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Journal of Photochemistry and Photobiology A: Chemistry 94 (1996) 163–166

Journal of PHOTOCHEMISTRY AND PHOTOBIOLOGY A:CHEMISTRY

Photochemical properties of systems containing cytochrome *c* and kojic acid

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Received 12 January 1995; accepted 21 September 1995

Abstract

Redox reversible kojic acid anions, denoted as K^- and irreversible oxalato anions ($C_2O_4^{2-}$) are bound to the positively charged protein part of ferric cytochrome *c* (Fe(III)cyt^{7+}) in basic aqueous solutions (pH 8.5). UV and visible irradiation of the ion pairs formed leads to the net photoreduction of ferric to ferrous cytochrome *c* (Fe(II)cyt^{6+}) when the ion pairs are "free" (solvated), as well as in systems containing the ion pairs Fe(III)cyt^{7+}–$(C_2O_4^{2-})_n$ embedded inside sodium dodecylsulphate micelles. Systems containing the pairs Fe(III)cyt^{7+}–$(K^-)_n$ inside the micelle cage and systems with anions K^- or $C_2O_4^{2-}$ outside the micelles containing Fe(III)cyt do not undergo any net observable photoredox process. The quantum yields of photoreduction of Fe(III)cyt increase with increasing average number of the anions attached to the surface of Fe(III)cyt and with increasing energy of the incident radiation.

Keywords: Photoredox; Cytochrome *c*; Kojic acid; Cage effect

1. Introduction

Due to its antimicrobial properties, insecticidal activity and chelate-forming ability [1–5], kojic acid (5-hydroxy-2-(hydroxymethyl)-4H-pyran-4-one), denoted as HK, is a commonly applied compound. HK itself and its anion K^- are photochemically stable (they are used in photoprotective mixtures and suntan oils [6,7]). In the presence of a redox reactive metal ion, HK can undergo spontaneous oxidation [8] or photo-oxidation [9,10] to yield the radical K$^{\bullet}$. In the presence of an electron donor, the radical K$^{\bullet}$ reverts to the anion K^- and no net change in the concentration of K^- is observed in such systems [9,10]. Ferric cytochrome *c* (Fe(III)cyt) is a suitable partner for many redox active reactants due to its well-known structure and properties [11].

In this paper, the photochemical properties of systems containing HK anions K^-, Fe(III)cyt and the anionic surfactant sodium dodecylsulphate (SDS) are investigated and compared with those containing oxalato anions ($C_2O_4^{2-}$) instead of K^-.

2. Experimental details

Horse-heart cytochrome *c* (Fe(III)cyt, Serva), SDS (Fluka, purissimum), potassium tris(oxalato)ferrate(III) ($K_3[Fe(C_2O_4)_3]$, Oxford Organic Chemicals) and 1,10-phenanthroline (Aldrich) were of analytical grade and were used without further purification. HK was available from previous work [9,10]. Water was doubly distilled prior to use. The other chemicals were from Lachema, all being of analytical grade and used as received.

The irradiated solutions were prepared by dissolving solid Fe(III)cyt, HK (or potassium oxalate) and SDS in water in a given order (see Table 1) so as to obtain solutions with the required concentrations of the compounds. The micelles containing Fe(III)cyt were prepared by a standard method [12]. The pH value of all investigated solutions was adjusted to 8.5 by addition of 0.01 M NaOH.

Steady state photolyses were performed in a three-chambered temperature-controlled (20 ± 2 °C) quartz photoreactor. As radiation source, a medium pressure Hg lamp (Tesla RVK, 125 W, radiation monochromatized by solution filters [13]) and a low pressure Hg lamp (Germicidal Lamp G8T5) were used. The solutions studied were deoxygenated by bubbling with argon 30 min prior to and during irradiation. The intensity of the incident light I_0 was determined by a ferrioxalate actinometer [13] to be of the order of 10^{18} quanta s^{-1} cm^{-2}.

The photoreduction of Fe(III)cyt to Fe(II)cyt was followed spectrophotometrically on the basis of the different

SSDI 1010-6030(95)04224-5

Table 1
Quantum yield of Fe(II)cyt formation (ϕ) for systems containing 6.3×10^{-5} M Fe(III)cyt, (0–5.0) $\times10^{-4}$ M HK and zero or 1.0×10^{-4} M SDS irradiated in aqueous solutions (pH 8.5) by monochromatized (λ_{irr}) radiation (standard deviation of ϕ values, less than $\pm10\%$)

System	c(HK) (M)	λ_{irr} (nm)	ϕ
Fe(III)cyt^{7+}	0	313	2.3×10^{-4}
Fe(III)cyt^{7+}–$(K^-)_n$	3.6×10^{-4}	254	8.1×10^{-3}
Fe(III)cyt^{7+}–$(K^-)_n$	3.6×10^{-4}	313	3.2×10^{-3}
Fe(III)cyt^{7+}–$(K^-)_n$	5.0×10^{-4}	313	5.6×10^{-3}
Fe(III)cyt^{7+}–$(K^-)_n$	3.6×10^{-4}	365	2.0×10^{-3}
Fe(III)cyt^{7+}–$(K^-)_n$	3.6×10^{-4}	436	3.6×10^{-4}
{Fe(III)cyt^{7+}–$(K^-)_n$} (in SDS)	3.6×10^{-4}	313	0
{Fe(III)cyt^{7+}} (in SDS) + K^-	5.0×10^{-4}	313	0

spectral properties of the cytochromes in the visible region [14,15].

Electronic absorption spectra were recorded on a Specord M-40 spectrophotometer (Zeiss, Jena, Germany). pH measurements were performed using a digital pH meter (Radelkis, Hungary).

3. Results and discussion

The initial concentration of Fe(III)cyt in all systems was 6.30×10^{-5} M. Irradiation of Fe(III)cyt in the absence of HK and SDS at 313 nm induces photoreduction to Fe(II)cyt, with a quantum yield ϕ of 2.3×10^{-4}. The corresponding spectral changes are in accordance with the low spin nature of both cytochromes. The photoreduction is associated with a decrease in the 695 nm band intensity, i.e. with the homolytic Fe(III)–S(methionine-80) bond splitting.

The addition of HK (up to 5.0×10^{-4} M, Table 1) to the system containing Fe(III)cyt leads to no substantial spectral changes in the visible region. The band at 695 nm is preserved, i.e. anions K^- do not enter the primary coordination sphere of Fe(III)cyt and do not substitute for the sulphur S(methionine) atom [15]. The electronic absorption spectrum of this system in the UV region is an overlap of the spectra of Fe(III)cyt and K^- anions. This is in accordance with the assumption that there is a strong interaction between the positively charged protein part of Fe(III)cyt (at neutral pH its charge is 7^+ [16]) and the negatively charged anions K^-. Within such a model, the radiation absorbing and photochemically reacting species can be represented (Fig. 1) as ionic pairs having various numbers of anions K^- bound to the protein part of Fe(III)cyt^{7+}.

Irradiation of this system leads to the photoreduction of Fe(III)cyt to Fe(II)cyt with a higher efficiency (quantum yield) than that in the absence of HK. The corresponding spectral changes are shown in Fig. 2. It follows from Table 1 that the higher the K^- concentration in the system, the higher the quantum yield of Fe(III)cyt photoreduction. Because in the UV region the incident radiation is absorbed by both Fe(III)cyt and K^- anions (due to pK_a(HK) = 7.88 [17], at pH 8.5 more than 80% of HK is in its anionic form K^-), the

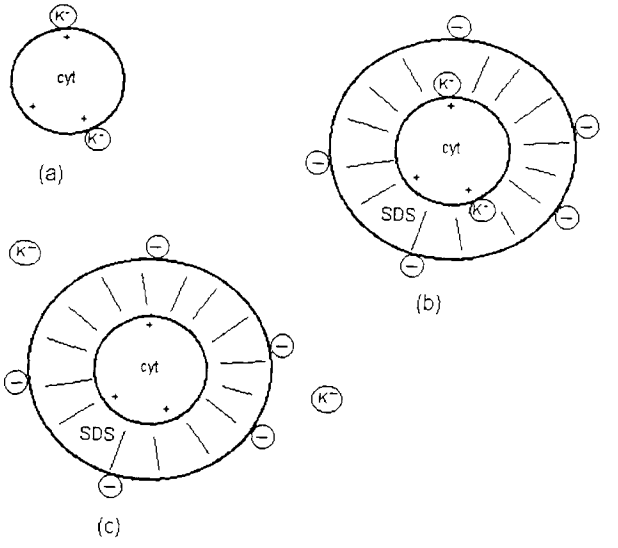

Fig. 1. Schematic model of the ion pair Fe(III)cyt^{7+}–$(K^-)_n$ (a), the ion pair in an SDS micelle {Fe(III)cyt^{7+}–$(K^-)_n$} (in SDS) (b) and K^- anions present outside an SDS micelle containing Fe(III)cyt^{7+} (c).

intensity of the incident radiation absorbed by Fe(III)cyt was corrected for the absorption by K^- anions and the quantum yields of photoreduction of Fe(III)cyt (ϕ) were calculated using Eq. (1) (see Appendix)

$$\phi = \mathrm{d}A_m/\mathrm{d}t \frac{V}{\Delta\epsilon_m l} \frac{c(\mathrm{cyt})\epsilon(\mathrm{cyt}) + c(\mathrm{HK})\epsilon(\mathrm{HK})}{c(\mathrm{cyt})\epsilon(\mathrm{cyt})I_0(1-10^{-A})} \quad (1)$$

where $\mathrm{d}A_m/\mathrm{d}t$ is the initial rate of absorbance change at the monitoring wavelength of 550 nm (the position of the peak maximum of Fe(II)cyt, ϵ_{550}(Fe(II)cyt) = 21 500 M^{-1} cm^{-1} [18]), $\Delta\epsilon_m$ is the difference between the molar absorption coefficient values of Fe(II)cyt and Fe(III)cyt at the monitoring wavelength ($\Delta\epsilon_{550}$ = 14 420 M^{-1} cm^{-1} [18]), l is the optical path length (l = 1 cm), V is the volume of the irradiated solution (V = 140 cm^3), A is the absorbance at the wavelength of incident radiation λ_{irr} (λ_{irr} = 254, 313, 365 or 436 nm) and ϵ(cyt) and ϵ(HK) are the molar absorption coefficients of Fe(III)cyt and HK at pH 8.5 respectively at λ_{irr}. Values of ϵ(cyt) and ϵ(HK), evaluated from the spectra of basic solutions of Fe(III)cyt and HK, are listed in Table 2. The calculated values of ϕ are given in Table 1.

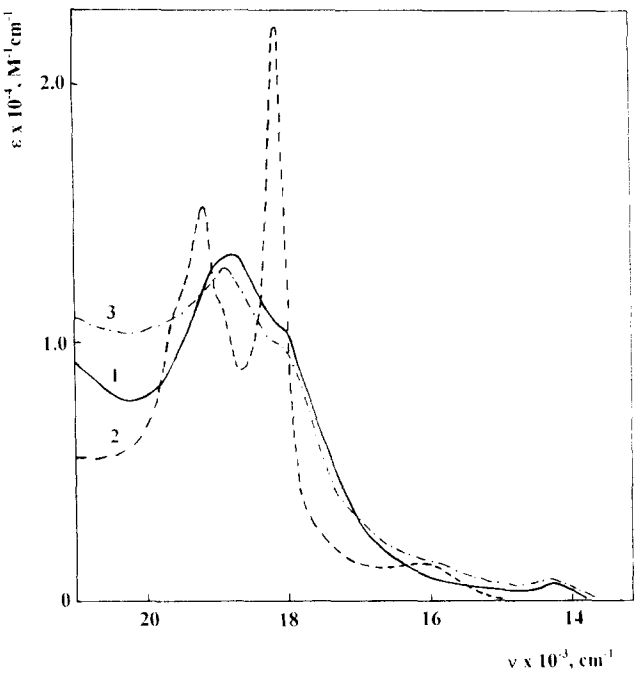

Fig. 2. Electronic absorption spectra of the ion pairs Fe(III)cyt^{7+}–$(K^-)_n$ (curve 1) and Fe(II)cyt^{6+}–$(K^-)_n$ (curve 2) and systems containing Fe(III)cyt^{7+}–$(K^-)_n$ encapsulated in SDS micelles after irradiation at 313 nm (curve 3).

Table 2
Molar absorption coefficients of ferric cytochrome *c* (ε(Fe(III)cyt)) and kojic acid (ε(HK)) (in $M^{-1}\,cm^{-1}$) at the wavelengths of incident radiation λ_{irr} (in nm)

ε	λ_{irr}			
	254	313	365	436
ε(Fe(III)cyt)	14000	10000	18600	27900
ε(HK)	4700	2100	0	0

Systems containing 5.0×10^{-4} M HK and 1.0×10^{-2} M SDS (critical micellar concentration cmc(SDS) = 1.5×10^{-3} M [12]) were prepared by two different procedures. In the first procedure, HK was added to a solution of Fe(III)cyt, and after ion pair formation, the pairs Fe(III)cyt^{7+}–$(K^-)_n$ were incorporated into SDS micelles. In the second procedure, Fe(III)cyt was first incorporated into SDS micelles and HK was subsequently added. In a simplified form, the structures of the systems containing HK and SDS are depicted in Fig. 1.

Irradiation of either of the above systems leads to no observable photoreduction of Fe(III)cyt; no characteristic sharp peaks of Fe(II)cyt are observed in the spectra (Fig. 2).

The nature of the preventative effect of SDS on Fe(III)cyt photoreduction is probably different for the two systems. The observation of no overall photoreaction in the system containing caged ion pairs Fe(III)cyt^{7+}–$(K^-)_n$ can be explained as a consequence of the known reversible properties of the redox pair K^-–$K^\bullet$ (the radical $K^\bullet$ does not undergo decomposition, and by abstraction of an electron from a surrounding molecule, it reverts to the anion K^- as found in many systems [10]). It may be assumed that there is primary electron transfer from surface-bound K^- to the central atom Fe(III) of excited Fe(III)cyt. Due to a certain rigidity of the SDS cage, the molecules of the geminate pair Fe(II)cyt and $K^\bullet$ cannot separate, thermodynamically driven back electron transfer from Fe(II)cyt to $K^\bullet$ occurs and the parent reactants Fe(III)cyt and K^- reappear.

The observation of no net photoreduction of Fe(III)cyt in the system with K^- anions present outside the SDS micelles containing Fe(III)cyt can be rationalized in terms of the highly negative surface charge of the micelles which impairs any approach of the anionic redox partner K^-. The distance between the possible reaction centres is too great for electron transfer (the inner diameter of SDS micelles, Stern layer included, is estimated to be $r > 1500$ pm [19]). Such an explanation is consistent with theoretical models on the relationship between the distance between the reaction centres and the kinetic parameters of the electron transfer process [20,21], and experimental observations of photoredox processes occurring in the absence or presence of surfactants [19,22]. It is worth noting that SDS itself is not redox reactive in our and similar [18,19] conditions. Also it is known [10] that HK and its anion K^- do not behave as quenchers towards excited Fe(III) complexes, and excited HK and K^- do not act as electron donors towards ground state Fe(III) complexes.

The interpretation of our experimental observations is in accord with that of other workers who found that the protein surrounding the iron porphyrin moiety in cytochrome *c* does not hinder electron transfer; however, in micellar systems electron transfer can be retarded [22].

The reliability of the above conclusions was checked using a strongly irreversible redox reactant, oxalate anion ($C_2O_4^{2-}$), instead of HK anion K^- in the systems containing Fe(III)cyt and SDS. It was found that irradiation of the system of ion pairs Fe(III)cyt^{7+}–$(C_2O_4^{2-})_n$ embedded in SDS micelles led to the photoreduction of Fe(III)cyt. For the system with $c(\text{Fe(III)cyt}) = 6.30\times10^{-5}$ M, $c(C_2O_4^{2-}) = 3.0\times10^{-4}$ M and $c(\text{SDS}) = 1.0\times10^{-2}$ M irradiated at 313 nm, the quantum yield of photoreduction of Fe(III)cyt is $\phi = 2.7\times10^{-4}$. The radical $C_2O_4^{-}$ formed in the primary electron transfer step does not therefore revert to its parent dianion $C_2O_4^{2-}$, and the net formation of Fe(II)cyt is observed. In the case when anions $C_2O_4^{2-}$ are present outside the micelles containing Fe(III)cyt, irradiation does not induce any observable photoreduction of Fe(III)cyt. The reason for this observation, the long distance between the anions $C_2O_4^{2-}$ and the central atom Fe(III), is obviously the same as that for the systems containing K^- anions.

4. Conclusions

The present and previous results [15,18,22] can be summarized as follows. The net photoreduction of Fe(III)cyt can

be observed in instances when at least one of the following conditions is fulfilled:

(1) an oxidizable ligand (e.g. N_3^-) is coordinated to the central atom Fe(III);
(2) an anionic ligand is bound to the positively charged protein part of Fe(III)cyt and the molecules of the geminate pair (such as Fe(II)cyt and radical K$^\bullet$) can diffuse away from each other and be separated by solvent molecules;
(3) a parent redox pair, embedded in a micelle or other cage, contains an irreversible redox partner (e.g. $C_2O_4^{2-}$).

Appendix

In our system, there are three radiation absorbing species. Two of them, namely Fe(III)cyt and Fe(II)cyt, absorb radiation at the monitoring wavelength (quantities at this wavelength are denoted by the subscript m). Fe(III)cyt and K^- absorb incident radiation at the initial time of irradiation. For simplicity, the species in our system are denoted as follows: Fe(III)cyt as A, Fe(II)cyt as B and K^- as C. A general equation for quantum yield calculation can be derived as follows.

The absorbance A_m at the monitoring wavelength is given by

$$A_m = c(A)\epsilon_m(A) + c(B)\epsilon_m(B) \quad (A1)$$

Since $c(A) + c(B) = \text{constant}$, the experimentally measured slope dA_m/dt can be expressed as

$$dA_m/dt = \Delta\epsilon_m l dc(B)/dt \quad (A2)$$

where $\Delta\epsilon_m = \epsilon_m(B) - \epsilon_m(A)$ and l is the path length of the cell. The number of moles of product B formed in unit time, $dn(B)/dt$, which is needed for the calculation of the quantum yield of product formation ϕ, is given by the relation

$$n(B)/dt = dA_m/dt \frac{V}{\Delta\epsilon_m l} \quad (A3)$$

where V is the volume of the irradiated solution.

The number of moles of incident photons I_{abs} absorbed by reactant A (in addition, incident radiation is absorbed by C), which is the second quantity needed for the quantum yield calculation, is obtained from the Beer–Lambert law

$$I_{abs} = I_0(1 - 10^{-A}) \frac{c(A)\epsilon(A)}{c(A)\epsilon(A) + c(C)\epsilon(C)} \quad (A4)$$

where the values of the absorbance A, molar absorption coefficients ϵ and total intensity of incident radiation I_0 are related to the wavelength of incident radiation. From Eqs. (A3) and (A4), a general relation for quantum yield calculation can be derived

$$\phi = dA_m/dt \frac{V[c(A)\epsilon(A) + c(C)\epsilon(C)]}{\Delta\epsilon_m l c(A)\epsilon(A) I_0(1 - 10^{-A})} \quad (A5)$$

which is consistent with Eq. (1) (see Section 3).

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