Electrochemical denature of urease by adsorption to the carbon electrode surface

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It is well known that some enzymes can be denatured by adsorption to the electrode surface such as carbon. However, the effect of the electrode potential on the extent of denature has not been clarified. In this research, the apparent numbers of electrons involved in the electrooxidatin of cysteine residues of urease have been measured by controlled potential coulometry using concentration-step method. The schematic structure of coulometric cell used in this experiment was described earlier [1]. The oxidation of SH groups can be performed at carbon electrode [2[. The oxidation current of enzyme involves not only the oxidation currents of thiol groups but also those of other oxidizable groups. Maleimide monomer can make only thiol groups electroinactive by the reaction of maleimide group and thiol group.

Coulometry of both native urease and maleimide reacted urease were carried out, and the effect of the electrode potential on the difference between the quantities of electricity flowed during the complete electrolyses of native urease and maleimide-bonded urease are shown in Fig.1. This result indicates that two SH groups located near surface of urease are oxidized to cysteinic acid (napp is 6 per one molecule of urease) at lower potential range below + 0.56 V because napp reached to 12, and it can be recognized that the additional oxidation of 10 SH groups to cysteinic acid are abruptly observed at a time at about + 0.6 V vs. SCE. This abrupt increase of electroactive SH groups suggests that the denature of urease takes place by electric energy. At the same potential range, the hydroxylase activity of urease was completely decreased and this fact supports the occurrence of electrochemical denature of enzyme based on oxidation of SH groups to strong anionic cycteinic acid. The oxidized urease did not exhibit the original hydroxylase activity. Then, the destroyed conformation of urease is not restructured. The change of hydroxylase activity by electric potential was measured by combining carbon felt electrode and ammonia electrode. The hydroxylase activity of adsorbed urease can be monitored by ammonia electrode response based on formation of ammonia from hydrolyzed urea. Fig. 2 shows the current vs. coulomb (I vs. Q) curves obtained by the coulometric oxidation of unease, and the further oxidation rate of SH groups is much slower than the oxidation rate of initial two SH groups, this difference reflects the feasibility of contact to the electrode surface.

[1] S.Uchiyama, et al Anal. Chem., 60, 1835 (1988)
[2]S.Uchiyama, et al., Electroanalysis 14, 1644 (2002)

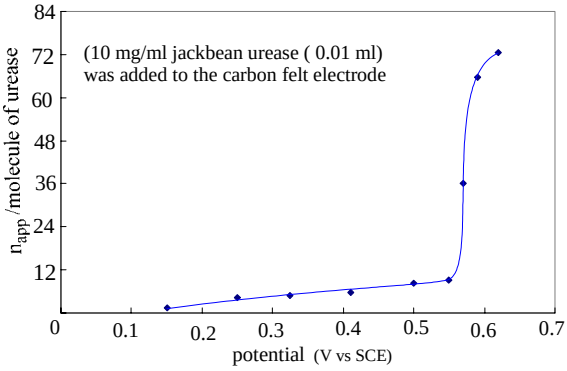

Fig.1 Effect of the electrode potential on the apparent electron number of oxidation of urease

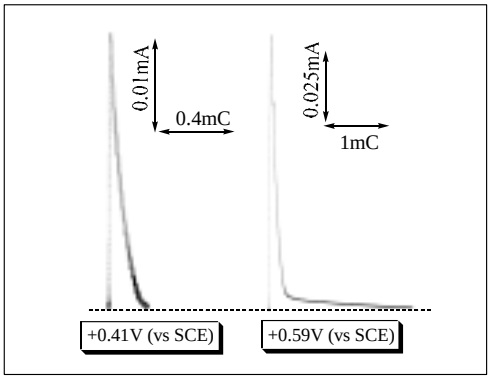

Fig.2 Current vs coulomb curves obtained by the oxidation of urease at each potential