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A REVERSIBLE AND SPECIFIC MULTIPLE BINDING OR ADSORPTION OF DITHIONITE ANION ON CATIONIC MICELLAR SURFACE

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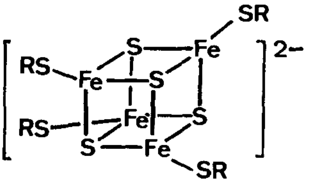
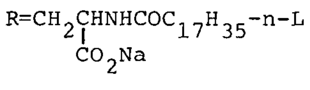
Iron-sulfur cluster incorporated into CTAB micelles was reduced by sodium dithionite at pH 8.5. Dependency of the observed rate constants on dithionite concentrations showed that the electron transfer reaction from dithionite ($SO_2^{-\bullet}$) bound on CTAB micelles to iron-sulfur cluster was much faster than that from dithionite ($SO_2^{-\bullet}$) in the bulk aqueous solution. From the detailed analysis of the kinetic and equilibrium data, the reaction parameters were determined as 19 ± 7, 1300 ± 400 M^{-1} and 2200 ± 300 $M^{-1/2}\cdot s^{-1}$ for the number of binding sites, binding constant and specific reduction rate constant respectively.

Recently, micelles, artificial liposomes and similar microheterogeneous systems have attracted much attention. This is mainly due to the fact that structure elucidation of the microheterogeneous ordered aggregates has made a great progress in these days. Small angle X-ray spectroscopy1), light scattering method2) with satisfactory precision, electron microscopy3) with much improved capacity, etc., have been applied to these microheterogeneous systems. The precise structure elucidation necessarily enhances understanding of often unique physical properties and chemical reactions of the oredered aggregates. During the course of the study on micelles modified with iron-sulfur cluster4), we happened to find out saturation kinetics for dithionite ($S_2O_4^{=} \rightleftarrows 2\cdot SO_2^{-\bullet}$)5).

Now we wish to report that dithionite was reversibly and specifically bound or adsorbed on the the cetyltrimethylammonium bromide (CTAB) micellar surface. The data obtained here are important not only for the analysis of the electron transfer reactions catalyzed by the iron-sulfur cluster but also for the understanding the (multiple)ion-exchange equilibrium on the surface of micelles6).





$$R{=}CH_2\underset{\displaystyle CO_2Na}{CH}NHCOC_{17}H_{35}\text{-n-L}$$

1

Fig. 1. The artificial iron-sulfur cluster.

Artificial iron-sulfur cluster, 1, was prepared and incorporated into CTAB micelles according to the previous report4) (see Fig. 1). The reduction rates of 1 in CTAB micelles at a variety of dithionite concentrations were followed by the decrease of absorption intensity at 400 nm of 1. To 2 mL of aqueous micellar solution of 1 in a quartz cell was added 0.4 mL of dithionite solution by a "syringe stopped flow" method7) (final concentrations were ; **1** : 3×10^{-5} M, $S_2O_4^{=}$: 0.25 - 8 $\times 10^{-3}$ M, CTAB : 5 mM, Tris-Cl : 100mM) pH 8.5 at 25°, where all solutions were prepared in a carefully deoxygenated dry box. The sodium dithionite solutions were titrated and standardized by the $K_3Fe(CN)_6$ method.

The reactions followed clear pseude first order kinetics and a saturation of the reduction rate constant ($k_{red.}$) at high dithionite concentration was observed as shown in Fig. 2. The maximum value of $k_{red.}$ was determined to be 0.36 ± 0.02 s^{-1}. Since the multiple equilibrium for binding of dithionite anion may be reasonably assumed (see eqn. 1,2), the theory developed by Edsall and Tanford8) for the noncooperative multiple binding was applied to the analysis of present kinetic behaviours, e.g.,

$$M(\mathbf{1}^{ox})D_{i-1} + D \xrightleftharpoons{K_i} M(\mathbf{1}^{ox})D_i \quad (i = 1,2, \ldots n) \qquad \text{eqn } 1^{9)}$$

$$M(\mathbf{1}^{ox})D_i \xrightleftharpoons{K_{diss.}} M(\mathbf{1}^{ox})(SO_2^{-\cdot})_{2i} \xrightarrow{2ik} M(\mathbf{1}^{red.})(SO_2^{-\cdot})_{2i-1} \qquad \text{eqn } 2$$

$$k_{red.} = kK_{diss.}^{1/2}(nKD_f/1 + KD_f)^{1/2} \qquad \text{eqn } 3$$

$$D_f = ((KD_o - nKM_t - 1) + ((KD_o - nKM_t - 1)^2 + 4KD_o)^{1/2})/2K \qquad \text{eqn } 4^{10)}$$

where k; single reduction rate constant ($M^{-1/2} \cdot s^{-1}$), n; number of binding sites, K; single equilibrium constant (M^{-1}), D_f; free [$S_2O_4^{=}$] (M), D_o; initial [$S_2O_4^{=}$] (M), M_t; total [micelle] (M) and K_{diss}; dissociation constant for the equilibrium of dithionite5). The value of M_t in the present condition is calculated to be 6.7 $\times 10^{-5}$ M by assuming the aggregation number and CMC to be 61 and 0.9 mM respectively11). Then, by use of non-linear curve fitting method, the kinetic parameters were determined to be; n = 19 ± 7, K = 1300 ± 400 M^{-1}, k = 2200 ± 300 $M^{-1/2} \cdot s^{-1}$ (Fig. 2).

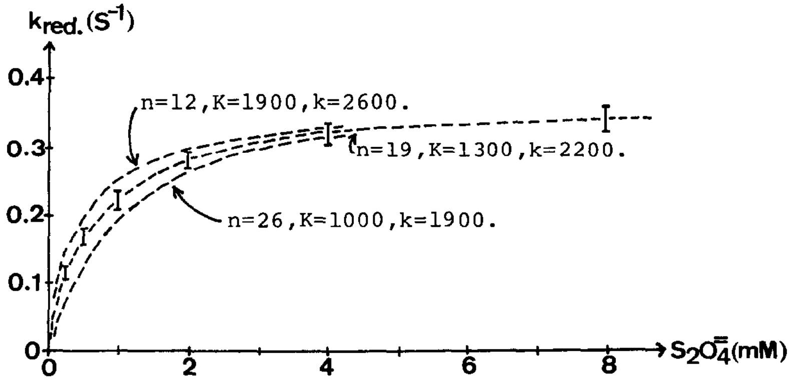


Fig. 2. Reduction rates of **1** by $S_2O_4^{=}$ (SO_2^{-}).

1 3×10^{-5}M, 5 mM CTAB, 100 mM Tris-Cl, pH 8.5 at 25°C under Ar.

I observed points, ------- simulation curves.

Thus, the present results clearly show that the limited binding sites of micelles for dithionite ion are available for the reduction of **1** incorporated into micelles, and the electron transfer reaction from the surface bound or adsorbed dithionite ($SO_2^{-\cdot}$) to **1** are much faster than the direct (collisional) electron transfer reaction from exterior aqueous dithionite ($SO_2^{-\cdot}$). The order of magnitude of nK is also confirmed by independent experiments of static adsorption of dithionite on the CTAB micelles. As shown in Fig. 3, the absorption λmax at 316.0 nm of ca. 2×10^{-5} M of $S_2O_4^{=}$ 12) was found to shift by the addition of the CTAB micelles and the largest redshift of 317.6 nm was observed in the presence of large excess of micelles (over 2.5×10^{-4} M). The double reciprocal plots of the Δλmax values toward the concentration of micelles give the nK value to be $(1.0 \pm 0.5) \times 10^4$ M^{-1} 13), which is in fairly good agreement with that obtained from the kinetic analysis (nK = (2.5 ± 1.5) 10^4 M^{-1}). The present observation of the pre-equilibrium behaviour in the dithionite reduction of the iron-sulfur cluster in CTAB micelles lead to the expectation of the inhibition by other anions, as is often observed in the electron transfer reaction of enzymes with inorganic electron donors or acceptors 14). Actually, the reasonable inhibition was observed when another anion was added to the present **1**-CTAB-$S_2O_4^{=}$ systems. For the typical examples, the reduction rate (0.22 ± 0.02 S^{-1}) of **1** at 1 mM of $S_2O_4^{=}$ was decreased to (0.10 ± 0.02 S^{-1}) by the addition of $S_2O_3^{=}$ (at $S_2O_4^{=}$ 1mM, $S_2O_3^{=}$ 50 mM).

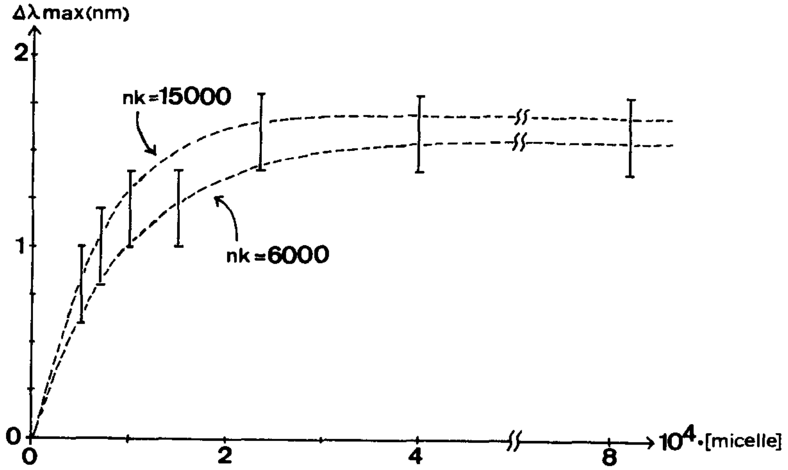


Fig. 3. The shifts of absorption maximum of $S_2O_4^{=}$ against the micellar concentration, $S_2O_4^{=}$ 2×10^{-5}M, 100 mM Tris-Cl, pH 8.5 at 25° under Ar.

In conclusion, CTAB cationic micellar surface has limited multiple binding sites for $S_2O_4^=$ anion, which number (n) was obtained to be 19 ± 7 from saturation kinetics of electron transfer from micellar surface bound or adsorbed $S_2O_4^=$ to micellar interior cluster **1**. The electron transfer reaction *catalyzed* by **1** is now under way.

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