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Enzymatic chemistry of ferrocenes: micellar tuning of the glucose oxidase reactivity toward solubilized electrochemically generated *n*-alkylferricenium cations

Alexander D. Ryabov *, Ekaterina S. Ryabova, Marina D. Reshetova

Department of Chemistry, Moscow State University, 119899 Moscow, Russia

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Abstract

Remarkable kinetic tuning of the glucose oxidase activity by the nature of micelle–alkylferrocene assemblies is realized during the electrochemical coupling between the enzyme reduced by D-glucose and the electrochemically generated alkylferricenium cations $H(CH_2)_nFc^+$ ($n = 0, 2, 4–8$) solubilized in micelles of neutral, positively and negatively charged surfactants (Triton X-100, CTAB and SDS, respectively) in the presence of D-glucose at pH 7. The relative electron-transfer rate increases 14 times for *n*-butylferrocene in CTAB and decreases by a factor of 3000 for *n*-pentylferrocene in SDS micelles relative to their reactivities in the neutral Triton X-100 medium.

Keywords: Ferrocene; Alkyl ferrocenes; Ferricenium ions; Glucose oxidase; Surfactants; Micelles; Bioelectrocatalysis; Kinetics; Electron transfer

1. Introduction

The impact of ferrocene on the development of modern chemistry and closely related fields is incredible. In addition to various chemical applications of this molecule highlighted in this issue, ferrocene should be emphasized as an important biochemically relevant organometallic compound [1–8]. When enzymes accept ferrocene, they display reactivity comparable to that of natural substrates of biocatalysts. This fact stimulated fundamental and applied studies aimed, in general, at creation of *functioning* assemblies of enzymes and ferrocenes [8,9]. Recent reports from this group [10–21] demonstrate that enzymatic chemistry of ferrocenes is very rich and the potential of this fairly simple organometallic molecule is enormous.

This paper discusses an example of the role of ferrocene in an enzymatic oxidation. In this connection ferrocene, as a water-insoluble electron-transfer mediator, can electrochemically be coupled with redox enzymes, if incorporated into micelles of various surfactants [13,22]. In particular, solubilized in micellar solutions, the ferricenium cation generated electrochemically from ferrocene is capable of reoxidizing reduced glucose oxidase, which is produced during enzymatic oxidation of D-glucose into D-gluconolactone (Scheme 1). As a result, cyclic voltammograms of the system GO–D-glucose–ferrocene are characterized by large catalytic currents [23]. The rate constants for the oxidation of the reduced enzyme by the ferricenium ion were independent of various surfactants such as cationic CTAB, nonionic Triton X-100, and anionic SDS. The rate constants of $(4.3–5.7) \times 10^5$ $M^{-1} s^{-1}$ (pH 7, 5% EtOH) are in accord with the mechanism referred to as the 'jumping off' ferricenium [13]. It implies that GO reduced by D-glucose captures HFc^+ in the rate-limit-

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

$$GO(red) + 2\,RFc^+ \rightarrow GO(ox) + 2\,RFc \quad \text{(solution)}$$

$$2\,RFc - 2\,e \rightarrow 2\,RFc^+ \quad \text{(electrode)}$$

Scheme 1.

Abbreviations: CTAB, cetyltrimethylammonium bromide; SDS, sodium dodecylsulfate; GO, glucose oxidase; HFc, ferrocene; RFc, *n*-alkylferrocene; $FADH_2$ and FAD, reduced and oxidized form of flavin adenine dinucleotide, respectively.

* Corresponding author. Present temporary address: Department of Chemistry, Carnegie Mellon University, 4400 Fifth Avenue, Pittsburgh, PA 15213, USA. Fax: +1-412-268-1061.

E-mail address: ryabov@andrew.cmu.edu (A.D. Ryabov).

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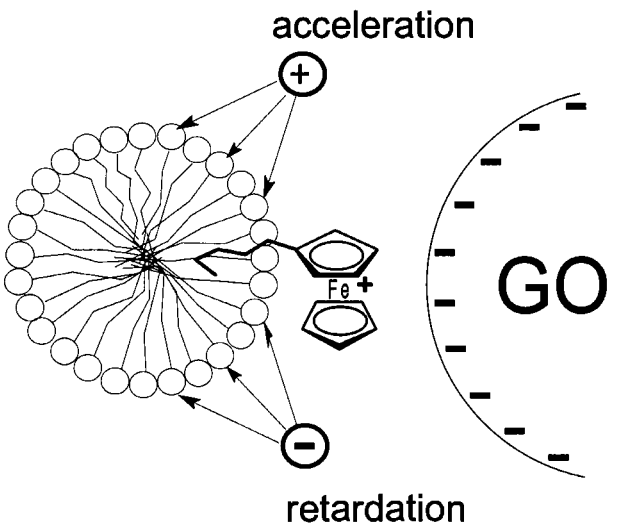


Fig. 1. Representation of a plausible mechanism of tuning the activity of reduced GO by micelle charge toward solubilized *n*-alkylferricenium cations. For details, see text.

ing step after fast reversible dissociation of the ferricenium ion from the micelle interior. This mechanism is in accord with the data of Kuwana et al. [24–26] on the titration of cytochrome *c* and cytochrome *c* oxidase by ferrocene solubilized in the nonionic surfactant Tween 20, which suggested that the ferricenium ion formed is not bound to the micelles. In order to enhance the role played by micelles in the interaction between ferricenium ions generated electrochemically from ferrocenes and negatively charged GO (pI = ca. 4 [27], total charge ca. -60 at pH 7 [28]), we increased the hydrophobicity of ferricenium cations by introducing *n*-alkyl groups, from methyl to dodecyl, on the ferrocene unit. It could be hypothesized that: (i) the alkyl group could serve as an 'anchor' or an interphase between the micelle and the electrochemically generated alkylferricenium ion; and (ii) the rate of electron transfer between GO reduced by D-glucose and alkylferricenium cation could be tuned by the negative, positive, or neutral micelles. On the basis of a simple electrostatic model shown in Fig. 1, the highest reactivity could be anticipated for the positively charged CTAB and the lowest for the negatively charged SDS micelles, while the neutral micelles of Triton X-100 being between the two extremes. Alternatively, other mechanisms associated with different binding of the ferricenium ions with positive, neutral and negative micelles could also be operative. Here, we report that regardless of mechanism, the micellar tuning of the reactivity is feasible with the most remarkable effects being observed for *n*-butyl- and *n*-pentylferrocenes.

2. Experimental

2.1. Materials

Alkylferrocenes $H(CH_2)_nFc$ used in this work were either commercially available ($n = 0$ or 4 (Aldrich) and 2 (Strem Chemicals) and 4) or were made using a two-step procedure involving acylation of HFc with the corresponding acyl chloride in the presence of $AlCl_3$ in CH_2Cl_2 followed by reduction of acylferrocene formed with zinc amalgam in a HOAc–HCl mixture ($n = 3$, 5–8, 12). The synthetic procedures are reported in detail elsewhere [29]. The $[HFc]^+PF_6^-$ was obtained from Aldrich, $[MeFc]^+PF_6^-$, $[EtFc]^+PF_6^-$, $[n\text{-PrFc}]^+PF_6^-$, and $[n\text{-BuFc}]^+PF_6^-$ were prepared as described elsewhere [29]. GO from *A. niger* was purchased from Serva and standardized with respect to the active FAD as described by Weibel and Bright [30]. Stock solutions of D-glucose (Sigma) were kept at least overnight for equilibration between the α and β anomers. All other chemicals were of the highest purity available.

2.2. Electrochemical measurements

Electrochemical measurements were made using a three-electrode cell with a pyrographite working electrode ($A = 1.2 \pm 0.2$ cm^2) at 25 °C. Cyclic voltammograms were obtained on a PC-interfaced potentiostat–galvanostat IPC-3 (Institute of Physical Chemistry, RAS, Moscow, Russia). The working electrode was polished with Al_2O_3 and then sonicated for 1–2 min in an Ultrasonic Type 07 bath before every new measurement. Potentials are with respect SCE throughout. Ethanol-free micellar solutions of alkylferrocenes were obtained after stirring a weighed amount of $H(CH_2)_nFc$ for 8–10 h in 0.1 M phosphate buffer (pH 7.0) containing 0.05 M CTAB or Triton X-100. Stable solutions could not be obtained in the case of SDS even at a lower phosphate concentration (0.01 M). Therefore, micellar solutions of SDS were prepared in the presence of 5% EtOH (by volume). It was, however, noticed that the activity of GO increases ca. 3–5-fold in the presence of 5% EtOH [14]. Micellar solutions of ferricenium cations were prepared from the corresponding alkyl ferricenium hexafluorophosphate. A weighed amount of ferricenium salt was first dissolved in 5 mM HCl in the presence of 0.01 M Triton X-100, as ferricenium cations are fairly stable in acidic media. The resulting solution was diluted with 0.1 M phosphate buffer, pH 7.0, containing 0.01–0.08 M Triton X-100 to give 1 mM solution of the ferricenium cation. The pH of the solution was adjusted to 7.0.

2.3. Determination of the rate constants

The rate constants for the oxidation of reduced GO(red) by electrochemically generated alkylferricenium cations were calculated using the procedure introduced by Bourdillon et al. [22,31]. In a typical experiment the values i_p/i_p^o were plotted against $([GO]/v)^{1/2}$, where i_p and i_p^o are the peak currents of alkylferrocenes in the presence and in the absence of GO and D-glucose, respectively, v is the scan rate (2–50 mV s^{-1}). Total concentrations of $H(CH_2)_nFc$ in the system were steadily lowered until the slope became concentration-independent and equal to $3.17 \times (k_3\delta RT/F)^{1/2}$, where $\delta = D_R/D_O$, is the ratio of the diffusion coefficients of reduced and oxidized alkylferrocene in micellar media. This occurred when the concentration reached ca. 1×10^{-4} M. For evaluation of the δ values, the apparent diffusion coefficients of alkyl ferrocenes and alkylferricenium cations at different concentrations of surfactants were determined using the Randles–Sevcik equation ($i_p = 2.69 \times 10^5 n^{3/2} A D^{1/2} c v^{1/2}$) for one-electron reversible systems [32,33]. For the determination of the exact electrode area, 1 mM solution of ferrocene in MeCN ($D = 2.4 \times 10^{-5}$ cm^2 s^{-1}) was used [34]. Apparent diffusion coefficients of reduced alkyl ferrocenes (D_R) and alkylferricenium cations (D_O) were obtained from the slopes of linear dependences of i_p versus $v^{1/2}$ in the range of surfactant concentrations 0.01–0.08 M. The D_R values for ferrocene in Triton X-100, CTAB and SDS were in a good agreement with the data of Mandal [35]. Because of lowered stability of *n*-propyl- and *n*-butylferricenium ions in micellar solutions, their concentration in solution was additionally checked by UV–vis spectroscopy (Shimadzu UV-160A).

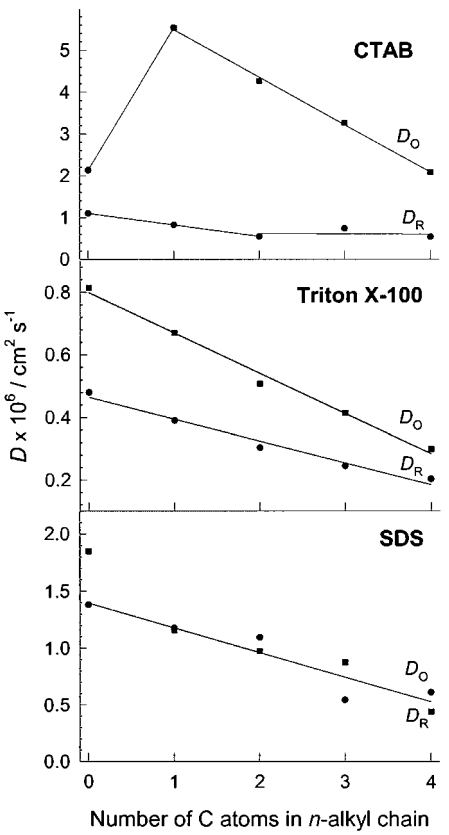


Fig. 2. Dependence of the apparent diffusion coefficients of *n*-alkylferrocenes (●, D_R) and *n*-alkylferricenium ions (■, D_O) against the number of carbon atoms *n* in $H(CH_2)_nFc$ in different micellar media. Conditions: pH 7.0, temperature 25 °C.

3. Results and discussion

3.1. Electrochemical properties of alkyl ferrocenes

Alkyl ferrocenes solubilized in buffered 0.1 M phosphate aqueous solution at pH 7 by charged and neutral micelles were electrochemically tested on a pyrolytic graphite electrode with its basal plane exposed to the aqueous solution. Such an electrode proved to be very convenient for electrochemical investigations of ferrocenes in micellar solutions [36] and all ferrocenes behave similar to HFc studied previously [13]. There is a linear dependence between the peak current i_p and the square root of the scan rate ($v^{1/2}$) in the range 2–50 mV s^{-1}; the difference between the anodic and cathodic maxima ($E_{ac} - E_{pc}$) increases only insignificantly, from 50 to 65 mV, with increasing scan rate. Cyclic voltammograms obtained at low scan rates in the SDS medium contain a small adsorptive anodic peak. Additionally, the cathodic peak current for ferrocenes with longer alkyl substituents (C5, C6, etc.) is 30–40% lower compared with the anodic one in SDS thus showing worse reversibility in this medium [37].

3.2. Estimation of the δ values

Values of the apparent diffusion coefficients of alkylferrocenes (D_R) and alkylferricenium cations (D_O) in 0.05 M micellar solutions of CTAB, Triton X-100 and SDS calculated from the cyclic voltammetry data using the Randles–Sevcik equation [32,33] are shown in Fig. 2. As seen, the most pronounced difference between D_R and D_O is observed for ferrocene and methylferrocene. In case of *n*-propyl- and *n*-butylferrocene, the variation becomes much less significant. This is expected because the hydrophobic interaction between an alkyl radical and micelle starts to play a decisive role, with the effect of the positively charged ferricenium head becoming less important. The D_R and D_O values were found to be almost similar in SDS reflecting the favorable coulombic interaction between the positively charged ferricenium head and the negatively charged micelle. The reverse explanation is applicable to account for the largest differences in the CTAB system. Extrapolation

Table 1
Values of $\delta = D_O/D_R$, measured by cyclic voltammetry using 1 mM solutions of RFc (D_O) or RFc^+ (D_R) in 0.1 M phosphate buffer (pH 7.0; 0.05 M surfactant; 25 °C)

Entry	Ferrocene	δ (Triton X-100)	δ (CTAB)	δ (SDS)
1	HFc	0.589	0.541	0.745
2	MeFc	0.582	0.149	~1
3	EtFc	0.598	0.128	~1
4	*n*-PrFc	0.518	0.226	~1
5	*n*-BuFc	0.678	0.258	~1
6	*n*-C_5H_{11}	0.737 [a]	0.628 [a]	~1 [a]
7	*n*-C_6H_{13}	~1 [a]	~1 [a]	~1 [a]

[a] Calculated values assuming linear dependence between D and n; for details, see text.

Table 2
Rate constants k_3 for the oxidation of reduced GO by *n*-alkylferricenium ions calculated using the δ values from Table 1 (pH 7.0; 0.1 M phosphate; 25 °C)

Entry	Ferrocene	Conditions	k_3 ($M^{-1}\,s^{-1}$)	$E^{\circ\prime}$ (mV vs. SCE)
1 [a]	HFc	SDS, 5% EtOH	$(7.7 \pm 1.7) \times 10^5$	150 ± 5
2 [a]	HFc	Triton X-100	$(1.4 \pm 0.4) \times 10^5$	210 ± 5
3 [a]	HFc	CTAB	$(4.8 \pm 1.0) \times 10^5$	220 ± 5
4	MeFc	Triton X-100	$(9.1 \pm 2.1) \times 10^4$	170 ± 5
5	EtFc	Triton X-100	$(6.8 \pm 0.4) \times 10^4$	195 ± 5
6	EtFc	CTAB	$(9.2 \pm 1.0) \times 10^5$	200 ± 5
7	EtFc	SDS, 5% EtOH	$(2.4 \pm 1.0) \times 10^4$	120 ± 5
8	*n*-BuFc	Triton X-100	$(3.4 \pm 0.4) \times 10^4$	255 ± 5
9	*n*-BuFc	CTAB	$(4.7 \pm 0.2) \times 10^5$	265 ± 5
10	*n*-BuFc	SDS, 5% EtOH	$(4.4 \pm 0.2) \times 10^2$	225 ± 5
11	*n*-C_5H_{11}Fc	Triton X-100	$(2.3 \pm 0.5) \times 10^4$	300 ± 5
12	*n*-C_5H_{11}Fc	CTAB	$(1.5 \pm 0.3) \times 10^5$	275 ± 5
13	*n*-C_5H_{11}Fc	SDS, 5% EtOH	$(5.01 \pm 4.36) \times 10$	250 ± 5
14	*n*-C_6H_{13}Fc	Triton X-100	$(1.66 \pm 0.02) \times 10^4$	315 ± 5
15	*n*-C_6H_{13}Fc	CTAB	$(4.81 \pm 0.02) \times 10^4$	310 ± 5
16	*n*-C_6H_{13}Fc	SDS, 5% EtOH	n.d. [b]	145 ± 5
17	*n*-C_7H_{15}Fc	Triton X-100	$(1.96 \pm 0.2) \times 10^4$	345 ± 5
18	*n*-C_7H_{15}Fc	CTAB	$(2.2 \pm 0.8) \times 10^4$	320 ± 5
19	*n*-C_7H_{15}Fc	SDS, 5% EtOH	n.d. [b]	145 ± 5
20	*n*-C_8H_{17}Fc	Triton X-100	$(8.4 \pm 0.4) \times 10^3$	350 ± 5
21	*n*-C_8H_{17}Fc	CTAB	$(9.8 \pm 1.2) \times 10^3$	350 ± 5
22	*n*-C_8H_{17}Fc	SDS, 5% EtOH	n.d. [b]	140 ± 5

[a] Data taken from Ref. [13].
[b] n.d.: too low to be determined.

of the linear portions of the dependencies of D against n at $n > 4$[1] suggests that the limit of $\delta = D_O/D_R$ is close to unity as n approaches infinity. The experimentally determined and calculated δ values are listed in Table 1.

The spike on the CTAB curve for MeFc (Fig. 2) might first seem surprising. Earlier we mentioned that it takes much less time to dissolve MeFc in micellar media compared to HFc, suggesting that somehow the asymmetry of the former increases its hydrophilicity. An extra positive charge introduces additional repulsion between a CTAB micelle and $MeFc^+$ accounting for an increased value of D_O.

3.3. Coupling of alkylferricenium cations with glucose oxidase

Cyclic voltammograms of micellar solutions of $H(CH_2)_nFc$ in CTAB, SDS, or Triton X-100 medium in the presence of GO and D-glucose indicate coupling between GO and ferrocenes (Scheme 1) [23], the efficacy of which is strongly determined by n. No coupling is observed when $n = 12$. The electrochemical data were used for the evaluation of the rate constants for the oxidation of the reduced enzyme, GO(red), by a ferricenium ion, Eq. (1), as suggested by Bourdillon et al. [22,31].

$$GO(red) + 2RFc^+ \rightarrow GO(ox) + 2RFc + 2H^+ \quad (1)$$

The stoichiometry of Eq. (1) is a combination of two successive $1e^-$ oxidations of $FADH_2$ by RFc^+ into FAD [31]: $FADH^- + RFc^+ \rightarrow FADH^\bullet + RFc$ (k_3) and $FAD^{\bullet -} + RFc^+ \rightarrow FAD + RFc$, and the first step is probably rate-limiting. It is noteworthy that when the surfactant concentration is as low as 0.05 M, the catalytic activity of GO is unaffected by CTAB, Triton X-100 or SDS [38]. The rate constants k_3 calculated using the δ values from Table 1 are summarized in Table 2 together with the formal redox potentials $E^{\circ\prime}$ of $H(CH_2)_nFc$ under the same conditions. The key find-

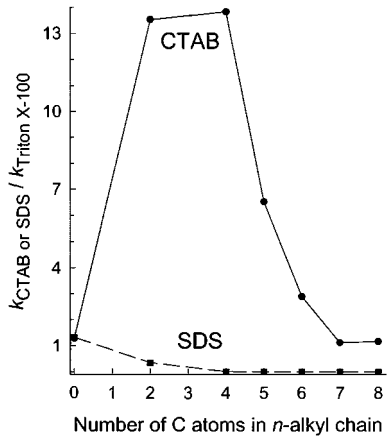


Fig. 3. Recognition by reduced GO of the charge of micelle with solubilized electrochemically generated *n*-alkylferricenium ions. For details, see text. Data are from Table 2.

[1] Such D_O values cannot be measured because of instability of alkylferricenium cations with $n > 4$ in aqueous micellar media.

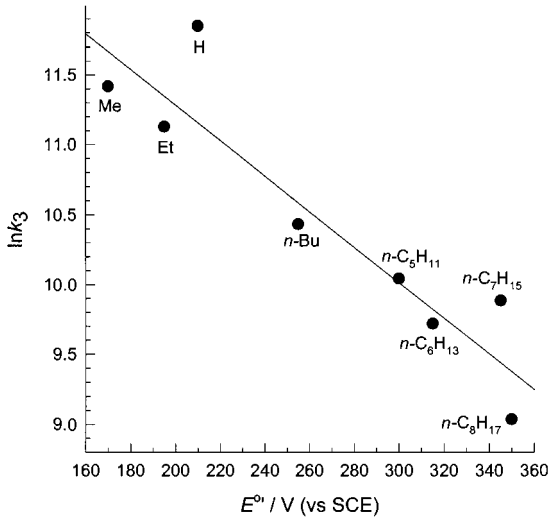


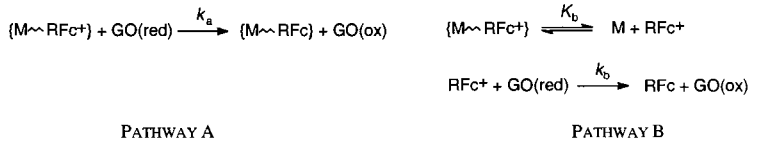
Fig. 4. Lowering of the rate constant for oxidation of reduced GO by ferricenium ions (k_3) with elongation of the alkyl radical of $H(CH_2)_nFc^+$. Data are from Table 2.

ings are depicted in Figs. 3 and 4. Fig. 3 shows the effect of the length of alkyl radical on the reactivity of the alkylferricenium cations toward GO(red). The normalized rate constants, viz. the ratio $k_{surf}/k_{X\text{-}100}$, where k_{surf} and $k_{X\text{-}100}$ are the second-order rate constants for the oxidation of reduced GO by RFc^+ in a given micellar medium and Triton X-100 micelles, respectively, are plotted against the number of carbon atoms in the ferrocene alkyl chain (n). It is clearly seen that although the ratio $k_{surf}/k_{X\text{-}100}$ is close to unity for $n = 0$, viz. for HFc in both CTAB and SDS media, the curves diverge appreciably on increasing the length of alkyl radical. The upper CTAB curve increases reaching a maximum at $n = 4$ and then decreases. The lower SDS curve decreases monotonically and the rate constant k_3 becomes too slow to measure for n-hexylferrocene. Thus, conditions have been found where a given n-alkylferrocene acquires different reactivity if incorporated into micelles of different charge.

The maximal 14-fold rate increase with respect to the rate constant measured in the Triton X-100 system is observed for n-butylferrocene in the positive CTAB micelles. The negative SDS micelles bring about 3000-fold retardation in the case of n-pentylferrocene. The 'cost' for the tuning of the reactivity of n-alkylferricenium cations towards GO(red) is a decrease in the rate constants k_3 on going to ferrocenes with longer alkyl radicals. The effect is demonstrated in Fig. 4, where the values of $\ln k_3$ observed in Triton X-100 are plotted against $E°'$. As seen, the rate constants decrease on increasing $E°'$. A similar graph is observed if $\ln k_3$ values are plotted against the number of carbons in the alkyl side-chain. Alternatively, this indicates that there should be a linear dependence between $E°'$ and n. In fact, this dependence was observed in the range of n 2–8 even in micelle-free solutions where the water soluble ferricenium salts $H(CH_2)_nFc^+PF_6^-$ were used [29].

3.4. Mechanism of micellar tuning

Although the behavior of redox species in surfactant solutions has been described extensively [39], the rationalization of the detailed mechanism reported here does not seem an easy task considering that the micellar system under study is complex. It should also be mentioned that the understanding of enzyme functioning in the presence of surfactants is in its infancy [40]. Since crucial differences are observed on going from ferrocene to n-butyl- or n-pentylferrocene, with other parameters of the system being kept unchanged, we assume that the effect arises from the mode of complexation micelle↔alkylferricenium. However, the mechanism with a rate-limiting dissociation of the alkylferricenium cation from the micelle surface can be eliminated on the following basis. The slope of the plot i_p/i_p^o against $([GO]/v)^{1/2}$, where [GO] is the concentration of the active enzyme, was found to be independent of the enzyme concentration. This was verified by the example of ferrocene and n-pentylferrocene in the GO concentration range $(0.8–5.6) \times 10^{-6}$ M. Two possible pathways for the oxidation of reduced enzyme by ferricenium cations should be considered (Scheme 2). Pathway A implies that alkylferricenium cations interact with GO(red) when bound to the micelle as shown in Fig. 1. Pathway B implies that alkylferricenium cations dissociate reversibly from the micelle interior (K_b) before the rate-limiting reoxidation of the reduced enzyme (k_b). The first step in Pathway B should be considered as a rapidly established equilibrium that is strongly shifted to the left. A rapid equilibration is suggested by our previous investigation, which showed that ferricenium ions fast dissociate from micelles [13], whereas the presence of a hydrophobic alkyl radical at the ferricenium core should increase profoundly the



Scheme 2.

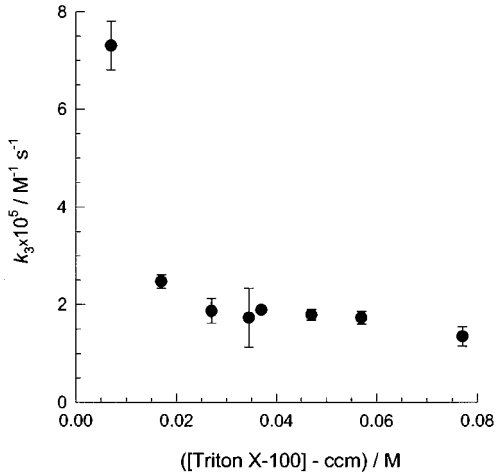


Fig. 5. Effect of Triton X-100 concentration on k_3 for oxidation of GO(red) by *n*-butylferricenium cation; 0.1 M D-glucose, 1.1×10^{-6} M GO, 0.1 M phosphate buffer, pH 7.0, 25 °C.

affinity of the cation to micelles. If Pathway B is operative and the conditions stated above hold, the expression for the rate constant k_3 is given by Eq. (2)

$$k_3 = k_b \times K_b \times [M]^{-1} \quad (2)$$

where [M] is the concentration of micelles: [M] = ([surfactant] ccm)/N (N is the aggregation number). Obviously, the rate constant k_3 should be independent of [M], if Pathway A is operative, and $k_3 \equiv k_a$. Therefore, if Pathway A is operative, the measured rate constants should not depend on the concentration of surfactant, while if Pathway B holds a reciprocal dependence on surfactant concentration should be observed. The effect of Triton X-100 concentration on the k_3 in the case of *n*-BuFc is demonstrated in Fig. 5. It is clearly seen that the experimental rate constants decrease as [Triton X-100] becomes higher giving mechanistic evidence in favor of Pathway B.

The nature of tuning becomes more clear in terms of the mechanism in Scheme 2B. Since the electron transfer occurs in the aqueous pseudo-phase, the k_b values should be basically independent of the surfactant nature. Of course, this is an idealized treatment that does not take into account possible medium effects brought about by CTAB, SDS, or Triton X-100. But in any case k_b must be less sensitive to their nature compared to the equilibrium constants K_b. Assuming that both the hydrophobic effect of the alkyl radical and the positive charge of the ferricenium moiety contribute to the overall binding of $H(CH_2)_nFc^+$ to the micelles, the former effect may be comparable for all the surfactants used, whereas the latter must be drastically different. The coulombic attraction in the case of SDS micelles is a crucial factor that strongly increases the binding between *n*-alkylferricenium cations and the anionic SDS micelles. In contrast, the repulsion is expected in the case of cationic CTAB micelles [41]. As a result, the following series in the dissociation constants is to be expected: $K_b^{SDS} \ll K_b^{X\text{-}100} < K_b^{CTAB}$. The strongest binding of *n*-alkylferricenium cations with the negative SDS micelles becomes more pronounced as far as the alkyl radical becomes longer. When it has 4–5 carbons, the additive effect consisting of the anchoring of the alkyl radical to the hydrophobic micelle interior and the favorable electrostatic interaction in the Stern micelle layer, could be responsible for the low K_b^{SDS} values. As a result, the efficacy of binding increases so much that the *n*-pentyl group inhibits completely the enzyme–ferrocene coupling.

If the same considerations were applied to CTAB and Triton X-100 micelles, the relationship $K_b^{X\text{-}100} < K_b^{CTAB}$ arises from the repulsion of the positive charges in the Stern layer. The relative rate increase in the CTAB medium observed for ethyl, *n*-butyl- and *n*-pentylferrocene disappears on going to ferrocenes with longer alkyl chains, e.g. *n*-heptyl- and *n*-octylferrocene. It seems likely that the coulombic repulsion is less important compared to the hydrophobic anchoring and the latter becomes the only factor governing the dissociation constants K_b. As a result, $K_b^{X\text{-}100} \approx K_b^{CTAB}$ when $n > 6$, the tuning disappears, and the ratio $k_{CTAB}/k_{X\text{-}100}$ becomes close to unity as in the case of HFc^+. It should be emphasized, however, that the unity when $n = 0$ and $n > 6$ has mechanistically different origin. In the case of HFc^+ the cation dissociates rapidly from micelle and then is reduced by GO(red). In other words, the equilibrium driven by K_b in Pathway B plays no significant role when $n = 0$. For *n*-alkylferrocenes, this equilibrium is a key mechanistic feature and the ratio $k_{CTAB}/k_{X\text{-}100} \approx 1$ is observed when $K_b^{X\text{-}100} \approx K_b^{CTAB}$.

4. Conclusions

We have demonstrated that the reactivity of an oxidized ferrocene towards reduced glucose oxidase can be controlled by proper design of micellar systems. The changes in reactivity can be rationalized in terms of either electrostatic (Pathway A) or hydrophobic interactions (Pathway B). Electrochemical coupling between reduced GO and alkylferricenium ions $H(CH_2)_nFc^+$ ($n = 0$, 2, 4–8) in systems of neutral, positively and negatively charged micelles (Triton X-100, CTAB and SDS, respectively) in the presence of D-glucose at pH 7 occurs with the discrimination by GO of the surfactant–alkylferrocene aggregates. In the case of *n*-butylferrocene the electron-transfer rate constant increases 14-fold and decreases by a factor of 3000 for *n*-pentylferrocene in the CTAB and SDS micelles, respectively, relative to the reactivity in the neutral Triton X-100 medium. The maximum distinction is observed for $H(CH_2)_nFc$ with $n = 4$ and 5, whereas the effect is much less pronounced for ferrocenes with higher *n*.

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