ENZYMIC INVESTIGATION OF SURFACTANT MICELLES BY MEANS OF TIME RESOLVED ANALYSIS: INFLUENCE OF SURFACTANT ON THE ACTIVITY OF GLUCOSE OXIDASE**KAZUO MIYAMURA, MASUHIRO OKADA and YOHICHI GOHSHI**

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Abstract - Deactivation of glucose oxidase activity by cationic surfactants, cetyltrimethylammonium bromide (CTAB), cetyltrimethylammonium chloride (CTAC), and dodecyltrimethylammonium bromide (DTAB) are investigated. In the concentration region below critical micellar concentration (cmc) of CTAB, the addition of CTAB increased the inhibition rate, while in that above cmc, the inhibition rate decreased. Addition of large amounts of nonionic surfactant to CTAB solution drastically reduced the inhibiting effect of CTAB.

Keywords enzyme, surfactant, inhibition, cmc

Enzymes have an outstanding selectivity towards various organic compounds as compared to synthetic chemical reagents[1]. Owing to their high selectivity, enzymes are now commonly used as highly selective analytical reagents for organic compounds in the field of clinical analysis[2]. Enzyme electrodes, which uses immobilized enzymes, are also used widely in clinical analysis[3].

In some cases, surfactants are added to stabilize the solution of biomolecules[4]. However, surfactants are known to inhibit some enzymic reactions[4,5]. Therefore the effect of surfactants present in solution towards the enzymes should be considered before applying enzymic analysis to the sample. This effect might become serious for immobilized enzymes, because the surfactant molecules tend to associate at the interface between the immobilized membrane and the sample solution.

We started our investigation of the inhibiting effect of the surfactant towards enzymes with the common surfactants, sodium dodecylsulphate (SDS) and CTAB. The enzyme, glucose oxidase obtained from *Aspergillus niger*, which is commercially available was also used. The anionic surfactant SDS, which is reported to be highly inhibiting for many enzymic reactions[4], did not exhibit any inhibiting effect but the cationic surfactant CTAB was highly inhibiting. In this paper, we report the CTAB concentration dependence of the enzyme inhibition rate (or rather the deactivation rate), using time resolved analysis. A method used to avoid the inhibiting effects of the surfactant molecules is also presented.

EXPERIMENTAL

Materials The glucose oxidase (*Aspergillus niger*, Lot AS01) was commercially available from Tokyo Kasei Kogyo Co., and used without further purification. The nonionic surfactant used is polyethyleneglycohol mono-p-nonylphenyl ether [$\text{HO}(\text{CH}_2\text{CH}_2\text{O})_5\text{C}_6\text{H}_4\text{C}_9\text{H}_{19}$] supplied also from Tokyo Kasei Kogyo Co. All other reagents were commercially available as reagent grade.

Preparation of Phosphate Buffer Solution and Glucose Oxidase Solution The phosphate buffer solution is prepared by dissolving 3.55 g of anhydrous disodium hydrogenphosphate and 3.35 g of potassium dihydrogenphosphate in 1 dm³ of water. The solution is oxygen saturated by bubbling air for 5 hr. The glucose oxidase ca. 20 mg is dissolved in 20 cm³ of phosphate buffer solution.

Measurement of Enzyme Activity, and Deactivation Rate Calculation The β -D-glucose (0.25 g) is dissolved into the oxygen saturated phosphate buffer solution (99 cm³). The surfactant (CTAB, DTAB, CTAC, and/or nonionic surfactant), MeTAB or KBr is added to the solution, and allowed to reach 25°C.

About 20 sec after the start of the measurement of the dissolved oxygen concentration, 1 cm³ of glucose oxidase solution is added to the glucose solution with stirring. The oxygen concentration data taken at the rate of 1 datum/sec for 2048 sec are then stored in a floppy diskette. The derivative calculation is performed by the Savitzky-Golay method[6] on an NEC PC-9801 personal computer.

Apparatus The dissolved oxygen concentration was measured by Toa DO-1B dissolved oxygen meter. The signal is A/D converted (12 bit A/D) and the data is sent to 8 bit computer Panasonic A1F. The reaction is carried out at 25°C using an Eyela UA-110 thermostat.

RESULTS AND DISCUSSION

Deactivation of Glucose Oxidase by Cationic Surfactants The glucose oxidase catalyzes the following reaction of β -D-glucose with dissolved oxygen to form gluconic acid and hydrogen peroxide[7].

By this reaction, the dissolved oxygen is consumed at a rate proportional to the glucose concentration. This principle is used to analyse the substrate concentration by the enzyme electrodes. Thus the consumption rate of the dissolved oxygen is a function of the glucose, oxygen, and enzyme concentration. The consumption rate of glucose equals that of oxygen, and is stated as follows:

$$d[\text{Glucose}]/dt = d[\text{O}_2]/dt = k[\text{Glucose}][\text{O}_2][\text{Enzyme}]$$

The use of a dissolved oxygen sensor enables one to measure the concentration and consumption rate of oxygen. Thus one can evaluate the activity of the enzyme as follows:

$$k[\text{Enzyme}] \propto (1/[\text{Glucose}])(1/[\text{O}_2])d[\text{O}_2]/dt$$

In Figure 1-a are shown typical oxygen consumption curves with the corresponding $(1/[\text{O}_2])d[\text{O}_2]/dt$ curves (derivative curve) for $[\text{Glucose}] \gg [\text{O}_2]$. At the initial stage of the reaction, the absolute value of the latter curves should be proportional to the glucose concentration. In Figure 1-b is plotted the initial $(1/[\text{O}_2])d[\text{O}_2]/dt$ value versus the glucose concentration. The curve is not linear indicating a slight saturation of the enzymic reaction at this concentration region. The following experiments, however, were carried out at a fixed glucose concentration of 2.5 g·dm⁻³ so as to obtain reproducible results.

Figure 1 Glucose concentration dependence of oxygen consumption due to enzymic reaction of glucose oxidase.

a: Oxygen consumption curve

b: Consumption rate vs. glucose concentration

When the cationic surfactant, cetyltrimethylammonium bromide (abbreviated as CTAB) is added to the solution, the oxygen consumption rate is found to decrease. In Figure 2-a are shown the oxygen consumption curves with the derivative curves with CTAB present at various concentrations. The slope of the latter curve shows that the consumption of the dissolved oxygen decreases. Therefore the slope corresponds to the inhibition rate of glucose oxidase. The slope increases as the CTAB concentration increases, indicating the elevated inhibiting (or rather deactivating) effect of CTAB towards glucose oxidase at high concentration.

In order to determine whether the bromide anion or the CTA cation is responsible for this inhibition, the effect of cetyltrimethylammonium chloride (CTAC) and KBr is examined. KBr did not affect the oxygen consumption rate while CTAC inhibited the reaction considerably. This observation indicates that it is the CTA cation and not the bromide anion that inhibits the reaction.

The effect of the alkyl chain length was also investigated by using tetramethylammonium bromide (MeTAB) and dodecyltrimethylammonium bromide (DTAB). MeTAB did not inhibit the reaction, while DTAB slightly inhibited the glucose oxidase reaction. This result demonstrates that the presence of long alkyl chains such as the cetyl group in the cationic surfactant is responsible for the glucose oxidase deactivation.

CTAB Concentration Dependence of Deactivation It was shown that CTAB inhibits the reaction of glucose oxidase drastically. By measuring the dependence of the slope in the derivative curve, one can determine the CTAB concentration in solution. In the concentration region from 0 to $0.1 \text{ g}\cdot\text{dm}^{-3}$, the slope of the derivative curve increases almost proportionally to the CTAB concentration as can be seen in Figure 2-a.

In the high concentration region of CTAB, however, the slope of the derivative curve tends to decrease as the CTAB concentration increases (Figure 2-b). This inhibition is most drastic at a CTAB concentration of ca. $0.50 \text{ g}\cdot\text{dm}^{-3}$ and additions of CTAB at this point decreases the inhibiting effect towards the glucose oxidase. This dependence is opposite to that reported for other surfactants[5]. The reported value of the critical micellar concentration (cmc), which is the concentration at which the surfactant begins to aggregate and form micelles, of CTAB in aqueous solution at 25 C is $0.34 \text{ g}\cdot\text{dm}^{-3}$ ($9.2 \times 10^{-4} \text{ mol}\cdot\text{dm}^{-3}$)[8]. Although the cmc of CTAB may vary according to the experimental conditions (i.e., phosphate salts, glucose, and enzymes are added in this case), the change in the CTAB concentration dependence of the enzyme inhibition rate occurs very close to the cmc concentration.

Figure 2 Surfactant (CTAB) concentration dependence of the inhibition of the enzymic reaction of glucose oxidase, a: at relatively low CTAB concentration, b: at relatively high CTAB concentration

This fact strongly suggests that the inhibition occurs mainly by the monomeric CTA cation and not or weakly by the CTAB micelle. Therefore the effect of the CTAB micelle is either that it protects the enzyme from attack by the CTA cation or that it decreases the effective concentration of the monomeric CTA cation, responsible for the inhibition. This phenomenon reminds us of the old Japanese proverb "Cure the Poisoned with Poison". It is known that certain cationic surfactants exhibit a protecting effect instead of inhibiting effect towards certain enzyme[9]. Therefore the phenomenon observed for CTAB can be understood as the result of both protecting and inhibiting ability of the CTAB towards glucose oxidase.

The result implies that the protecting effect of any micelle can be used to decrease the inhibiting ability of CTAB. In Figure 3 is shown the effect of $1 \text{ g} \cdot \text{dm}^{-3}$ of nonionic surfactant, which does not seriously inhibit the reaction, added to the solution with $0.2 \text{ g} \cdot \text{dm}^{-3}$ of CTAB. A drastic recovery of the glucose oxidase activity is observed. Therefore, the micelles of nonionic surfactant formed either remove the monomeric CTA cation or protect the glucose oxidase from attack by the CTA cation.

Figure 3 Protective effect of nonionic surfactant towards enzymic reaction of glucose oxidase

It was shown that the addition of non- or less-inhibiting surfactants with the concentration above cmc excluded the inhibition effect of the surfactants. The activity of the enzyme in the presence of substrates, impurities, etc. in high concentration is still an unresolved matter[10]. Although this phenomenon has been studied only for the glucose oxidase, we suggest that a similar examination should be made for other enzymic reactions.

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