Nonenzymatic NADH-Dependent Reduction of Keggin-Type 12-Tungstocobaltate(III) in Aqueous Medium

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Abstract: The kinetics of the electron transfer reaction of NADH with 12-tungstocobaltate(III) has been studied over the range $5.07 \leq 10^4$ [NADH] ≤ 15.22 mol dm⁻³, $7.0 \leq \text{pH} \leq 8.0$ and $20 \leq t \leq 35$ °C in aqueous medium. The electron transfer reaction showed first-order dependence each in [NADH]_T and [12-tungstocobaltate(III)]_T. The products of the reaction were found to be NAD⁺ and 12-tungstocobaltate(II). The activation parameters $\Delta H^\ddagger$ (kJ mol⁻¹) and $\Delta S^\ddagger$ (JK⁻¹mol⁻¹) of the electron transfer reactions were found to be 64.4 ± 1.8 and -48.86 ± 6.0 . Negative value of $\Delta S^\ddagger$ is an indicative of an ordered transition state for the electron transfer reaction.

Keywords: Electron transfer, NADH, 12-Tungstocobaltate(III), Nonenzymatic

Introduction

Nicotinamide adenine dinucleotide (NADH) is a coenzyme. It is oxidized to NAD⁺ in presence of enzymes. In metabolism NAD⁺ is involved in redox reactions, carrying electrons from one reaction to other. The coenzyme is therefore found in two forms in cells: NAD⁺ as an oxidizing agent and NADH as a reducing agent. The redox potential of the NAD⁺/NADH couple is -0.32 volts¹, which makes NADH a strong reducing agent. The reaction is reversible⁸, this means the coenzyme can continuously cycle between NAD⁺ and NADH forms. Studies on the electron transfer reactions of NADH and its synthetic analogues have been reported²⁻⁸. Reduction of methemoglobin⁹, orthovanadate and vanadyl sulphate¹⁰, ferric siderophores¹¹ and heteropolytungstovanadophosphates¹² by NADH has been reported.

The oxidant Keggin type 12-tungstocobaltate(III) (Co^{III}W₁₂O₄₀⁵⁻ or Co^{III}W⁵⁻) cluster has a reduction potential of 1.0V¹³, hence it can accept electrons from NADH. The above cluster is known for photochemical hydrogen generation and is involved in multielectron reduction

processes¹⁴⁻¹⁶. The redox behavior of the above cobalt cluster with few biomolecules like ascorbic acid¹⁷, glutathione¹⁸, citric acid¹⁹, *DL*-methionine²⁰ and cystine²¹ have been investigated. In order to examine the redox behavior of 12-tungstocobaltate(III), its reaction with NADH has been investigated.

Experimental

[Co^{III}W₁₂O₄₀]⁵⁻ was prepared by the reported method¹⁷ and was characterized spectrophotometrically²³ (at 388 nm $\epsilon_{388} = 1150 \pm 2 \text{ dm}^{-3} \text{ mol}^{-1} \text{ cm}^{-1}$). The chemicals used were of A.R grade and doubly distilled water was used throughout the experiment. The electron transfer reaction was studied at pH = 7.5 ± 0.01 using a pre-standardised Elico (India) digital pH meter equipped with glass electrode. The pH of the solution was maintained using phosphate buffer.

The absorbance measurements for the kinetic studies were done using CECIL CE 7200 UV-VIS scanning spectrophotometer equipped with CE 2024 thermoelectric controller. Reaction progress was monitored at 380 nm, pseudo first order conditions were maintained throughout the runs by using large ($\geq$ five fold) excess of [NADH]. The rate constants (k_{obs}) were obtained from the slope of $-\ln(A_t - A_\infty)$ versus 't' (min) plots (Figure 1).

$$[\text{Co}^{\text{III}}\text{W}_5]_T = 9.93 \times 10^{-5} \text{ mol dm}^{-3}, [\text{NADH}]_T = 14.3 \times 10^{-4}, \text{pH} = 7.5 \text{ Temp} = 25^\circ \text{C}$$

Figure 1. Determination of k_{obs} value

$$-\ln(A_t - A_\infty) = k_{\text{obs}} t + C \quad (1)$$

Where A_t and A_∞ are the absorbance of the reaction mixture at time 't' and at equilibrium respectively. The reported rate data represented as an average of duplicate runs were reproducible to within $\pm 3\%$. The correlation coefficient of the plots used to determine k_{obs} were found to be 0.99 in most cases.

Results and Discussion

Co^{III}W₁₂O₄₀⁵⁻ has absorbance maxima at 388 nm and on mixing with NADH the peak is found to be decrease with time (Figure 2) with simultaneous increase of new peaks at 625 nm (Figure 3) due to the formation of Co^{II}W₁₂O₄₀⁶⁻ and a peak at 260 nm (Figure 5) due to the formation of NAD⁺ is observed. The decrease of absorbance at 380 nm is followed for the reduction of Co^{III}W₁₂O₄₀⁵⁻ by NADH.

$[Co^{III}W^{5-}]_T = 9.93 \times 10^{-5} \text{ mol dm}^{-3}$, $[NADH]_T = 5.07 \times 10^{-5} \text{ mol dm}^{-3}$, $pH = 7.5$, $Temp = 30^\circ C$, $a = 0 \text{ min}$; $b = 10 \text{ min}$; $c = 20 \text{ min}$; $d = 30 \text{ min}$; $e = 40 \text{ min}$; $f = 50 \text{ min}$; $g = 60 \text{ min}$; $h = 70 \text{ min}$; $i = 80 \text{ min}$

Figure 2. Overlay scan of reaction mixture with respect to time

$[Co^{III}W^{5-}]_T = 9.93 \times 10^{-5} \text{ mol dm}^{-3}$, $[NADH]_T = 5.07 \times 10^{-4}$, $pH = 7.5$, $Temp = 30^\circ C$

Figure 3. Scan of reaction mixture with respect to time showing $[Co^{III}W]^{6-}$ formation

Effect of $[NADH]$ on reaction rate

The effect of varying $[NADH]$ on the reaction rate was studied at fixed pH (7.5) and between 20–35 °C. The concentration of $Co^{III}W^{5-}$ was kept constant and $10^4 [NADH]$ was changed from 5.07 to 15.22 mol dm^{-3} .

The plot of k_{obs}/s^{-1} vs. $[NADH]_T$ is linear at four different temperatures (20–35 °C) (Figure 4). This fact indicates that the reaction is first order with respect to $[NADH]_T$. Since the second order rate constant does not change appreciably at a given temperature (Table. 1). The overall order of the electron transfer reaction is two. Hence the order with respect to the $[Co^{III}W^{5-}]_T$ must be one.

Table 1. Values of k_{obs} at different temperatures $[\text{Co}^{\text{III}}\text{W}^{5-}]_{\text{T}} = 9.93 \times 10^{-5} \text{ mol dm}^{-3}$ and $\text{pH} = 7.5$

$10^4 [\text{NADH}]$ mol dm^{-3}	$10^4 k_{\text{obs}} / \text{s}^{-1}$			
	293 K	298 K	303 K	308 K
5.07	0.33	0.50	0.89	1.42
7.61	0.43	0.71	1.15	1.86
10.15	0.57	0.90	1.34	2.01
14.31	0.77	1.16	1.66	2.40
15.22	0.82	1.22	1.75	2.51
$k_{2(\text{average})}$ $\text{mol}^{-1} \text{dm}^3 \text{s}^{-1}$	0.057 ± 0.004	0.088 ± 0.0079	0.1380 ± 0.0254	0.212 ± 0.050

$$\Delta H^\ddagger = 64.4 \pm 1.8 \text{ kJ mol}^{-1}, \Delta S^\ddagger = -48.86 \pm 6.0 \text{ JK}^{-1} \text{mol}^{-1}$$

Rate dependence on pH

At 298K with $[\text{Co}^{\text{III}}\text{W}^{5-}]_{\text{T}} = 9.93 \times 10^{-5}$ and $[\text{NADH}]_{\text{T}} = 5.07 \times 10^{-4} \text{ mol dm}^{-3}$, the pH was varied from 7.0 to 8.0 using phosphate buffer ($\text{KH}_2\text{PO}_4 + \text{Na}_2\text{HPO}_4$) and $10^4 k_{\text{obs}} (\text{s}^{-1})$ was found to be fairly constant (1.11 ± 0.38). The electron transfer reaction does not show any pH dependence in the pH range 7.0 to 8.0. The electron transfer reaction could not be studied at higher pH because oxidant cobalt(III) clusters will undergo base hydrolysis.

Rate dependence on temperature

The electron transfer reaction was followed in the temperature range 20–35 °C. The oxidant concentration was kept constant at $9.93 \times 10^{-5} \text{ mol dm}^{-3}$ and the reductant NADH concentration was varied from 5.07×10^{-4} to $15.22 \times 10^{-4} \text{ mol dm}^{-3}$. The k_{obs} was found to increase with increase in temperature (Figure 4). The second order rate constant $10^2 k_2$ changed from 5.7 ± 0.4 to 21.2 ± 5.0 when temperature was changed from 20–35 °C. The values of $\Delta H^\ddagger$ and $\Delta S^\ddagger$ were calculated using eyring equation and were found to be $64.4 \pm 1.8 \text{ kJ mol}^{-1}$ and $-48.86 \pm 6.0 \text{ JK}^{-1} \text{mol}^{-1}$. The negative value of activation entropy indicates an ordered transition state for the electron transfer reaction.

Plots of $10^4 k_{\text{obs}}$ versus $10^4 [\text{NADH}]$ at various temperatures**Figure 4.** Rate dependence on temperature

Polymerization study

The participation of free radicals was examined as follows. The reaction mixture, to which a known quantity of acrylonitrile scavenger had been added initially, was kept in an inert atmosphere for 5 h. When the reaction mixture was diluted with methanol, a white precipitate resulted. The above experiment supports the formation of free radical. Blank experiments with either $[\text{Co}^{\text{III}}\text{W}]^{5-}$ or NADH with acrylonitrile did not give polymerization under the same conditions as those used for the reaction mixture.

Stoichiometry and characterization of product

The stoichiometry of the reaction was studied at 30 °C where $[\text{Co}^{\text{III}}\text{W}^{5-}]_{\text{T}}$ was kept constant at $9.93 \times 10^{-5} \text{ mol dm}^{-3}$ and $[\text{NADH}]_{\text{T}}$ at $5.07 \times 10^{-4} \text{ mol dm}^{-3}$. Formation of a peak at 260 nm after 3 half lives (Figure 5) indicates the formation of NAD^+ . The formation of a peak at 625 nm (Figure 3) indicates the formation of $[\text{Co}^{\text{II}}\text{W}]^{6-}$. The stoichiometry of the reaction can be written as

Basing on the above observations and experimental results, the probable mechanism may be delineated as in Scheme 1.

$[\text{Co}^{\text{III}}\text{W}^{5-}]_{\text{T}} = 9.93 \times 10^{-5} \text{ mol dm}^{-3}$, $[\text{NADH}]_{\text{T}} = 5.07 \times 10^{-4}$, $\text{pH} = 7.5$, $\text{Temp} = 30^\circ \text{C}$

Figure 5. Scan of reaction mixture with respect to time showing NAD^+ formation after 24 h.

Scheme 1. The rate law for the electron transfer reaction can be written as $\text{Rate} = k_2 [\text{NADH}] [\text{Co}^{\text{III}}\text{W}^{5-}]$ $k_2 = 2^{\text{nd}}$ order rate constant

The above mechanism involves multiple electron-proton-electron transfer steps. Initially an electron is transferred from NADH to $[\text{Co}^{\text{III}}\text{W}]^{5-}$ producing NADH^+ and $[\text{Co}^{\text{II}}\text{W}]^{6-}$. The solvent water molecule abstracts the proton from NADH^+ to produce $\text{NAD}^{\cdot}$. For such a mechanism either electron transfer from NADH to $[\text{Co}^{\text{III}}\text{W}]^{5-}$ or proton transfer from NADH^+ to water may be rate limiting step. As reported earlier^{12,22} the initial electron transfer step appears to be the rate limiting step in the oxidation of NADH by $[\text{Co}^{\text{III}}\text{W}]^{5-}$.

References

1. Campbell M K and Farrell S O, *Biochemistry*, 6th Edn., Thomson Brooks/cole, Canada, 2008.
2. Rao A V S and Ramasarma T, *Biochem Biophys Acta*, 2000, **1474**, 321-330.
3. Shi X and Dalal N S, *Arch Biochem Biophys.*, 1993, **307**, 336-341.
4. Patole M S, Gullapalli S and Ramasharma T, *Free Rad Res Commun.*, 1988, **4**, 201.
5. Patole M S, Gullapalli S and Ramasarma T, *J Neurochem.*, 1988, **51**, 491.
6. Khandke L, Gullapalli S, Patole M S and Ramasarma T, *Arch Biochem and Biophys.*, 1986, **244**, 742-749.
7. Ramasarma T, Mac Keller W and Crane F L, *Indian J Biochem Biophys.*, 1980, **17**, 1638.
8. Vijaya S and Ramasarma T, *J Inorg Biochem.*, 1984, **20**, 247-254.
9. Tomoda A, Yubisui T, Tsuji A and Yoneyama Y, *J Biol Chem.*, 1979, **254**, 3119-3123.
10. Keller J R, Coulombe A R, Sharma P R, Grover A T and Piette H L, *Arch Biochem Biophys.*, 1989, **271**, 40-48.
11. Adjimani J P and Owusu E, *J Inorg Biochem.*, 1997, **66**, 247-252.
12. Sami P and Rajasekaran K, *J Chem Sci.*, 2009, **121**, 155-161.
13. Ebersson L, *J Am Chem Soc.*, 1983, **105**, 3192.
14. Ioannidis A and Papaconstantinou E, *Inorg Chem.*, 1985, **24**, 439.
15. Pope T M and Papaconstantinou E, *Inorg Chem.*, 1967, **6**, 1147.
16. Pope T M and Varga Jr M G, *Inorg Chem.*, 1966, **5**, 1249.
17. Amjad Z, Brodovtch C J and McAuley A, *Can J Chem.*, **55**, 1977, 3581.
18. Ayoko A G and Olatunji A M, *Inorg Chim Acta*, 1983, **80**, L15-L17.
19. Saha K S, Ghosh C M and Banerjee P, *Int J Chem Kinet.*, 2004, **20**, 699.
20. Satpathy K P, Dash C G, Acharya S and Mohanty P, *J Indian Chem Soc.*, 2006, **83**, 891.
21. Satpathy P K, Dash G C and Mohanty P, *Indian J Chem.*, 2008, **47A**, 1199-1203.
22. Keita B, Essaadi K, Nadjo L, Contant R and Justum Y, *J Electro Anal Chem.*, 1996, **404**, 271.
23. Rasmussen G P and Brubaker H L, *Inorg Chem.*, 1964, **3**, 977.