



Proposed formation mechanism and active species of hydrogen molecules generated from a novel magnesium–citric acid–hydroxypropyl cellulose coating (MgCC) material

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ARTICLE INFO

Article history:

Received 9 July 2015

Received in revised form

21 October 2015

Accepted 26 October 2015

Available online 11 November 2015

Keywords:

Molecular hydrogen

Deuterium

Magnesium

Gas chromatography mass spectrometry

Electron spin resonance

Transition state

Density functional theory

ABSTRACT

The presence of acids is known to accelerate the reaction ($\text{Mg} + 2\text{H}_2\text{O} = \text{Mg}(\text{OH})_2 + \text{H}_2$). We developed a novel Mg–citric acid coating (MgCC) material produced by milling Mg powder coated with hydroxypropyl cellulose (HPC); because of its H_2 generation, this material could be used in antioxidant therapy and antiaging applications. After milling in the presence of citric acid, this material produced H_2 -rich water upon addition to cooled water. Although the reaction was considered to involve a two-electron transfer from Mg to $2\text{H}_2\text{O}$, the role of the acid in H_2 generation remains incompletely understood. To clarify the reaction mechanism, we performed studies on the deuterium kinetic isotope effects (KIE) and electron spin resonance (ESR). We observed differences in the concentration ratios, such as $\text{H}_2/\text{D}_2 > 1$ and $\text{H}_2/(\text{H}_2 + \text{D}_2 + \text{HD}) > 1$, involved in H_2 , D_2 , and $(\text{H}_2 + \text{D}_2 + \text{HD})$ production, and found that adducts with hydrogen atoms (H^\bullet) were not obtained from the spin-trapping reaction between 5-(2, 2-Dimethyl-1,3-propoxy cyclophosphoryl)-5-methyl-1-pyrroline N-oxide (CYPMPO) and the MgCC material. The H_2 , D_2 , and HD produced from MgCC were identified by using a gas chromatograph connected to a mass spectrometer. The spin-trapping techniques showed that the H^\bullet adducts formed by the reaction of NaBH_4 with CYPMPO could not be observed from reaction of MGCC with CYPMPO in H_2O . The data suggest that the rate-controlling step and proposed transition state (TS) exist in the reaction pathway of the O–H bond cleavage and H–H bond formation. A TS of a structure such as $[\text{Mg}(\text{OH})_2]^\ddagger$ could be expected in the reaction pathway between Mg and $2\text{H}_2\text{O}$ by density functional theory calculations. Also, these results show that H_2 generation is accelerated in the presence of acids because the activation energy of the TS is significantly smaller than that of H_2O .

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1. Introduction

Reactive oxygen species (ROS), such as hydroxyl radical (HO^\bullet), superoxide ($\text{O}_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and singlet oxygen (O_2), exhibit high oxidative abilities, abstract electrons and hydrogen radicals from biological targets, and cause oxidative damage *in vitro*. In our organelles, their excess production is implicated in the pathogenesis of various diseases, including cancer, inflammation, diabetes mellitus, and neurodegenerative

diseases [1–3]. They have also been shown to play a critical role in aging. To understand the mechanisms of oxidative damage and to design and synthesize effective antioxidants, many studies have been performed in recent years. Our previous studies reported the development of new antioxidants based on catechol and quinine rings as effective scavengers of ROS and free radicals [4–6]. On the other hand, it is known that the hydrogen molecule (H_2), with its low molecular weight of 2, is the smallest antioxidant, and its antioxidant activity is considered high because it only scavenges HO^\bullet .

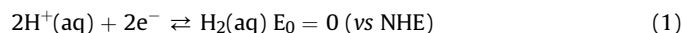
Ohta et al. have recently proposed H_2 as a new antioxidant that can reduce the oxidative state in the body [7,8]. A previous study

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using a rat stroke model indicated that hydrogen molecules may be effective for treating myocardial infarction, against which existing antioxidants are ineffective. Therefore, H₂ may be used in the treatment and prevention of inflammation [9], cancer, diabetes, Parkinson's disease, Alzheimer's disease, and cardiovascular diseases.

Hydrogen molecules are very weakly acidic (pK_a = 35) and hardly dissociate in solution [10]. Hydrogen molecule-rich water exhibits negative oxidation–reduction potential (ORP), whereas purified degassed water and tap water present positive ORPs.



The method used to obtain hydrogen-saturated water involves the electrolytic reduction of water (Eq. (1)). Hydrogen atoms overlap on the electrode surface, and bind together to form hydrogen molecules [11]. Hydrogen molecule-rich water and electrolyzed-reduced water slow down the aging process and scavenge harmful ROS. Hydrogen molecule-rich water containing 0.55–0.65 mM H₂ has previously been produced by placing a magnesium metal (Mg) stick (Doctor SUIOSUI[®]; Friendear, Tokyo, Japan) in drinking water [12].

In this paper, we developed a new powder-like form of Mg–citric acid–HPC (MgCC) material by milling Mg powder coated with hydroxyl propyl cellulose (HPC) in the presence of citric acid. When added to cooled water (6 ml), this material (18 mg) could produce H₂ (5.1 ppm) effectively in cooled water in 20–30 min. It is known that the production of H₂ can be accelerated by the presence of weak organic acids, but the mechanism by which weak acids promote H₂ production remains unknown. In this work, D₂ and HD were easily generated from MgCC in D₂O and H₂O–D₂O solution. The H₂, D₂, and HD gases generated from MGCC were identified by using a gas chromatograph connected to a mass spectrometer (GCMS).

To better understand the mechanism by which weak acids accelerate the H₂ production, deuterium kinetic isotope effect (KIE) [13] measurements were conducted for the H₂, D₂, and HD produced by the MgCC materials. When the ratio of generated H₂ to generated D₂ (H₂/D₂) was examined, it was suggested that the significant difference (H₂/D₂ > 1) results with the KIE and the rate-controlling step and a transition state (TS) exists in the reaction pathway. Moreover, the electron spin resonance (ESR) signal of the spin-trapping adducts of 5-(5,5-Dimethyl-2-oxo-1,3,2-dioxaphosphinan-2-yl)-5-methyl-1-pyrroline N-oxide (CYPMPO) [14] with H radical were not observed when using the MGCC material-based system. Based on these findings, we performed computational studies using a density functional theory (DFT) method to elucidate whether the transition state exists for the H₂ production pathway between Mg and 2H₂O, and proposed a structure of the TS. These results showed that acids (H₂O as weak acid) decreased the activation energy (ΔG[‡]) of the TS, and thus provide insight into the catalytic role of weak acids in H₂ production.

2. Materials and methods

2.1. Materials

Metal magnesium powder, citric acid, malic acid, and sodium borohydride (NaBH₄) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Deuterium oxide was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). CYPMPO was purchased from SHIDAI Systems Co. (Satte, Saitama, Japan). All experimental solutions were prepared with degassed redistilled water. All other chemicals used were of the highest grade available.

Table 1
Concentration of H₂, HD, and D₂ produced from MgCC systems.

Compounds	Concentration (ppm ± SD) ^{a,b}
H ₂	5.20 ± 0.058
D ₂	4.20 ± 0.152
HD + H ₂ + D ₂	3.57 ± 0.057 ^c

A total sum concentration of HD, H₂, and D₂.

^a Concentration of H₂ generated using 18 mg of MgCC system in H₂O (6 ml).

^b All values were expressed as means ± standard deviation (SD) (n = 5).

^c In 1:1 H₂O/D₂O (molar ratio) mixture.

2.2. Preparation of the Mg–citric acid–coating material

The Mg–citric acid–coating material was prepared by the physical mixing of Mg powder (particle size: 212–600 μm), citric acid, and the coating agent hydroxypropyl cellulose (HPC) in a 1: 7: 0.08 weight (%) ratio. The resulting rough powder was pulverized in a bead mill (Ashizawa Finetech Ltd., Chiba, Japan), and a fine powder (particle size: 75–150 μm) was isolated using a sieving machine. From 1 g of MgCC materials, 80 ml of gaseous H₂ were generated by this method.

2.3. Quantification of H₂ and D₂ generated using the MgCC material in several solutions

H₂ and D₂ generated using the MgCC powder (18 mg) were quantified by titration using a methylene blue–platinum colloid (MbPt) indicator (Mizu Co., Tokyo, Japan) [16] in degassed H₂O and D₂O (6 mL) for 25 min, respectively. One drop of indicator corresponds to 0.1 ppm. Similarly, H₂, D₂, and HD produced from the MgCC material (18 mg) were also measured by titration using a MbPt indicator in a degassed 1:1 H₂O/D₂O mixture (6 mL) at 23 °C for 25 min. The amount of H₂ generated using the Mg powder (4 mg) was estimated by titration in cooled water for 25 min (6 mL). The results are expressed as the means ± standard deviation (S.D.) (n = 5) (Table 1 and Fig. 1).

2.4. Gas chromatograph mass spectrometry

The mass spectra of gases H₂ and D₂ were collected using a model 6890N network gas chromatography system (Agilent Technologies Japan Inc., Tokyo, Japan) connected to a Model JMS-700 mass spectrometer (JEOL Ltd., Tokyo, Japan). The insoluble gaseous H₂, D₂, and HD produced from MgCC material in H₂O, D₂O, and H₂O–D₂O (1:1 M ratio), respectively, were collected and identified using a gas over water and injected into the helium carrier gas directly. DB-5MS columns (30 m × 0.25 mm, coated with 0.25 μm thicknesses of silicon film) (Agilent Technologies Japan Inc.) were employed for the helium carrier gas. Here, gas chromatography was

Table 2
Comparison of total energies of CYPMPOs, **1a** and **b**, and their H radical adducts, **2a** and **b**.

Compound ^a	Total energies (E, eV) ^b	ΔE (kcal/mol) ^b	Dihedral angle ^c (deg.)	
			Calcd.	lit. ¹⁴⁾
1a	–29636.10	0	162.05	164.57
1b	–29635.97	+3.0	51.11	52.8
2a	–29652.43	0	163.04	–
2b	–29652.38	+1.2	49.02	–

^a **1a**; A–CYPMPO, **1b**; G–CYPMPO, **2a**; A–CYPMPO–H and **2b**; G–CYPMPO–H.

^b From B3LYP/6-311G(d) calculations.

^c Dihedral angle: O¹–P¹–C⁶–N¹.

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