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Hofmeister effects on the glucose oxidase hydrogel-modified electrode

Aimi Suzuki, Seiya Tsujimura[1,*]

Division of Materials Science, Faculty of Pure and Applied Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8573, Japan

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ABSTRACT

We describe the consistent effect of salts in the electrolyte solution on glucose oxidation current production in the redox hydrogel-modified electrode containing glucose oxidase as an electrocatalyst and Os complex mediator. The ions affect not only on the electron transfer between the enzyme and the Os complex, but also on the hydrogel structure. This study found that the degree of the effect can be characterized by Hofmeister series. The relative decrease in oxidization current is the lowest in the middle of the Hofmeister series, and increases monotonically on either side. An increase of ionic strength inhibits the electron transfer from the active site of glucose oxidase to Os complex. In addition to this, the kosmotropic anions, which are strongly hydrated, caused hydrogel deswelling (shrinking). The more chaotropic an ion is, the more it adsorbs to uncharged parts of polymer/enzyme with dispersion force, and the swelling of the hydrogel decreases the catalytic current. This study impacts the design of hydrogel electrode and selection of electrolyte ions for bioelectronic applications.

1. Introduction

Redox hydrogel technology, pioneered by A. Heller in the 1990s, is still commonly used to immobilize redox enzyme and redox mediator jointly on an electrode surface [1–5]. They are immobilized on the electrode surface by electrostatic interaction and covalent cross-linking to form a hydrogel, in which the polymer–enzyme matrix stores the electrolyte solution. In hydrogel film, Os complexes tethered to polymer transfer electrons by electron hopping, so that the enzyme generates catalytic current efficiently regardless of its orientation. The technology allows fast and efficient electron transfer from the active site of the enzyme to the electrode surface through the Os complex coordinated to the polymer backbone, due to the highly concentrated enzyme and mediator, as well as the high self-electron exchange rate of the Os complex. This technology can be applied to bio-electrochemical devices such as biosensors [6], especially implantable continuous glucose monitoring systems and one-compartment type biofuel cells [7].

The reaction is easily affected by the ionic conditions in electrolyte solution. It has been reported that the current decrease as a function of NaCl concentration is caused by countering electrostatic interaction between the negatively charged enzyme and the positively charged redox polymer; and coiling occurs because of screening positive repulsion within the redox polymer itself [2,4,5,8]. However, we consider that this phenomenon could be explained not only by the effect of ionic strength based on the Debye-Hückel theory alone, but also by the complex interactions among (macro) molecules involving van der Waals force, hydrophobic/hydrophilic interaction, hydration of water and so on.

The objective of this study is to elucidate the consistent effect of electrolyte salts on the GOx hydrogel modified electrode reactions based on “Hofmeister effect”. In 1888, Hofmeister studied the effect of different salts on the aggregation of egg white proteins [9]. The Hofmeister series, which is the order of cations/anions in promoting the precipitation (salting-out) or the solubility (salting-in) of a protein in aqueous solution, has attracted considerable attention [10–14]. Anions appear to have stronger effect than cations and are usually ordered as follows, from the strongly hydrated “salting-out” to the more hydrophobic “salting-in” anions:

$$\text{Citrate}^{3-} > SO_4^{2-} > HPO_4^{2-} > F^- > Cl^- > Br^- > NO_3^- > ClO_4^-$$

A similar series for cations was also reported:

$$Mg^{2+} > Li^+ > Na^+ > K^+ > NH_4^+$$

The Hofmeister series is widely observed in the order of the ionic influence on chemical and biological phenomena associated with aggregation or structural change of macromolecules,

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[*] Corresponding author. Tel.: +81 29 853 5358; fax: +81 29 753 4490.
E-mail address: seiya@ims.tsukuba.ac.jp (S. Tsujimura).

[1] ISE member.

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especially on enzymatic stabilities and activities [11,14]. Recently, Carucci et al. reported that the effect of electrolyte on the electro-enzymatic reaction follows Hofmeister series, from observing the glucose oxidation current catalyzed by glucose oxidase (GOx) dissolved in buffer solution with the aid of dissolved ferrocene methanol mediator [15].

In this study, glucose oxidation catalytic reaction of electrode with redox hydrogel composed of GOx and a redox polymer, poly (1-vinylimidazole) complexed with [Os(bipyridine)$_2$Cl] was investigated. Various anions and cations were used to obtain the consistent effect of salts on the GOx hydrogel modified electrode.

2. Experimental

All chemicals used in this study were of analytical reagent grade and all solutions were prepared with distilled water. The biocatalyst solution was composed of GOx (from *Aspergillus niger*, Wako Pure Chemical, 40 mg mL^{-1}), the redox polymer poly(1-vinylimidazole) complexed with [Os(bipyridine)$_2$Cl] (PVI-Os, E° = 0.22 V vs. Ag|AgCl, 6 mg mL^{-1}), and a crosslinker, poly(ethylene glycol) diglycidyl ether (PEGDGE, molecular weight 500, Sigma–Aldrich, 8 mg mL^{-1}) at a mass ratio of GOx/PVI-Os/PEGDGE, 45:45:10. PVI-Os was synthesized according to an earlier report [2] and partially quaternized [16] to improve its water solubility. This biocatalyst solution was pipetted onto the glassy carbon surface, which was conferred with hydrophilicity by prior modification via plasma oxidation (10 min). The total hydrogel loading on the electrode surface was fixed at 600 μg cm^{-2}. After the modification, the electrode was dried at 4 °C for 18 h. The pH 7.0 electrolyte solution was prepared by mixing 0.1 M disodium hydrogen phosphate and sodium dihydrogen phosphate solutions containing 0.5 M specific salt. The pH of the electrolyte solution was checked before the electrochemical measurement.

Rotating-disc cyclic voltammetry (CV) was performed on an electrochemical analyzer (BSA 50 W). The electrode was rotated at 2000 rpm using a rotator (RDE-2, BAS). Platinum wire counter electrode and Ag|AgCl|KCl (sat.) reference electrode were used. All measurements were performed in phosphate buffer at 25 °C in a total volume of 20 mL. A scan rate of 20 mV s^{-1} was used in all experiments.

3. Results and discussion

3.1. Effect of anion on the GOx-hydrogel reaction

The effect of anions on the GOx hydrogel electrode was elucidated by comparing the rotating-disk CV results with and without various sodium salts. Curve (1) of Fig. 1(A) corresponds to phosphate buffer containing 0.2 M glucose, and displays a steady-state glucose oxidation current of 2 mA cm^{-2} above 0.3 V vs. Ag|AgCl. Subsequently, the electrode was washed with distilled water and dipped in phosphate buffer containing 0.2 M glucose and 0.5 M NaF. The observed steady-state current in curve (2) was 86% of the value in curve (1). It should be noted that this current decrease did

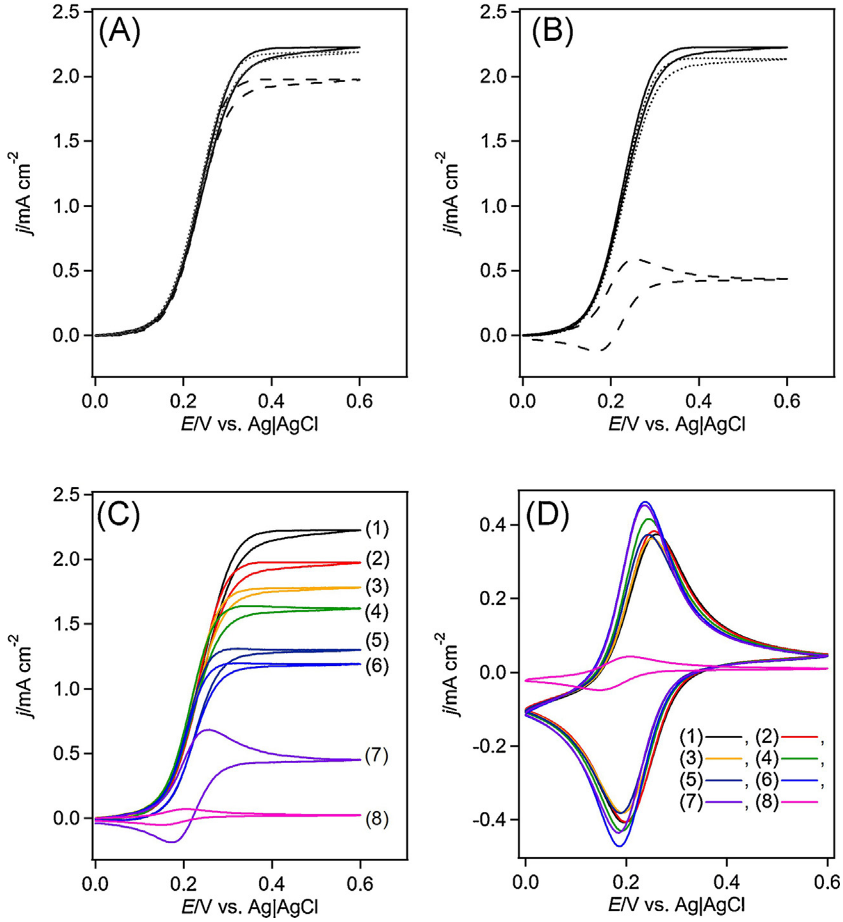

Fig. 1. (A, B) Cyclic voltammograms for glucose oxidation performed sequentially on the same electrode in phosphate buffer with 0.2 M glucose (solid curve), with 0.5 M NaF (A, dashed curve) or 0.5 M NaNO$_3$ (B, dashed curve), and without addition of salts again (dot curve). (C, D) Cyclic voltammograms performed in phosphate buffer with 0.2 M glucose (C) and without glucose (D) in the presence of 0.5 M various sodium salts; (1) base solution without additive salts, (2) fluoride, (3) phosphate,(4) sulphate, (5) citrate, (6) chloride, (7) nitrate, (8) perchlorate.