intermolecular NOE signals (enclosed by circles) were recognized in addition to strong intramolecular NOE signals, and the assignment of correlated protons between NAD^+ and $\text{Rh}(\text{terpy})_2^{3+}$ were shown by linkage of lines in Fig. 3. The intermolecular NOE signals indicate that some aromatic protons of NAD^+ are close to those of $\text{Rh}(\text{terpy})_2^{3+}$ within 3 Å and that NAD^+ interacts with $\text{Rh}(\text{terpy})_2^{3+}$ stereospecifically. On the basis of both the results obtained here and previous works⁸⁾ which describe the folded form of NAD^+ in aqueous solution, space filling molecular model was used to define the structure of complex, and probable candidates were proposed to be four kinds of structures as are shown in Fig. 4. Judging from the weak NOE signals and flexibility or movement in solution, it is the proper interpretation that NAD^+ and $\text{Rh}(\text{terpy})_2^{3+}$ are in equilibrium between free molecules and those complexes or between each complexes.

In those models, it is recognized that nicotinamide group in four structures lie in geometry where 4-position of nicotinamide is at close range



NOE	N-C4H \leftrightarrow T-C4H N-C5H \leftrightarrow T-C3H	N-C4H \leftrightarrow T-C4H N-C5H \leftrightarrow T-C3H A-C8H \leftrightarrow T-C6H	N-C2H \leftrightarrow T-C3H N-C5H \leftrightarrow T-C6H	N-C2H \leftrightarrow T-C3H N-C5H \leftrightarrow T-C6H
Upfield Shift	A-C2H A-C1'H		A-C2H A-C1'H	A-C1'H

Fig. 4. Proposed structure of complexes and evidences assisting these proposals. Aromatic group of NAD^+ was described with dark gray, terpy was with light gray, and rhodium was described as a black sphere. Nitrogen atoms were omitted except for nicotinamide.

In conclusion, we proposed that the aromatic group of NAD^+ interacts with $\text{Rh}(\text{terpy})_2^{3+}$ to give a complex stereospecifically as in Fig. 4, and this is the principal reason for regioselective reduction of NAD^+ .

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