Electrochemical Protein Immobilization through Genetical Introduced Tag

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Protein immobilization technique has been very much required for utilization of functional proteins. Most of these methods are based on either chemical covalent bond or affinity assembly. However, most of them are not able to control both molecular orientation and detachability. The author found that the metal coordinated histidine homo peptide can be immobilized electrochemically on an electrode surface. In this paper, we investigate the novel method of controllable protein immobilization using genetical introduced ECtag.

In the experiment, the peptidic ligand (hexa histidine; EC tag sequence) is synthesized and is tagged to protein structural sequence on a plasmid. Protein A (B subunit) is employed as model protein for immobilization. It is tagged to the hexa histidine sequence (ECtag sequence) on plasmid pkkB5. The plasmid is transformed into *E. coli* BL21 and is expressed. The purified polyhistidine-protein A is dissolved in 1ml of phosphate buffer at a final concentration of 0.3 mg /ml. Nickel chloride added into the protein solution at a final concentration of 200 mM in order to form Ni^{2+}: hexa His-Protein A complex. Then free nickel ion was removed from bulk solution by dialysis against phosphate buffer (0.1M, pH 7.4). Electrochemical immobilization/removal of EC tag tagged protein is controlled by applied potential to the micro electrode. After electrochemical immobilization of ECtag-molecule, the micro electrode is washed and sonicated in buffer solution. And then it is analyzed by enzymatic luminescence assay in order to determine the amount of immobilized ECtag-protein A molecule.

In the results, electrochemical immobilization of ECtag-protein A is performed by controlled potential application on a platinum disc microelectrode. Amount of immobilized ECtag-protein A is increased when cathodic current is applied (Fig.1). In the other hand, immobilization is never done when higher potential than -200 mV vs. AgAgCl is applied. Oxidation-reduction potential of Ni^{2+} ←→ Ni is at about -200 mV in neutral pH condition. Therefore, Ni^{2+} reduction to Ni is only performed when -200 mV or lower potential is applied. The result indicated that the immobilization of ECtag-protein A in only observed when a reducing potential of Ni^{2+} is applied. Figure 2 shows a passed current profile when immobilization of ECtag-protein A is performed by application of -200 mV for 5 min. In the case of cathodic potential application to the micro electrode in Ni^{2+} coordinated ECtag-Protein A solution, remarkable cathodic faradaic current is clearly observed. In the contrast, any remarkable passed current is not observed in ECtag-Protein A (without Ni^{2+} coordination) solution. The passed cathodic faradaic current indicates that the Ni^{2+} is reduced to Ni in ECtag ligand on an electrode surface. In the state, ECtag-Protein A is immobilized on an electrode surface (Fig.2B). The immobilized ECtag-protein A is detached from an electrode when +300 mV potential is applied. In that time, anodic oxidation current is observed (Fig.2C). Further investigation as for the novel molecular immobilization will be presented.

All the results indicates that a neutral Ni:hexa histidine complex is formed on a solid electrode surface electrically as:

$$\text{ECtag} : Ni^{2+} + e^- \leftrightarrows \text{ECtag} : Ni \text{ (on an electrode)}$$

In the present study, it is clear that the ECtag and its fused molecule can be immobilized on an electrode surface reversibly through electrochemical reaction. The technique can be applied for either biosensor, protein chip or other protein application. Formation of stable neutral metal:N complex will probably be an attractive reaction, because the novel reversible immobilization procedure can be done through genetically introducible tag. It will be applied for practical molecular modulation.

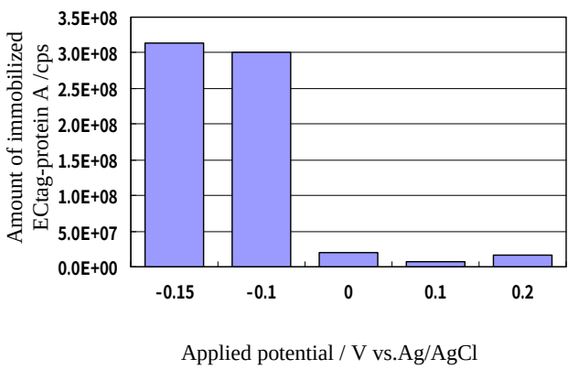

Fig.1 Applied potential dependent of immobilized EC tag-Protein A.

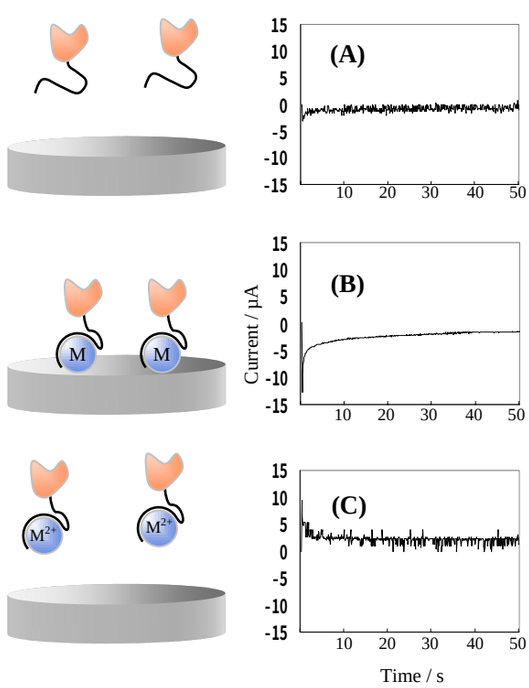

Fig.2 Passed current profile on electrolysis of ECtag tagged protein A. (A); -300 mV applied to the Pt micro electrode in ECtag-Protein A solution without Ni^{2+} coordination. (B); -300 mV applied to the Pt micro electrode in ECtag-Protein A solution with Ni^{2+} coordination. (C); +300 mV applied to the ECtag-Protein A immobilized Pt micro electrode (prepared in B) in buffer solution.