Integrated Research Center for Carbon Negative Science

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1. Introduction

In order to achieve carbon neutrality by 2050, we need to develop new energy systems that include active carbon dioxide fixation processes in addition to "zero-emission" technologies. Our research center is conducting research on such carbon negative technologies. To be specific, we are working on the conversion of carbon dioxide into useful materials by using renewable energy and biomass, etc.

In this fiscal year, we have investigated the conversion of CO_2 into carbon material by molten salt electrochemical process. We have also studied CO_2 fixation reaction which is facilitated by an enzyme called ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO).

2. Conversion of CO_2 into Carbon Material by Molten Salt Electrochemical Process

As one of the Carbon dioxide Capture and Utilization (CCU) technologies, the electrochemical synthesis of carbon in molten salts is attracting much attention [1]. By using molten salts containing O^{2-} ions, CO_2 can be captured as CO_3^{2-} ions.

$$CO_2 + O^{2-} \rightarrow CO_3^{2-}$$

Then, CO_3^{2-} can be reduced to carbon as follows.

$$CO_3^{2-} + 4e^- \rightarrow C + 3O^{2-}$$

Many allotropes of carbon, for example, diamond [2], amorphous carbon, graphite, carbon nanotubes, and carbon nanofibers have been electrodeposited in molten salt. In this fiscal year, the electrodeposition of carbon from molten LiCl–KCl–K_2CO_3 was conducted at various potentials (0.6–1.0 V vs. Li^+/Li) and temperatures(773–973 K) to investigate the effect of electrolysis conditions on carbon deposition.

Fig. 1 shows the transitions of current density during electrolysis. The current density increased as the potential became negative. The photograph of a typical sample on a Ni substrate is shown in Fig. 2. The black deposits were attached to the substrate and the black powders were obtained after washing. Fig. 3 shows the Raman spectrum of the sample obtained at 0.80 V. Broad bands attributed to the D-band (around 1350 cm^{-1}) and G-band (around 1570 cm^{-1}) are observed. These spectra are specific to amorphous carbon, which has both sp^2 and sp^3 hybrid orbital. There is no significant difference in the electrodeposited carbon at the different potentials and temperatures.

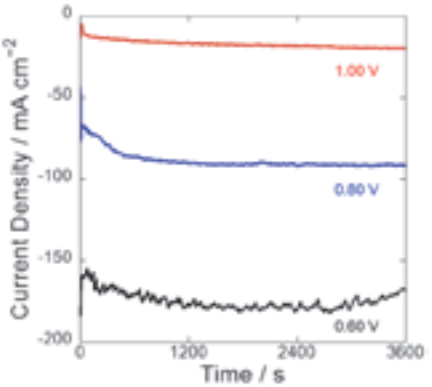

Fig. 1. Transitions of current density during the electrolysis at 0.60 V, 0.80 V, and 1.00 V in molten LiCl–KCl–K_2CO_3 at 973 K.

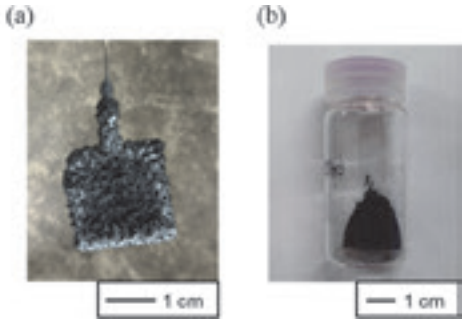

Fig. 2. Photographs of the sample electrodeposited by potentiostatic electrolysis at 0.6 V for 30 min at 873 K in molten LiCl–KCl–K_2CO_3. (a) As electrodeposited, (b) after washed.

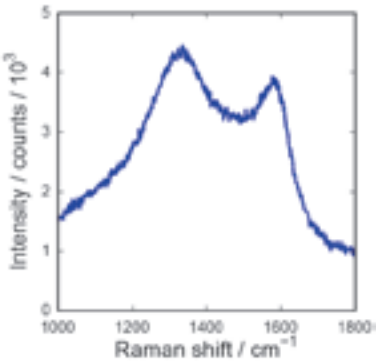

Fig. 3. Raman spectra of carbon electrodeposited at 0.80 V in molten LiCl–KCl–K_2CO_3 at 973 K.

Table 1 Current efficiencies in the electrodeposition of carbon in molten LiCl–KCl–K_2CO_3 at various electrolysis conditions.

	0.4 V	0.6 V	0.8 V
773 K	97%	94%	98%
873 K	89%	93%	91%
973 K	61%	71%	72%

Table 1 summarizes the current efficiencies of carbon deposition under various potentials and temperatures. High current efficiencies of 89–98% were achieved at 773 and 873 K. On the other hand, the current efficiencies tended to decrease at 973 K. This may be because, at 973 K, the current used for carbon monoxide generation instead of carbon deposition increased.

3. Novel Process for Carbon Fixation Reaction

RuBisCO enzyme, the most abundant enzyme on earth, catalyzes the primary CO_2 fixation reaction in plants, algae, and bacteria via the Calvin–Benson–Bassham (CBB) pathway. This enzyme catalyzes two competing reactions: (1) carboxylase reaction producing 3-phosphoglycerate (3-PGA) from ribulose 1,5-bisphosphate (RuBP), CO_2, and H_2O, and (2) oxygenase reaction adding O_2 to RuBP, resulting in 3-PGA and 2-phosphoglycolate. The oxygenase reaction reduces photosynthesis efficiency. Furthermore, RuBisCO is an enzyme that has low turnover efficiency.

As one of RuBisCOs, *T. kodakaraensis* KOD1, or *Tk*-RuBisCO enzyme shows extreme thermostability, high carboxylase activity, and specificity at a high-temperature range. This suggests that the enzyme's stable protein scaffold can tolerate higher degrees of mutations at ambient temperatures compared to other types. These outstanding properties make *Tk*-RuBisCO an attractive target for structure-function studies and protein engineering to improve CO_2 fixation activity.

By using the recombinant *Tk*-RuBisCO, an initial examination as to whether a *Tk*-RuBisCO could extend the substrate rather than RuBP. A *Tk*-RuBisCO was expressed in *E. coli* and used for CO_2 fixation activity after purification (Fig. 4). Activity was investigated and confirmed with two different substrates such as RuBP and ribulose 5-phosphate (Ru5P) via the NADH-linked spectrophotometric assays using GAPDH coupling enzymes for measuring RuBisCO activity.

Fig. 5 shows the enzymatic activities of *Tk*-RuBisCO that 3-PGA was produced. Along with the native RuBP substrate, significant levels of CO_2 fixation activity were observed as a result of the increase in 3-PGA production (Fig. 5a). On the other hand, the 3-PGA production detected from an alternative Ru5P substrate (Fig. 5b) was lower than that of the native substrate one. That could be a lack of affinity for Ru5P compared to RuBP. Thus, the activity could be observed only at a high concentration of Ru5P.

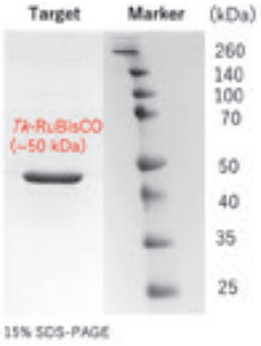

Fig. 4. SDS-PAGE analysis of *Tk*-RuBisCO.

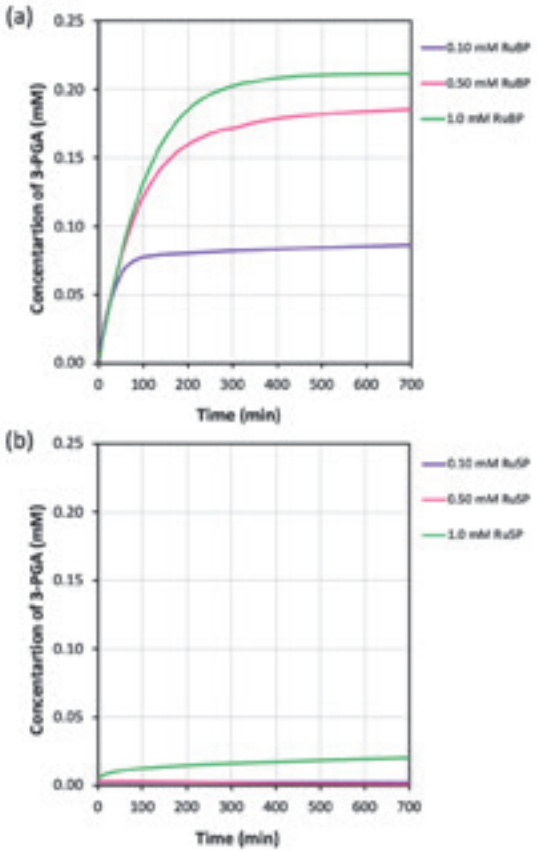

Fig. 5. The production of 3-PGA from CO_2 fixation reaction is catalysed by *Tk*-RuBisCO using native RuBP (a) and alternative Ru5P (b) substrates.

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