Regulatory role of surfactants in the kinetics of glucose oxidase-catalyzed oxidation of D-glucose by ferrocenium and *n*-butylferrocenium ions

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The effect of micelles of different surfactants (cationic, anionic, and neutral) on the kinetics of the glucose oxidase-catalyzed reduction of ferrocenium cations RFc^+ (R = H, Bu^n) by D-glucose was studied by spectrophotometry. In micellar media of Triton X-100 and sodium dodecyl sulfate (SDS), the Michaelis dependence of the reaction rate on the HFc^+ concentration is observed, while this dependence has an extreme character in cationic micelles of cetyltrimethylammonium bromide (CTAB). The nature and concentration of surfactants of all types have a slight effect on the rate of reduction of HFc^+. The level of enzymatic activity is approximately equal in the case of Triton X-100 and CTAB and is considerably lower in the SDS micelles. On going from HFc^+ to Bu^nFc^+, the reaction rate is maximum in the cationic CTAB micelles, the anionic SDS micelles exhibit almost no activity, and the activity has an intermediate value in neutral micelles of Triton X-100. The conditions are presented under which the micellar medium controls the catalytic activity of glucose oxidase with respect to ferrocenium cations.

Key words: surfactant, sodium dodecyl sulfate, cetyltrimethylammonium bromide, Triton X-100, alkylferrocenium cations, glucose oxidase, kinetics, catalysis, micellar effects.

Recently, the question on the specificity of biocatalysts, including redox enzymes in reactions with substrates of inorganic and organometallic nature, has attracted increasing attention.[1] The reactions of glucose oxidase (GO) with ferrocenium and alkylferrocenium cations (RFc^+) have been studied previously.[2—5] The biological function of GO is the oxidation of β-D-glucose to D-gluconolactone accompanied by the reduction of the enzyme, which is then reoxidized by oxygen[6]

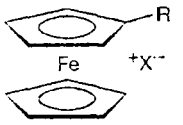
$$GO(ox) + \beta\text{-D-glucose} \rightarrow GO(red) + \text{D-gluconolactone}, \quad (1)$$

$$GO(red) + O_2 \rightarrow GO(ox) + H_2O_2. \quad (2)$$

Inorganic and organometallic substrates are capable of efficiently reoxidizing the reduced form of GO. In the case of ferrocenium at stage (2), the two-electron oxidation of GO(red) by the RFc^+ cations is observed

$$GO(red) + 2\ RFc^+ \rightarrow GO(ox) + 2\ RFc + 2\ H^+, \quad (3)$$

and the overall rate of the two-electron process limits the transfer of the first electron to RFc^+. The reaction occurs in the presence of dioxygen, which has almost no effect on the rate of this process (see Ref. 5 and cited herein). We have studied in detail[5] the kinetics of the GO-catalyzed oxidation of β-D-glucose by ferrocenium hexafluorophosphate **1**



1: R = H, X = PF_6;
2: R = Bu^n, X = PF_6

and the "ping-pong" type mechanism of the process, which is typical of oxidases, has been proposed on the basis of the data obtained:

$$GO(ox) + \beta\text{-D-glucose} \rightleftharpoons \{GO(ox),\beta\text{-D-glucose}\} \quad (k_1, k_{-1}), \quad (4)$$

$$\{GO(ox),\beta\text{-D-glucose}\} \rightarrow GO(red) + \text{D-gluconolactone} \quad (k_2), \quad (5)$$

$$GO(red) + RFc^+ \rightleftharpoons \{GO(red),RFc^+\} \quad (k_3, k_{-3}), \quad (6)$$

$$\{GO(red),RFc^+\} \rightarrow GO(ox) + RFc \quad (k_4). \quad (7)$$

Translated from *Izvestiya Akademii Nauk. Seriya Khimicheskaya*, No. 10, pp. 1795—1801, October, 1997.
1066-5285/97/4610-1700 $18.00

Kinetic Eq. (8) corresponds to the proposed mechanism (4—7)

$$v = \frac{k_2 k_4 [E]_o [Gl]_o [HFc^+]_o}{k_4 K_m^{Gl} [HFc^+]_o + k_2 K_m^{Fc} [Gl]_o + (k_2 + k_4)[Gl]_o [HFc^+]_o}, \quad (8)$$

where $[E]_o$, $[Gl]_o$, and $[HFc^+]_o$ are the overall concentrations of GO, D-glucose, and ferrocenium cation, respectively; $K_m^{Fc} = (k_4 + k_{-3})/k_3$ and $K_m^{Gl} = (k_2 + k_{-1})/k_1$. In this work, the effect of different surfactants, which are usually added to a solution to prevent the precipitation of the reaction product (water-insoluble ferrocene), on the run of this reaction was studied. However, this is not the only effect of surfactants.

When the system contains a surfactant capable of micelle formation, a sufficiently hydrophobic substrate can bind to micelles resulting in a change in the enzyme activity with respect to ferrocenium cations. In the case of ferrocenium cation **1**, great affinity to the hydrophobic core of the micelle is improbable; however, this possibility cannot be ruled out for alkylferrocenium cations of type **2**. When the {alkylferrocenium—micelle} associate is considered, the diphilic nature of the alkylferrocenium molecule should be taken into account. Its hydrophobic alkyl "tail" can bind to the hydrophobic core of the micelle. Hydrophilic ferrocenium (carrier of the "enzymatic activity) can be located either in the near-surface layers of the micelle or outside the micelle (Fig. 1). Since GO is negative for the commonly used values of pH 6—7 (the isoelectric point of the protein is equal to 4),[6] it can be expected that the activity of the enzyme at stage (3) depends on the micellar charge. In the case of a positively charged surfactant, the rate of reduction of ferrocenium ions by the enzyme can be maximum due to purely electrostatic reasons, even taking into account that the positive charges of both the micelle and enzyme (see Fig. 1) are

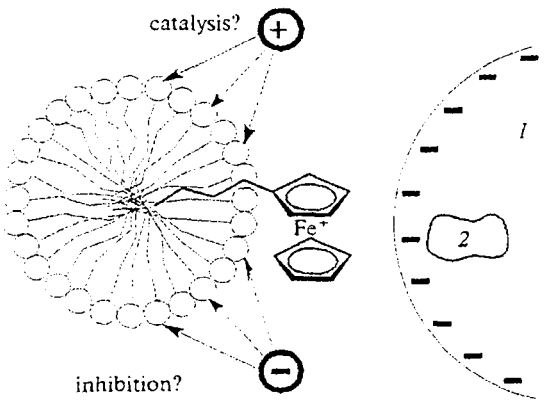


Fig. 1. Schematic representation of the surfactant molecule with the solubilized *n*-butylferrocenium molecule and the possible mechanism of the electrostatic effect of this associate on the effectiveness of stage 3: *1*, glucose oxidase; *2*, flavin adenine dinucleotide.

60—70% neutralized by counterions.[7] In the case of a negatively charged surfactant, a decrease in the reaction rate can be expected. In this work, the effect of cationic CTAB micelles, anionic SDS micelles, and neutral micelles of Triton X-100 on the kinetics of the GO-catalyzed reduction of ferrocenium and *n*-butylferrocenium cations by β-D-glucose was studied to verify these assumptions. Since kinetic Eq. (8) describing the enzymatic reaction[5] is complicated, the micellar effects were mainly analyzed by the Michaelis—Menten equation.

Experimental

Instruments and materials. The following reagents were used: surfactants SDS (Fluka), CTAB (Fluka), and Triton X-100 (Sigma); salts for preparing buffer solutions KH_2PO_4 and $Na_2HPO_4 \cdot 12H_2O$ (Reakhim); D-glucose (Reakhim), GO from *Aspergillus niger* (SU 1.1.3.4) with activity 220 unit mg^{-1} (Serva), ferrocene, *n*-butylferrocene, and potassium hexafluorophosphate (Aldrich). Ferrocenium (**1**) and *n*-butylferrocenium hexafluorophosphates (**2**) were synthesized according to the previously described procedure.[8,9] Kinetic studies were carried out on Specord M-40 and Shimadzu U-160A spectrophotometers. A BO detector (Estonian Republic) was used to study the effect of surfactants on dioxygen absorption in the presence of GO and D-glucose.

Preparation of ferrocenium solutions. Solutions of HFc^+ were prepared to study the dependences of the rate of the GO-catalyzed reduction of HFc^+ by D-glucose on the concentration of the surfactant (Triton X-100, CTAB, and SDS). Ferrocene (0.2 g, $1 \cdot 10^{-3}$ mol) was oxidized by atmospheric oxygen in conc. H_2SO_4 (5 mL) and let to stand over 1 h. Then water (20 mL) was added according to the known procedure,[8] and the resulting solution was brought to standard experimental conditions. For this purpose, the solution (2 mL) was added to a 0.01 *M* phosphate buffer (20 mL, pH 6.7), and the pH of the resulting solution was brought to 2.1 (pH of the solution when **1** is dissolved in $5 \cdot 10^{-3}$ *M* HCl). This method for generation of HFc^+ is required instead of using the synthesized salt **1**, because in the presence of compound **1** and CTAB, a precipitate is formed (presumably cetyltrimethylammonium hexafluorophosphate).

Kinetic measurements. The corresponding amount of a 0.01 *M* phosphate buffer (pH 6.7) containing a surfactant, a solution of ferrocenium ions (0.05—0.6 mL) prepared according to the aforementioned method, a solution of glucose (0.18 mL, 0.7 *M*), and a solution of GO (0.02 mL, $6.25 \cdot 10^{-6}$ *M*) were added to a spectrophometric cell with an optical path length of 1 cm. After the last component was added to the cell with the Teflon cap, the solution was thoroughly stirred by shaking. The optical density at 617 nm ($\varepsilon = 260$ mol^{-1} L cm^{-1}) was detected during 1 min at 10-s intervals. The values of the initial rates presented in the figures are the average values of at least three measurements. The calculation of effective parameters of the Michaelis—Menten equation (K_M, V_M) and all the other calculations were performed on a personal computer using the Sigma Plot 2.01 program package.

Preparation of *n*-butylferrocenium solutions containing Triton X-100 and SDS and kinetic measurements. We prepared aqueous solutions of glucose (0.7 *M*) and GO ($6.25 \cdot 10^{-6}$ *M*), a solution of **2** ($7.6 \cdot 10^{-3}$ *M*) in $5 \cdot 10^{-3}$ *M* HCl, and a 0.01 *M*

phosphate ($NaH_2PO_4 \cdot 12H_2O$) buffer (pH 6.7). Micellar solutions of Triton X-100 and SDS with the concentrations of $(2.6—78) \cdot 10^{-3}$ mol L^{-1} were prepared in this buffer. The kinetics of the reduction rate of *n*-butylferrocenium ions at different concentrations of the surfactant was studied as follows. A solution of **2** (0.4 mL, $7.6 \cdot 10^{-3}$ mol L^{-1}), a 0.01 *M* buffer (1.9 mL, pH 6.7) containing the surfactant (Triton X-100 or SDS) with a concentration of $(2.6—78.0) \cdot 10^{-3}$ mol L^{-1}, a solution of glucose (0.18 mL), and a solution of GO (0.02 mL) were successively added to a 1-cm cell. The change in the optical density was monitored spectrophotometrically at the wavelength of the absorption maximum of **2** (λ = 623 nm, ε = 196 mol^{-1} L cm^{-1}) in the thermostatted (25 °C) chamber of the spectrophotometer. The optical density was detected during 1 min at 10-s intervals. The values of the initial rates presented in figures are the average values of at least three measurements.

Preparation of *n*-butylferrocenium solutions in the presence of CTAB. Since Bu^nFc^+ cannot be generated by the simple "sulfuric acid" method because of its fast spontaneous reduction, the kinetics in CTAB micelles was studied by a different method. *n*-Butylferrocene was oxidized to *n*-butylferrocenium by the peroxidase method:[10] a 0.012 *M* solution of H_2O_2 (0.28 mL) and a solution ($8.1 \cdot 10^{-6}$ mol L^{-1}) of peroxidase (PH) from horse radish roots (Sigma) were added to a solution of Bu^nFc (10 mL, $8.7 \cdot 10^{-4}$ mol L^{-1}) in a 0.01 *M* phosphate buffer (pH 6.7) containing CTAB (0.01 *M*). The oxidation of *n*-butylferrocenium to the corresponding cation, which occurs at 25 °C during several min, was monitored spectrophotometrically at 623 nm. This reaction results in the oxidation of 60% *n*-butylferrocenium. The solution of Bu^nFc^+ thus prepared was used for studying the dependence of the rate of reaction (3) on the CTAB concentration. The kinetic experiment was carried out according to the previously described procedure.

Effects of different surfactants on the rate of dioxygen absorption. A 0.7 *M* solution of D-glucose was prepared in a 0.01 *M* phosphate buffer (pH 6.7) containing the corresponding surfactant (0.015 *M*; Triton X-100, SDS, or CTAB). The solution of glucose containing the surfactant and a solution of GO (0.5 mL, $1.56 \cdot 10^{-6}$ mol L^{-1}) were successively added to a 80-mL cell. The rate of dioxygen absorption in the presence of GO and D-glucose was monitored by the oxygen concentration ~5 s apart.

Results and Discussion

Effect of surfactants on the rate of enzymatic absorption of oxygen. To interpret correctly the data obtained on the effect of the surfactants on the kinetics of the GO-catalyzed reduction of the HFc^+ and Bu^nFc^+ cations, we should consider first of all, that the surfactant has no effect on the enzymatic activity. For this purpose, we studied the rate of absorption of the dioxygen dissolved in water in the GO—D-glucose system in the presence of the surfactants of three types used in the work but in the absence of ferrocenium cations, *i.e.*, the effect of the surfactants on the rate of enzymatic oxidative half-reaction (2) was studied. It can be seen in Fig. 2 that the surfactants taken in these concentrations do not affect the rate of the dioxygen absorption. In addition, the rate of the dioxygen absorption does not change in the absence of the surfactant. Thus, the surfactants do not change the inherent catalytic activity of GO.

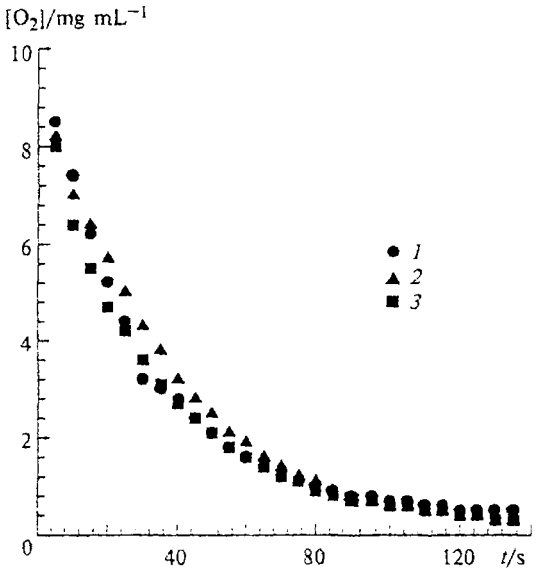


Fig. 2. Effects of Triton X-100 (*1*), CTAB (*2*), and SDS (*3*) on the oxygen absorption rate in the GO—D-glucose system (20±2 °C, pH 6.7, 0.01 *M* phosphate, [Surfactant] = 0.015 mol L^{-1}, [Gl] = 0.05 mol L^{-1}, [GO] = $1 \cdot 10^{-7}$ mol L^{-1}.

Effect of ferrocenium concentration on the rate of its enzymatic reduction. In the previous report,[5] only the micellar medium of Triton X-100 has been studied in detail. In this work, similar measurements were performed in the presence of CTAB, SDS, and Triton X-100 micelles using a sufficiently more active reagent, GO. The experiments were performed with a high concentration of D-glucose (0.05 *M*), when [Gl] > K_M^{Gl} (0.01—0.03 mol L^{-1}).[6]

The results obtained are shown in Fig. 3. It can be seen that for HFc^+ concentrations of up to $5 \cdot 10^{-4}$ mol L^{-1}, the rate of the GO-catalyzed reduction of ferrocenium in the presence of different micelles is almost the same. The differences begin to appear at higher concentrations of HFc^+. In the presence of Triton X-100 and SDS micelles (see Fig. 3), a Michaelis type dependence on the concentration of ferrocenium ions is observed, and when $[HFc^+] > 1 \cdot 10^{-3}$ mol L^{-1}, the reduction rate in the anionic micellar solution of SDS is retarded by 2.5—3.0 times. Both dependences can be satisfactorily approximated by the Michaelis—Menten equation ($v = V_M[S]/(K_M + [S])$, $S = HFc^+$), whose parameters are presented in Table 1.

A different dependence is observed in micelles of the cationic detergent CTAB (see Fig. 3). When the HFc^+ concentrations are low, the reaction rate almost coincides with those for the two previous cases, but when $[HFc^+] > 1.2 \cdot 10^{-3}$ mol L^{-1}, the rate of the enzymatic reaction decreases sharply as the substrate concentration increases. A similar dependence can be described by the formal Scheme 1.[11]

Table 1. Observed parameters of the Michaelis—Menten equation (V_M and K_M) and Eq. (9) for the GO-catalyzed reduction of the ferrocenium cation by D-glucose (0.05 *M*) in the presence of micelles of different surfactants (~0.01 *M*) ([GO] = $5 \cdot 10^{-8}$ mol L^{-1}, 25 °C, pH 6.7)

Surfactant	V_M /mol L^{-1} s^{-1}	$K_M(K_S)$ /mol L^{-1}	K_{iS}[a]	V_M/K_M /s^{-1}
Triton X-100	$(5.8\pm1.4) \cdot 10^{-5}$	$(2.0\pm0.9) \cdot 10^{-3}$	—	$2.9 \cdot 10^{-2}$
SDS	$(1.2\pm0.1) \cdot 10^{-5}$	$(0.42\pm0.01) \cdot 10^{-3}$	—	$2.9 \cdot 10^{-2}$
CTAB[a]	$(1.5\pm0.2) \cdot 10^{-5}$ [b]	$(0.7\pm0.1) \cdot 10^{-3}$	$K_{2S} = (3.4\pm0.3) \cdot 10^{-4}$ $K_{3S} = (4.1\pm0.4) \cdot 10^{-7}$	$2.1 \cdot 10^{-2}$

[a] Calculated from Eq. (9) for K_{2S} in mol L^{-1}, for K_{3S} in (mol L^{-1})^2.
[b] Product k[E], Eq. (9).

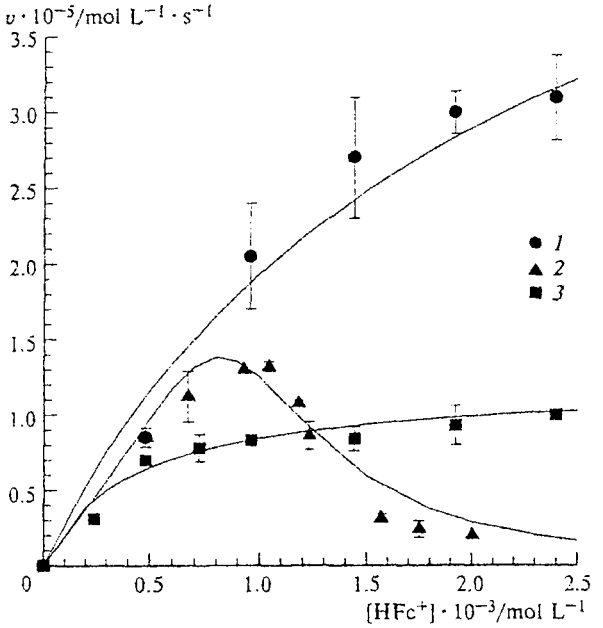


Fig. 3. Dependence of the steady-state rate of the GO-catalyzed reduction of HFc^+ by D-glucose in the presence of Triton X-100 (*1*), CTAB (*2*), and SDS (*3*) micelles on the concentration of HFc^+ (25 °C, pH 6.7, 0.01 *M* phosphate, [Surfactant] = $9.95 \cdot 10^{-3}$ mol L^{-1}, [Gl] = 0.05 mol L^{-1}, [GO] = $5 \cdot 10^{-8}$ mol L^{-1}). Solid lines show the dependences that were theoretically calculated using the kinetic parameters presented in Table 1.

Scheme 1

$E + S \rightleftharpoons ES$	K_S
$ES + S \rightleftharpoons SES$	K_{2S}
$SES + S \rightleftharpoons SES_2$	K_{3S}
$ES \rightarrow E + P$	k

Scheme 1 results in Eq. (9) for the steady-state rate of the enzymatic reaction.

$$v = \frac{k[E][S]}{K_{2S} + [S] + [S]^2/K_{2S} + [S]^3/K_{3S}} \quad (9)$$

The values of the kinetic parameters calculated from the data in Fig. 3 according to Eq. (9) are presented in Table 1. However, this description of the extreme dependence in Fig. 3 is formal, because no inhibition by the substrate is observed under conditions that differ only in the nature of the surfactant (*cf.* similar dependences for Triton X-100 and SDS in Fig. 3). No doubt the nature of CTAB plays an important role in the determination of the shape of the [HFc^+]—rate profile for the cationic micelles. The effect observed can be explained by the specific interaction of the cationic micelles with the negatively charged protein globule of GO, which results in a slight change in the spatial orientation of several near-surface groups of the globule creating additional binding centers for the second and third ferrocenium cations. When in the nonreactive binary and triple complexes the location of a molecule of the reduced flavin adenine dinucleotide (FAD) is unfavorable for the transfer of electron to the ferrocenium cation, this can explain the substrate inhibition observed.

Comparison of the values of the parameters of the enzymatic reaction (V_M and K_M obtained in micelles of different surfactants with the approximately same concentration of 0.01 *M*) (see Table 1) shows that the maximum rate V_M is observed in neutral micelles of Triton X-100. This result should be expected for this electroneutral surfactant. The maximum rates are somewhat lower in cationic CTAB and anionic SDS micelles. For low concentrations of ferrocenium, this is compensated by lower values of the Michaelis constants M_M. The V_M/K_M ratios, which characterize the reactivity for low concentrations of an organometallic substrate, are constant. This makes the comparative study of the effect of surfactants under these conditions possible.

Effect of surfactants on the kinetics of reduction of the ferrocenium cation. The dependences of the steady-state rate (normalized to [HFc^+]) of the GO-catalyzed reduction of the ferrocenium cation on the concentration of Triton X-100, CTAB, and SDS are presented in Fig. 4.

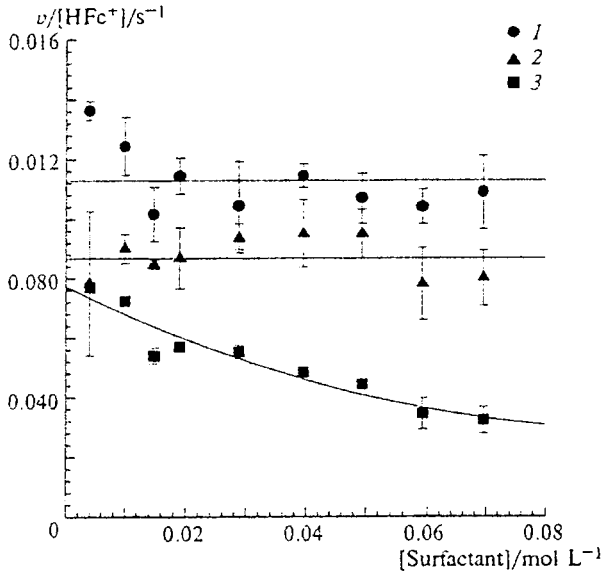


Fig. 4. Normalized steady-state rate of the GO-catalyzed reduction of HFc^+ by D-glucose as a function of the surfactant concentration: Triton X-100 (*1*), CTAB (*2*), and SDS (*3*) (25 °C, pH 6.7, 0.01 *M* phosphate, [Gl] = 0.05 mol L^{-1}, [GO] = $5 \cdot 10^{-8}$ mol L^{-1}).

The following facts are noteworthy: (1) the observed levels of the enzymatic activity in Triton X-100 and CTAB micelles almost coincide; (2) the activity in a solution of SDS is somewhat lower; and (3) in the intervals of surfactant concentrations used, the reaction rates are almost independent of the concentration of Triton X-100 or CTAB and only slightly depend on the SDS concentration. These facts agree with the previous observation for HFc^+ and Triton X-100 micelles[5] and the data in Fig. 3, which show that the maximum activity is observed in the presence of neutral Triton X-100 micelles.

The weak dependence of the steady-state rate of the ferrocenium cation reduction on [Surfactant] agrees qualitatively with the previous assumption[12] that the HFc^+ cations do not react, *e.g.*, with CTAB micelles. However, the obtained dependences do not allow one to assert with certainty that in the case of a surfactant of different nature, ferrocenium cations do not interact with the micellar phase. It can be assumed that these interactions exist and most likely concern external levels of micelle organization (Stern and Gouy—Chapman).[13] In the case of Triton X-100, these interactions can be easily detected from the spectra: the position of the absorption maximum of the HFc^+ cation in the UV region depends fairly strongly on the concentration of Triton X-100 in the solution (Fig. 5). There is even some correlation between the change in the enzymatic activity and the position of the absorption maximum. It can be distinctly seen in Fig. 5 that the spectral changes correspond to the concentration range of $3 \cdot 10^{-3}$—

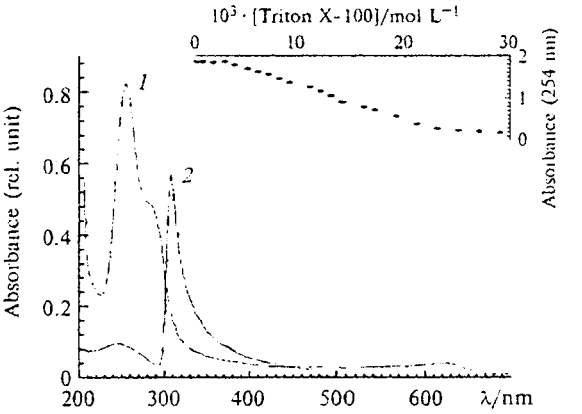


Fig. 5. Absorption spectra of the HFc^+ ion in the absence (*1*) and presence of Triton X-100 ($3 \cdot 10^{-2}$ mol L^{-1}) (*2*). The change in the absorption of the ferrocenium ion at 254 nm at different concentrations of Triton X-100 is shown in insertion.

$25 \cdot 10^{-3}$ mol L^{-1}. It follows from Fig. 4 that the insignificant decrease in the activity ends when [Surfactant] $\approx 25 \cdot 10^{-3}$ mol L^{-1}. Thus, weak micelle—ferrocenium interactions, which probably exist, have no decisive effect on the efficiency of the oxidation of the reduced GO by HFc^+.

Effect of surfactants on the kinetics of reduction of n-butylferrocenium. The effect of different surfactants on the normalized rates ($k = v/[E][Bu^nFc^+]$) of the GO-catalyzed reduction of 2 (Triton X-100 and SDS) and *n*-butylferrocenium generated by the "peroxidase method" under the action of D-glucose is shown in

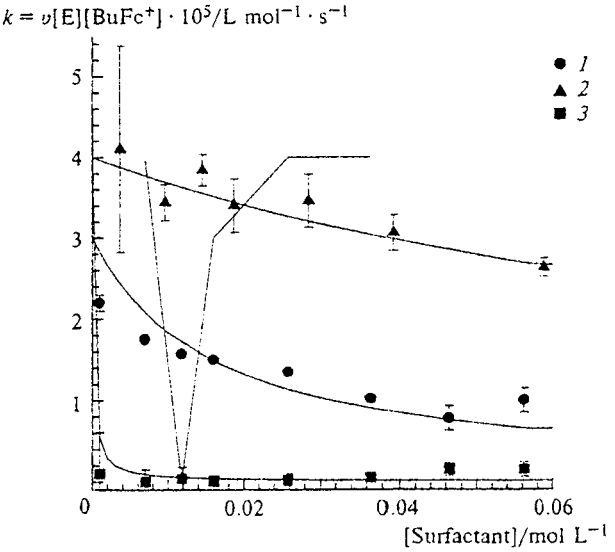


Fig. 6. Dependence of the normalized steady-state rate of the GO-catalyzed reduction of Bu^nFc^+ by D-glucose on the surfactant concentration: Triton X-100 (*1*), CTAB (*2*), and SDS (*3*) (25 °C, pH 6.7, 0.01 *M* phosphate, [Gl] = 0.05 mol L^{-1}). Solid lines show the dependences that were theoretically calculated using the parameters presented in Table 2.

Fig. 6. A comparison of Figs. 4 and 6 shows clearly that the processes of enzymatic reduction of HFc^+ and Bu^nFc^+ differ substantially. First, only the *residual* activity is observed for the reduction of Bu^nFc^+ in anionic SDS micelles when [SDS] > 0.002 *M*. Cationic CTAB micelles exhibit both a maximum rate and a weak dependence of the surfactant concentration. As the surfactant concentration increases in a solution of neutral Triton X-100 micelles, a more noticeable decrease in the enzymatic reduction of Bu^nFc^+ is observed. The observed regularities can be easily explained at the qualitative level. The stability of poorly reactive micelle—*n*-butylferrocenium associates increases on going from cationic to neutral and then to anionic micelles. The hydrophobic alkyl radical in the Bu^nFc^+ cation acts as a peculiar anchor, which performs a "positive" interaction between the cation and hydrophobic core of the micelle (see Fig. 1). When the micelle is negatively charged, an additional electrostatic interaction between the cationic "head" of the Bu^nFc^+ molecule with the anionic sulfo groups of SDS should occur in the Stern layer of the micelle. In the case of cationic CTAB micelles, the positive "core—tail" interaction is neutralized to one or another extent by the "negative" cation(micelles)—cation(Bu^nFc^+) interaction. The latter can be, first, a reason for the fact that among the three surfactants studied, the charged ferrocenyl head is the most accessible to the reduced GO. Second, this is likely the case of the mechanism of electrostatic acceleration of the reaction, which is schematically presented in Fig. 1. In the case of neutral Triton X-100 micelles, electrostatic interactions of *n*-butylferrocenium with the micelle are reduced to a minimum, and the stability of the corresponding associates is mainly determined by the "core—tail" interaction. Thus, the effectiveness of the micelle—*n*-butylferrocenium interaction, which does not favor, according to the data in Fig. 6, the enzymatic reduction of ferrocenium, should increase in the CTAB < Triton X-100 < SDS series.

The dependences of the steady-state concentration of the reduction of the *n*-butylferrocenium cation on the surfactant concentration (see Fig. 6), especially in the case of Triton X-100 and SDS micelles, strongly resemble the analogous profiles, which have been obtained for the surfactant effects on the rate of the PH-catalyzed *oxidation* of water-solubilized alkylferrocenes by hydrogen peroxide:[10]

$$RFc + H_2O_2 + 2\,H^+ \xrightarrow{PH} RFc^+ + 2\,H_2O. \qquad (10)$$

It should be mentioned that reaction (10) is more favorable for kinetic studies, because, first, alkylferrocene molecules are hydrophobic and, hence, do not contain cene cations and, hence, should bind rather strongly to the hydrophobic core of the micelle. Second, reaction (10) has a first kinetic order with respect to RFc, which simplifies the formal kinetic description of the micellar effects observed. The same approach can be used for reaction (3) when we operate with the data obtained for the concentrations of RFc^+ when the kinetic order with respect to RFc^+ is sufficiently close to the first order.

In the case of reaction (10), according to the pseudophase model of micellar catalysis, the dependence of the second-order rate constant ($v = k[RFc][E]$) on the surfactant is described by the equation[14]

$$k = \frac{k_m P_E P_S C V + k_w}{1 + P_S C V}, \qquad (11)$$

where P_S and P_E are the partition coefficients of the substrate (RFc in the case of PH and RFc^+ in the case of GO) and enzyme (PH or GO) between the micellar and aqueous phases, respectively; C is the concentration of the surfactant minus CMC; k_m and k_w are the reaction rate constants in the micellar and aqueous phases, respectively; and V is the molar volume of the surfactant. When a similar approach is used in the case of catalysis by GO, based on the data in Fig. 6, we can draw the following conclusions supported by mathematical simulation. First, the reduction of Bu^nFc^+ in micellar media of Triton X-100 and SDS most likely does not occur in the micellar pseudo-phase, *i.e.*, $k_m \approx 0$. This is indicated, in particular, by the sharp (especially pronounced for SDS) decrease in the catalytic activity as [Surfactant] increases. Thus, in this case, Eq. (11) can be transformed into the form

$$k = \frac{k_w}{1 + P_S C V}. \qquad (12)$$

Equation (12) can be used for determining the values of P_S if the data obtained for CTAB micelles are used for the estimation of k_w, because the dependence of the reaction rate on the concentration of this surfactant has the least sharp character, and the determination of this kinetic parameter seems to be the most exact in our case. Determining k_w and assuming that its magnitude, which reflects the interaction in an aqueous medium, should depend strongly on the surfactant nature and is almost the same in all three micellar media, we estimated the P_S values for CTAB, Triton X-100, and SDS. The values of CMC equal to $3 \cdot 10^{-3}$, $0.35 \cdot 10^{-3}$,[15] and $3 \cdot 10^{-3}$ mol L^{-1} for SDS, CTAB, and Triton X-100, respectively, and $V = 0.3$ dm^3 mol^{-1} were used.[14] The obtained values combined with the previously determined values of P_S for *n*-butylferrocene[10] are presented in Table 2. The systematic and, which is significant, expected change in the P_S values on going from butylferrocene to butylferrocenium depending on the nature of micelles is noteworthy. In particular, the distribution coefficient for Bu^nFc^+ in micellar solutions of SDS is especially great, which completely agrees (see above) with the results of qualitative analysis of the kinetic data obtained. The appearance of a charge in the Bu^nFc^+ molecule affects slightly its binding with neutral molecules of Triton X-100. In the case of cationic CTAB micelles, by contrast, the positive charge de-

Table 2. Rate constants k_w and distribution coefficients P_S for Bu^nFc^+ and Bu^nFc between micellar and aqueous phases

Surfactant	k_w/mol^{-1} L s^{-1}	P_S ($BuFc^+$)	P_S (BuFc)[10]
CTAB	$(4.0\pm0.2)\cdot10^5$	29±7	381
Triton X-100	$3\cdot10^5$	210.6±30	395
SDS	$4\cdot10^5$	$(2.1\pm0.2)\cdot10^4$	274

Note. Calculated from Eq. (12) under assumption that k_w(Triton X-100) ≈ k_w(SDS) ≈ k_w(CTAB) (25 °C, pH 6.7, 0.01 *M* phosphate).

creases sharply the capability of Bu^nFc^+ to bind with micelles.

The results of our study showed that the "micellar" control of the enzymatic activity in the GO-catalyzed reduction of ferrocenium cations by D-glucose becomes possible when the cation is hydrophobized on going from nonsubstituted ferrocenium to *n*-butylferrocenium. The butyl anchor, providing the "basic" interaction with the hydrophobic core of the micelle, in fact controls the reactivity of cationic, anionic, and neutral surfactants in micellar solutions due to both different in effectiveness binding to the micelle and "electrostatic" tuning of the reactivity of the {ferrocenium—micelle} associate.

The authors are grateful to N. K. Zaitsev for the possibility to measure the dioxygen absorption rates and to V. N. Goral′ for help in the experiments.

This work was financially supported by the Russian Foundation for Basic Research (Project No. 96-03-34328a).

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Received February 24, 1997;
in revised form April 21, 1997