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REDOX POTENTIALS IN HYDRO-ORGANIC MEDIA AT NORMAL AND SUBZERO TEMPERATURES

FERRO-FERRICYANIDE AND CYTOCHROME *c* AS MODELS

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Summary

Redox potentials of ferro-ferricyanide and cytochrome *c* were measured in water/ethylene glycol and water/dimethylsulfoxide (volume ratio from 100/0 to 50/50) between 25 and −25°C. For both systems, the midpoint potential decreases in the presence of organic solvents and increases by cooling. The magnitude of these variations is larger in dimethylsulfoxide than in ethylene glycol; moreover in the same solvent mixture it is larger with ferro-ferricyanide than with cytochrome *c*, so that the difference between the redox potentials of these two systems can be strongly affected and even reversed. While in pure water (cacodylate buffer pH 7.0, NaCl 0.1 M) they are respectively +388 and +265 mV, in 50% dimethylsulfoxide at 25°C they decrease to +112 and +208 mV. Reduction of cytochrome *c* by ferro-ferricyanide, in this mixture, is then expected and was indeed observed. On the other hand, as $(\partial E/\partial T)^T$, ($E$ being the redox potential) is higher for ferro-ferricyanide than for cytochrome *c*, the oxidative power of the former for the latter is expected to increase as temperature decreases. This effect was observed in 50% ethylene glycol at −16°C.

Organic solvents and large temperature variations appear then as powerful perturbants of redox reactions. Their effects should be taken into account in studies of redox reactions carried out in cooled hydro-organic media.

Introduction

Cryobiochemistry developed in this laboratory [1] is based on the idea that the investigation of enzyme systems in hydro-organic media at subzero temperatures requires not only the development and adaptation to these conditions of analytical techniques [2—6], but also the knowledge of physicochemical data for these media, including density, viscosity, dielectric constant, and pro-

ton activity of buffers [7]. Such data allow one to control changes in the essential properties of enzymes [8,9] as well as to preserve, by suitable readjustments of the physico-chemical parameters of the solution, the capacity of water as a solvent of biological macromolecules in the presence of high concentrations of organic solvents at any temperature.

This wealth of data has allowed a number of investigators to perform enzyme studies in these conditions. In recent reports it was shown that the electron transport chain of chloroplasts [10—13] and mitochondria [14,15] can function in mixed solvents at subzero temperatures. There was no evidence for any qualitative change in the behaviour of chloroplast down to —35°C. However some apparent changes in the properties of the photosynthetic electron transport chain at this temperature were observed. Such observations raise the problem of the effect of both organic solvents and temperature on the redox potential of biological molecules. Because of our lack of information in this domain, we decided to undertake the present work.

Two redox systems were chosen: ferro-ferricyanide, and ferro-ferricytochrome *c*. The ferro-ferricyanide couple is one of the most widely used oxidation-reduction potential buffers for the determination of the redox potential of biological systems [16—23] as well as for the studies of the electron transfer reaction between these systems [24—27]. Its midpoint potential has been determined under the influence of various parameters including pH, ionic strength [28], nature of buffer [29], nature of the electrolyte present in the solution [30]. Any additional information on this system may thus be useful for further studies. On the other hand, cytochrome *c* was shown to be highly resistant to organic solvents [31] and therefore constitutes a suitable model compound of hemoproteins in these conditions.

Experimental values for the midpoint potential of these systems, at pa_H 7.0, in two series of hydro-organic solvents, water/ethylene glycol and water/dimethylsulfoxide of different volume ratio, at normal and low temperatures are reported.

It appears that both organic solvents and temperature can induce strong variations in this parameter. According to these results, some predictions might be made concerning the perturbations which organic solvents and large temperature variations can bring about in the redox reaction between ferro-ferricyanide and cytochrome *c*. These perturbations were indeed observed and illustrate clearly the usefulness (or the inconvenience) of studying redox reactions in hydro-organic media at low temperature.

Materials

The two salts $K_4Fe(CN)_6$, $K_3Fe(CN)_6$ (Prolabo) were used without further purification. Their solutions, prepared daily, were kept in the dark.

Cytochrome *c* from horse heart (Sigma, type VI) was used without further purification. Stock solutions were prepared in phosphate buffer 20 mM, pH 7.2; (cytochrome *c* concentration about 10^{-3} M). Cytochrome *c* was reduced by excess of dithionite (sodium salt) and chromatographed on Sephadex G-25. Kept in the dark at 0°C, under a nitrogen atmosphere, its reoxidation was very slow. Its concentration was determined spectrophotometrically

on the reduced form, using for the molar absorption coefficient, $\epsilon = 27.7 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$ at 550 nm [32]. The ratio (oxidized form)/(reduced form) was derived from spectral recordings obtained with a Beckman Acta III spectrophotometer, equipped for low temperature experiments [3].

The various salts and buffers (sodium and potassium chloride, sodium cacodylate and phosphate) were from Merck. Ethylene glycol and dimethylsulfoxide from Merck, were freshly distilled under vacuum before use. Appropriate correction was made for the density change [7] when expressing molar concentrations in the various hydro-organic media.

Buffered solutions have been prepared according to recently published tables [6,7] in order to perform all the experiments described in this paper at a constant pa_H 7.0 ($pa_H = -\log a_H$, a_H proton activity in a hydro-organic medium).

Potentiometry

(1) Basic principle

According to the classical procedure, determinations of redox potentials are performed by measuring the e.m.f. generated by the following cell: platinum electrode, sample solution "X" | reference electrode

([OX]/[R])

The vertical line represents a liquid junction. If E is the measured e.m.f. of the cell, $E(X)$ the potential of the sample solution ([OX] and [R] being respectively the concentrations of the oxidized and reduced forms), E_J the e.m.f. introduced by the junction and E_R the potential of the reference electrode:

$$E = E(X) + E_J - E_R$$

$$E = E(X) - E_{RJ} \quad E_{RJ} = E_R - E_J \tag{1}$$

E_{RJ} is the potential of the reference electrode including the junction.

The midpoint potential of X, E_0 (X) can be derived from the Nernst equation:

$$E(X) = E_0(X) + \frac{RT \ln 10}{nF} \log \frac{[OX]}{[R]} \tag{2}$$

In the present work, where redox potentials have to be measured in hydro-organic media of varying composition (from pure water to water organic mixture 50/50 in volume) at normal and low temperatures, the basic principle of the measurements remains the same. However several problems occur: (i) Choice of a reference electrode, (ii) is the e.m.f. introduced by the junction dependent on organic solvent concentration and on temperature? (iii) calibration of the reference electrode.

(2) Apparatus

The potentiometric measurements were made with a platinum (Tacussel) calomel electrode assembly, placed in a Faraday cage. A hydrogen electrode (Pt.H.131 Tacussel; hydrogen N 55 air liquide, $O_2 < 1.5$ ppm) was used for control experiments. The measuring cell and the calomel electrode were sepa-

rately thermostated, the former between 25 and $-25^{\circ}C \pm 0.2^{\circ}C$, the latter at $25^{\circ}C \pm 0.1^{\circ}C$, respectively with Colora and Julabo (Juchheim Labortechnik) thermostats. The calomel electrode and measuring cell have already been described elsewhere [6]. A slow flow of Argon (N 55 Air Liquide) was maintained inside the measuring cell. The potential determinations were made on a Mettler Titrator assembly (DK 10, DK 11, DK 12) the information being sent through a digital multimeter Tektronic DM 501, to a Tektronic calculator (Tek 31-53) and displayed every minute according to an appropriate program. It was then possible to follow the establishment of the equilibrium of the system, which usually required between 5 and 10 min at both normal and low temperatures.

(3) The reference electrode

The standard potential of various cells has been already determined in hydro-organic media [33—34]. However the measurement of redox potentials in hydro-organic media of variable composition and their comparison with respect to pure water raises the problem of a standard state of reference.

For this purpose we used in this work, as in a previous one [6], calomel electrodes prepared either in pure water or in a given hydro-organic solvent (water/ethylene glycol 50/50 for ethylene glycol mixtures; water/dimethylsulfoxide 50/50 for dimethylsulfoxide mixtures), with a liquid junction of 0.1 M KCl in the same mixture. This low KCl concentration allowed us to minimize the ion contamination of the sample solutions, especially when working at very low ionic strengths, arising from diffusion through the porous stone of the junction.

From the relation (1) we see that this procedure is possible, only when it can be demonstrated that E_J is not dependent either on organic solvent concentration in the sample solution, or on temperature.

(4) Organic solvent and temperature effect on E_J

Some authors [35—36] have evaluated the solvent contribution to the e.m.f. of the liquid junction between different pure solvents. This contribution was found to be small. In our conditions, where the junction is established between partly aqueous media (the water concentration on both sides being in any case no lower than 50% in volume i.e. approximately 25 M) it is reasonable to expect this solvent contribution to be negligible.

To verify this assumption we prepared a cell,

Hg, Hg_2Cl_2; KCl 0.1 M	$Fe^{III}(CN)_6$-$Fe^{II}(CN)_6$	KCl 0.1 M, Hg_2Cl_2, Hg
water/ethylene glycol 50/50	in different media	water/dimethylsulfoxide 50/50

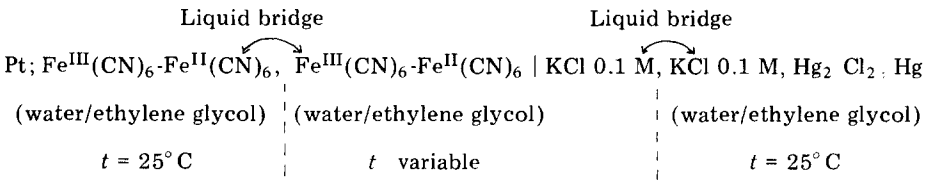
(cell I)

consisting of two calomel electrodes in water/ethylene glycol and water/dimethylsulfoxide, connected through their respective junctions by a solution of ferro-ferricyanide. The solvent composition of this solution was varied, so that both junctions were exposed to different media. In the absence of organic solvent effect on the e.m.f. introduced by the junctions, the e.m.f. of the cell

should remain a constant. This is what we observed: (1) ferro-ferricyanide in pure water, $E = +18 \pm 2$ mV, (2) ferro-ferricyanide in water/ethylene glycol 50/50, $E = +18 \pm 2$ mV, (3) ferro-ferricyanide in water/dimethylsulfoxide 50/50, $E = +20 \pm 2$ mV.

Accordingly in our conditions, ethylene glycol and dimethylsulfoxide (from 0 to 50%) do not significantly affect the e.m.f. introduced by the junction between the reference electrode and the sample.

To measure redox potentials as a function of temperature, the procedure we used was to keep the reference electrode at a constant temperature, 25°C, and to apply temperature variations only to the measuring cell. However, because of the particular geometry of the measuring cell, the junction was also exposed to variable temperature. It was therefore necessary to evaluate the temperature effect on the e.m.f. of the junction. A cell in which only the temperature of the junction can be varied, was prepared for this purpose



(cell II)

The e.m.f. of this cell was determined for different temperatures of the junction (between 25 and −25°C). A typical series of experiments is reported (Table I). As temperature decreases from +25 to −22 and increases from −22 to +25°C, the e.m.f. of the cell is not affected. It can then be concluded that the e.m.f. of the junction, with our conditions and solvents, is insensitive to temperature variations.

(5) Calibration of the reference electrodes

Potential of the calomel electrodes, including the e.m.f. of the junction (E_{RJ}, Eqn. 1), was determined from cells of the type:

Pt, $Fe^{II}(CN)_6$-$Fe^{III}(CN)_6$ | KCl 0.1 M, Hg_2Cl_2, Hg

(water) in water, water/ethylene glycol 50/50, or water/dimethylsulfoxide 50/50

(cell III)

A solution of ferro-ferricyanide in pure water was used as a standard. Its potential was calculated from the data published by Hanania [28] taking into

TABLE I

e.m.f. OF CELL II AS A FUNCTION OF TEMPERATURE

See text for details.

t (°C)	+25	+4	−13	−22	−3	+24
E (mV)	−16	−16	−15	−14	−15	−16

account the ionic strength contribution (see Eqn. 4 in the next section). We found:

$E_{RJ}^{25°C}$ (water) = +318 ± 2 mV

$E_{RJ}^{25°C}$ (ethylene glycol 50%) = +288 ± 2 mV

$E_{RJ}^{25°C}$ (dimethylsulfoxide 50%) = +270 ± 2 mV.

Results and Discussion

The midpoint potential values presented in this paper have been derived from the classical equation

$$E_{meas.} = E_i + \frac{RT \ln 10}{nF} \log \frac{[OX]}{[R]} \tag{3}$$

where $E_{meas.}$ is the measured value of the potential of the half cell containing the redox system at a finite ionic strength I. E_i is the midpoint potential of the system at the same ionic strength. RT has its usual significance; n is the number of electrons transferred from the reduced to the oxidized form; F is the Faraday; [OX] and [R] are the respective molar concentrations of the oxidized and the reduced forms.

For ionic strengths lower than 10 mM, the Debye-Hückel limiting law [37] was applied:

$$E_i = E + a\sqrt{I} \tag{4}$$

with

$$a = 1.825 \cdot 10^6 \cdot \frac{RT \ln 10}{nF} \cdot \left(\frac{\rho}{\epsilon^3 T^3}\right)^{1/2} \cdot (Z_{OX}^2 - Z_R^2) \tag{5}$$

where E is the midpoint potential of the system at zero ionic strength; I the ionic strength is given by the relation:

$$I = \tfrac{1}{2} \sum_i C_i Z_i^2 \tag{6}$$

ρ is the density of the solution, ϵ the dielectric constant, Z_{OX} and Z_R are the charge number of oxidized and reduced forms.

(1) Organic solvent and temperature effects on the midpoint of ferro-ferricyanide system and cytochrome c

The ferro-ferricyanide system. Midpoint potential values, extrapolated to zero ionic strength, E (derived from experiments realized at low ionic strength, 4.8 mM, using Eqns. 3 to 6) and at high ionic strength, E_i, (from Eqn. 3) in a cacodylate buffer pa_H 7.0, 0.1 M NaCl, obtained in two series of hydro-organic solvents at 25°C, are quoted in Table II. These data represent a mean value over several determinations from solutions of different [OX]/[R] ratios. The constant a (Eqn. 4) has been calculated according to Eqn. 5, using published tables of physicochemical constants [7]. For instance, at 25°C, a = 0.209 in pure water [28]; in the mixtures water/ethylene glycol and water/dimethylsul-

TABLE II

MIDPOINT POTENTIAL OF THE FERRO-FERRICYANIDE SYSTEM AND CYTOCHROME *C* AS A FUNCTION OF ORGANIC SOLVENT CONCENTRATION AT 25°C

% (v/v) Solvent	0	10	20	30	40	50	60	70
Ethylene glycol								
Molarity	0	1.8	3.6	5.4	7.2	9.0	10.8	12.5
E (mV) I = 0	355	339	319	297	277	254	219	193
E_i (mV) I = 0.1 M in cacodylate buffer pa_H 7.0	388	372	356	330	314	289		
E_m (mV) I = 0.1 M cytochrome *C* in cacodylate buffer, pa_H 7.0	265		249			238		
Dimethylsuffoxide								
Molarity	0	1.4	2.8	4.1	5.6	7.0		
E (mV) I = 0	355	302	254	197	141	79		
E_i (mV) I = 0.1 M in cacodylate buffer, pa_H 7.0	388	332	286	226	170	112		
E_m (mV) I = 0.1 M cytochrome *C* in cacodylate buffer, pa_H 7.0	265		242			208		

foxide 50/50 (by vol.), $a = 0.305$ and 0.236 at 25°C, and 0.230 and 0.190 at −20°C, respectively.

The midpoint potential of the ferro-ferricyanide system decreases as the concentration of the organic solvent increases. The variation is linear over the range 0 to 50% and is further amplified for higher organic solvent contents. The variation of redox potential in cacodylate buffer 0.1 M NaCl, closely parallels that observed at zero ionic strength so that it can be concluded that neither ionic strength nor buffer influence the organic solvent effect on the midpoint potential. Moreover it must be noticed that the effect of dimethylsulfoxide is much stronger than that of ethylene glycol.

Plots of the midpoint potential of the system extrapolated to zero ionic strength as a function of reciprocal of temperature in the various hydro-organic mixtures, are shown in Fig. 1. The midpoint potential increases linearly with the reciprocal of the absolute temperature. Moreover, the slope of the plots appears to be significantly affected by the nature and the concentration of the organic solvent. From the curves E against T (not reported here) which are roughly linear between 20 and −20°C, we derived a temperature coefficient of the midpoint potential: $(\partial E/\partial T)^T$; $-(\partial E/\partial T)^{25^\circ C} = 1.87\ mV \cdot K^{-1}$ in pure water, 1.87, 1.56 and 1.48 respectively in 10, 30 and 50% of ethylene glycol, and 0.87 in 50% dimethylsulfoxide.

Cytochrome c. Midpoint potential of cytochrome *c*, $E_{m(7.0)}$, obtained in different hydro-organic media in the presence of cadodylate buffer (pa_H 7.0, NaCl 0.1 M), at 25°C, is given in Table II. These data were derived from oxidative-reductive titrations of cytochrome *c* (30 μM) by the ferro-ferricyanide system; their plots shown in Fig. 2 reasonably fit the theoretical straight lines whose slope is given by (RT/nF) ln 10, assuming $n = 1$. $E_{m(7.0)}$ was deduced from these plots according to Eqn. 3.

Midpoint potential of cytochrome *c* decreases linearly as the organic solvent

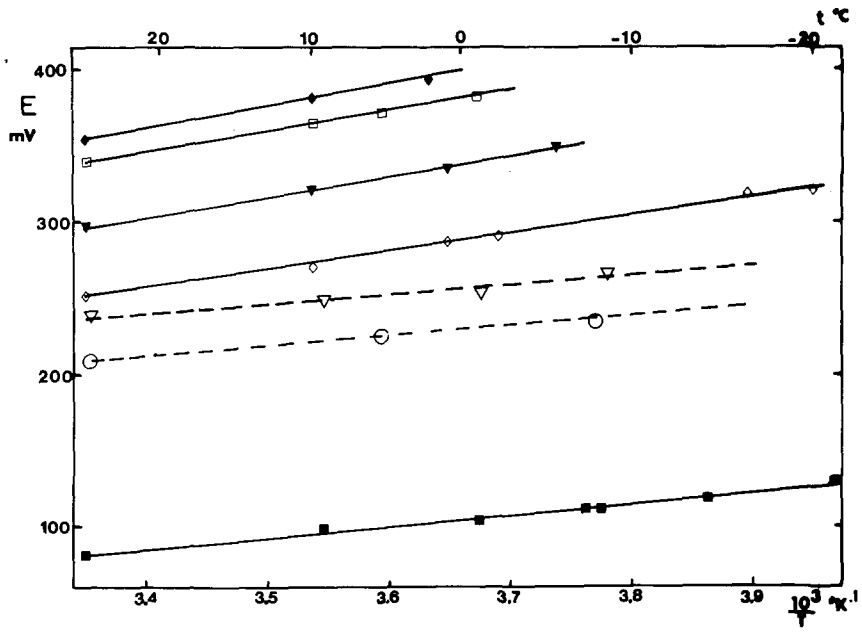


Fig. 1. Midpoint potential of the ferro-ferricyanide system and cytochrome *c* as a function of the reciprocal of absolute temperature. ♦———♦, Ferro-ferricyanide (extrapolated at I = 0) in water; ethylene glycol, □———□, 10%; ▼———▼, 30%; ◇———◇, 50%; ■———■, in dimethylsulfoxide 50%. Cytochrome *c* (in cacodylate buffer, pa_H 7.0 NaCl 0.1 M) ▽———▽, in ethylene glycol 50%; ○- - - - -○, in dimethylsulfoxide 50%.

concentration increases. However, these variations are lower than those reported for the ferro-ferricyanide system. Here again, the effect of dimethylsulfoxide is stronger than that of ethylene glycol. Let us mention that in the present conditions cytochrome *c* is not denatured [31], a finding further confirmed by the linearity of the plots of midpoint potential versus organic solvent concentration, and by the absence of any detectable spectral change.

Plots of midpoint potential of cytochrome *c* derived from plots of Fig. 2 and Eqn. 3 as a function of the reciprocal of absolute temperature are shown in Fig. 1, for two mixtures: water/ethylene glycol and water/dimethylsulfoxide 50/50 (by vol.).

Here again a temperature coefficient was derived from the curves E_m against $T \cdot -(\partial E/\partial T)^{25^\circ C} \simeq 0.83$ and 0.96 in 50% ethylene glycol and dimethylsulfoxide respectively, although these organic solvents exhibit very different properties. It seems therefore likely that contrary to ferro-ferricyanide systems, the organic solvents do not strongly affect $(\partial E/\partial T)^T$ in the case of cytochrome *c*.

According to these two series of results it is clear that the variation of the midpoint potential of both systems depends strongly on the nature of the organic solvent. Whereas ethylene glycol involves a moderate effect, dimethylsulfoxide dramatically affects the redox potential of both ferro-ferricyanide and cytochrome *c* systems although their molar concentration in the mixtures are comparable. This observation cannot be accounted for by the variation of dielectric constant since as we showed previously [7], ethylene glycol decreases

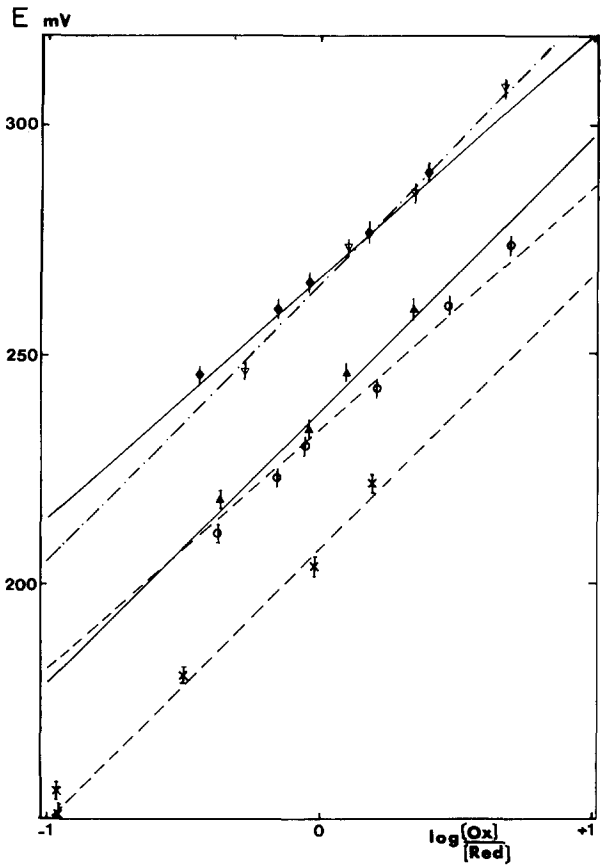


Fig. 2. Oxidative-reductive titrations of cytochrome *c* by ferro-ferricyanide in different conditions of solvent and temperature. Pure water, ▽· — · —▽, 25°C; in 50% ethylene glycol, ▲———▲, 25°C; ◆———◆, 9°C; in 50% dimethylsulfoxide, X- - - - -X, 25°C; ○- - - - -○, —8°C. The theoretical straight lines (slope: RT/nF ln 10, $n = 1$) are drawn through the corresponding series of experimental points. The [OX]/[R] ratios were determined spectrophotometrically at 550 nm. Only reductive titrations could be performed in dimethylsulfoxide 50%. E_m was determined potentiometrically, the ferro-ferricyanide present in the medium being used as a mediator. Further addition of a typical mediator (2,6-dichlorophenolindophenol for instance) does not improve the experiments.

the dielectric constant of water, while dimethylsulfoxide leaves it unaffected until 50% in volume. It could be more likely ascribed to the perturbation of the solvation shells of the systems, since with a different physico-chemical process, ionisation of usual buffer molecules [7], similarly we found an effect of dimethylsulfoxide much stronger than that of ethylene glycol. Further supporting evidence in favor of this view is the finding that the organic solvent action is stronger on the ferro-ferricyanide system than on cytochrome *c*. Such a difference could result from the partial protection provided, in the case of cytochrome *c*, by the protein moiety to the heme iron atom against the organic solvents, whereas in both ferro- and ferricyanide, the iron atom is more directly exposed to these agents.

Let us now focus on the consequences that the use of organic solvents and large temperature variations could have on electron transfer reactions between

ferro-ferricyanide and cytochrome *c*, as suggested by a critical examination of the results reported here.

(2) Reduction of cytochrome c by the ferro-ferricyanide system in water/dimethylsulfoxide 50/50 (by vol.), at 25°C

In water at 25°C, the couple ferro-ferricyanide is an oxidative titrant for cytochrome *c*, according to their respective midpoint potentials 388 and 265 (in cacodylate pH 7.0, NaCl 0.1 M). However in the hydro-organic solvents the redox potential of these systems is modified: whereas that of cytochrome *c* is slightly decreased, the redox potential of the ferro-ferricyanide system is affected to such an extent that in 50% dimethylsulfoxide it becomes lower than the midpoint potential of cytochrome *c* (Table II). Reduction of cytochrome *c* by the ferro-ferricyanide couple must accordingly be expected in these conditions. This is what we observed by the experiments reported in Fig.

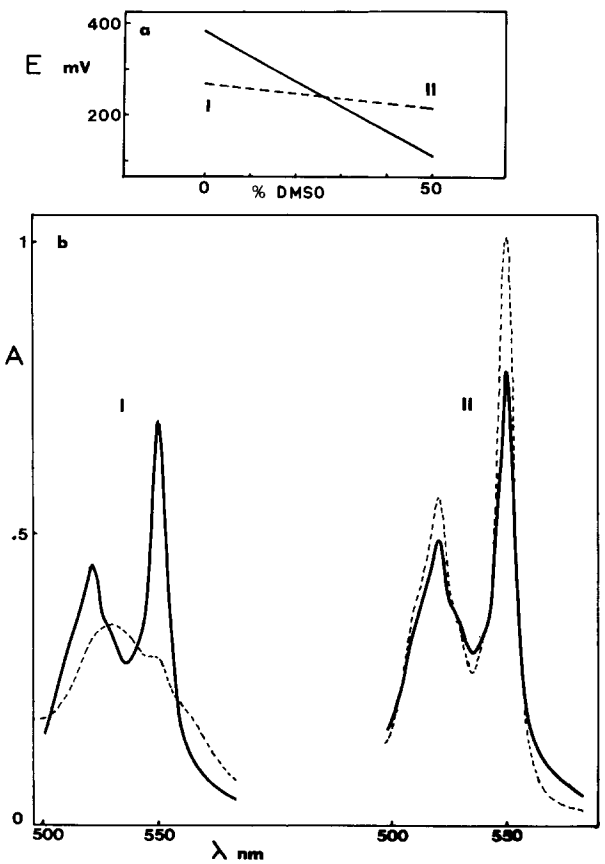


Fig. 3. Reduction of cytochrome *c* by ferro-ferricyanide in dimethylsulfoxide (DMSO) 50% at 25°C. a, Midpoint potential of both reactants as a function of dimethylsulfoxide concentration. ———, Ferro-ferricyanide; - - - - - -, ferro-ferricytochrome *c*. b, Spectral changes resulting from the action of ferro-ferricyanide (2.8 μmol of ferrocyanide and 0.2 μmol of ferricyanide) on cytochrome *c* (30 μM, 65% of reduction) in pure water (I), and in dimethylsulfoxide 50% (II) (cacodylate pa_H 7.0, NaCl 0.1 M, total volume of 1.5 ml). ———, Spectrum of cytochrome *c* alone; - - - - - -, spectrum of cytochrome *c* taken 30 s after addition of ferro-ferricyanide. These spectra were stable for at least 15 min.

3. In these experiments a known amount of ferro- and ferricyanide is added to a solution of cytochrome *c*, whose fraction of reduced form is 0.65: in pure water (I) nearly complete oxidation (monitored by the spectral change) is obtained, the fraction of the remaining reduced form being only 0.06; in 50% dimethylsulfoxide (II) complete reduction is obtained. In order to make easier the reading of these observations, the corresponding variation of the midpoint potential of both reactants, in water/dimethylsulfoxide mixtures, is shown in Fig. 3a (I and II).

(3) Combined organic solvent (ethylene glycol)-temperature effect on the oxidation-reduction reaction between ferro-ferricyanide and cytochrome c

From the results in Table II it is apparent that in 50% ethylene glycol the

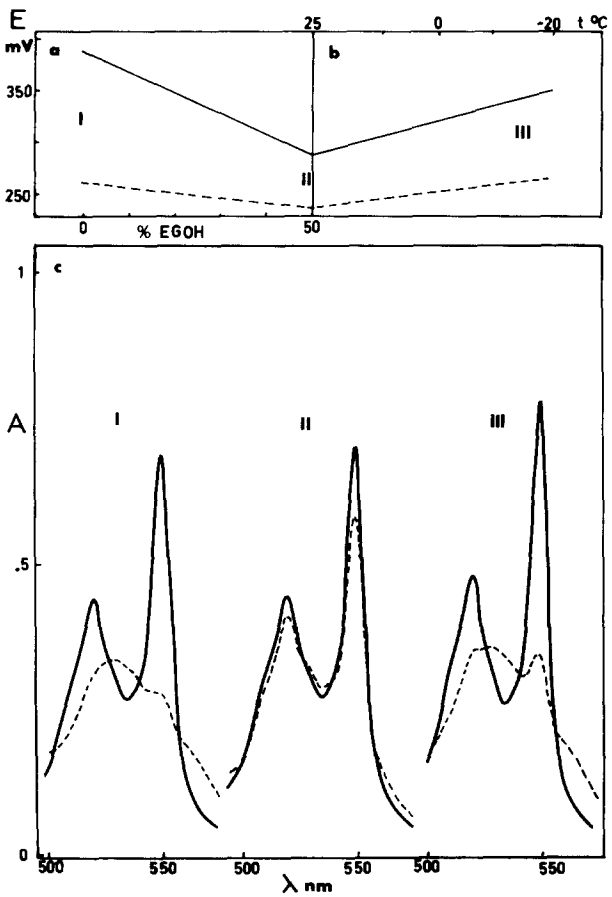


Fig. 4. Combined organic solvent-temperature effects on the oxidation of cytochrome *c* by ferro-ferricyanide. a, Midpoint of both reactants as a function of ethylene glycol concentration at 25°C. ———, Ferro-ferricyanide; - - - - - -, ferro-ferricytochrome *c*. b, Midpoint potential of both reactants as a function of decreasing temperature, in the water/ethylene glycol, 50/50. c, Spectral changes resulting from the action of ferro-ferricyanide (2.8 μmol of ferrocyanide and 0.2 μmol of ferricyanide) on cytochrome *c* (30 μM, 65% of reduction) in pure water (I), water/ethylene glycol 50/50 at 25°C (II), and water/ethylene glycol 50/50 at −16°C (III) (cacodylate pa_H 7.0, NaCl 0.1 M, total volume 1.5 ml). ———, Spectrum of cytochrome *c* alone; - - - - - -, spectrum of cytochrome *c* taken 30 s after addition of ferro-ferricyanide. These spectra were stable for at least 15 min.

oxidative power of ferro-ferricyanide with respect to cytochrome *c*, is significantly decreased. Moreover, Fig. 1 shows that temperature variations influence the midpoint potential of the two systems to a different extent in that mixed solvent. It is thus possible, by modifying temperature, to amplify (or to restrict) the difference between these redox potentials.

The consequences of these observations are shown by the experiments described in Fig. 4. In Fig. 4a we reproduce the variation of the midpoint potential of ferro-ferricyanide and cytochrome *c*, as a function of increasing concentration of ethylene glycol and in Fig. 4b, in the mixture water/ethylene glycol 50/50 (v/v), as a function of decreasing temperature. In Fig. 4c are presented the spectral changes accompanying the reaction between cytochrome *c* and ferro-ferricyanide, for three experimental conditions denoted I, II and III. In pure water (I) starting from a solution of cytochrome *c*, whose fraction of the reduced form is 0.65, a known amount of ferro-ferricyanide involves a nearly complete oxidation, 0.06 being the fraction of remaining reduced cytochrome *c*. When the same experiment is repeated in 50% ethylene glycol the fraction of the reduced form is only decreased to 0.47 (II). Decreasing temperature to −16°C in this mixed solvent restores a nearly complete oxidation, the fraction of reduced cytochrome *c* being 0.12 (III).

Conclusion

Organic solvents and temperature appear as powerful perturbants of redox reaction between cytochrome *c* and ferro-ferricyanide, according to their effect on the midpoint potential of these systems. It is not unreasonable to suspect that these effects will occur with other redox systems. Accordingly, in the interpretation of results derived from studies of redox reactions in cooled hydro-organic media, these possible solvent-temperature perturbations should be taken into account. Finally, this work suggests that organic solvents as well as temperature could be used as tools in the investigation of these reactions.

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