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Unfolding pathway of cytochrome *c* oxidase induced by ionic surfactants: Circular dichroism and picosecond time-resolved fluorescence studies

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Abstract. The unfolding of the membrane protein, cytochrome *c* oxidase (CcO) induced by ionic surfactants have been studied by using circular dichroism, optical absorbance and time resolved tryptophan fluorescence spectroscopic methods. Ionic surfactant cetyltrimethyl ammonium bromide (CTAB) was found to cause denaturation of this membrane protein leading to release of both, the heme *a* residues from CcO indicated by both CD and optical titration. Upon dissociation of the hemes from the protein matrix; the tryptophan fluorescence intensity of CcO increased drastically and the fluorescence lifetimes became much longer compared to the short lifetimes observed in the native protein. The shortest lifetime of 70 ps observed in the native protein due to strong quenching (energy transfer) of the heme groups, increased ~ 10-fold in the CTAB-unfolded protein indicating complete removal of the heme groups from the protein matrix. Remarkable differences were observed between the mode of actions of ionic surfactants and the commonly used denaturant guanidine hydrochloride. Improved data analysis of maximum entropy method showed that the lifetime distribution pattern in the two cases of unfolding were very different. The lifetimes in guanidine hydrochloride unfolded CcO were much shorter and more widely distributed indicating that the hemes are probably not separated away from the protein matrix and that the unfolded state is highly heterogeneous. Our results further showed that the lauryl maltoside inhibits denaturation of CcO by the ionic surfactant and the initial step of the denaturation possibly involves quantitative replacement of the lauryl maltoside by the ionic surfactant at the surface of the enzyme.

Keywords. Cytochrome *c* oxidase; unfolding; tryptophan fluorescence; maximum entropy method; ionic detergent.

1. Introduction

Cytochrome *c* oxidase (CcO, EC 1·9·3·1) is the terminal oxidoreduction complex of the respiratory chain which catalyzes the transfer of four electrons from its substrate ferrocytochrome *c* to molecular oxygen and simultaneously pumps protons across the inner mitochondrial membrane from the matrix to the cytosolic side [1–3]. In mammalian CcO, 13 different polypeptides have been discovered and these polypeptide units interact with each other with a varying amount of stabilization energy to form the

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complex. CcO contains two heme *a* moieties (named as heme *a* and heme a_3) and two copper centers (bimetallic Cu_A, and Cu_B) as prosthetic groups [4]. Heme a_3 and Cu_B interact with each other and form the binuclear oxygen-binding site. Heme *a* and heme a_3-Cu_B binuclear sites are located in subunit I while Cu_A resides in subunit II 4. The domain structure of CcO is indicated to comprise of two domains namely, matrix and cytosolic domains [5]. Denaturation studies with guanidine hydrochloride [6] indicated that the cytosolic domain is less resistant to denaturation than the matrix domain.

Surfactants are known to induce change in protein conformation even at very low concentrations [7]. The altered conformation of the protein has been proposed to have more affinity than the native protein to bind to the surfactants, leading to cooperativity in the surfactant binding. Ionic surfactants, such as sodium dodecyl sulphate (SDS), are commonly used for denaturation of proteins [8-10]. The mode of interaction of ionic surfactants may vary from one protein to other. Surfactants can disrupt the structure of a hemeprotein either by interacting directly with the heme cavity, or by binding elsewhere on the protein surface. It was earlier shown by us that surfactants, both cationic and anionic, can cause release of heme-NO species from nitric oxide complex of myoglobin [11]. The released heme complexes have been shown to be encapsulated as monomers inside aqueous micellar phase. Involvement of both charge and hydrophobic interactions exerted by the ionic surfactants, have been proposed to cause the denaturation. However, the molecular mechanism of the interaction of surfactants with proteins that causes unfolding is however not understood. Very few studies have been performed [12] with the aim of understanding the molecular mechanism of the surfactant-induced unfolding process.

Previous studies have shown that membrane proteins are generally more resistant to denaturation than their water-soluble counterparts [6,13]. CcO being a membrane protein, exhibits different behaviour towards denaturation by commonly used denaturants like guanidine hydrochloride or urea, compared to water soluble proteins. Neutral surfactants such as lauryl maltoside, Triton X-100 etc., are known to stabilize this enzyme in aqueous solution [14], while ionic surfactants were recently shown to denature it by removing both the heme *a* residues from the enzyme [15]. The mechanism of the action of ionic surfactants towards denaturation however, is not yet known. The amount of surfactant required to bring about the denaturation was shown to be much less than that of guanidine hydrochloride and it is expected that these two types of denaturations follow different mechanisms. Hence it is very important to study the interaction of ionic surfactants with CcO.

We report here detailed steady-state and time-resolved fluorescence, UV-visible absorption, circular dichroism and stopped-flow kinetic studies on interaction of the ionic surfactant, cetyltrimethyl ammonium bromide (CTAB) with CcO. CTAB was shown to induce a single phase unfolding transition in CcO structure while significant portion of the secondary structure remained intact. Studies were carried out at different concentrations of lauryl maltoside (LM) used to solubilise the enzyme, which showed that increasing concentration of LM tends to resist the unfolding of CcO by CTAB.

2. Materials and methods

Reagents

Cetyl trimethyl ammonium bromide (CTAB), sodium dodecyl sulfate (SDS), N-acetyl tryptophanamide (NATA), guanidine hydrochloride (Gdn.HCl) were purchased from

Sigma. Lauryl maltoside (LM) was a Fluka product. Other reagents used were of purest grade available commercially. Stock solutions of CTAB (5–10%) were prepared in 50 mM sodium phosphate, *p*H 7·4 and it was incubated at around 30°C before use.

Extraction of cytochrome c oxidase

Bovine cytochrome *c* oxidase (CcO) was extracted from beef heart muscles following the method of Yonetani [16]. The pellets obtained after repeated ammonium sulphate fractionation steps were dissolved in surfactant solution. For the experiments reported here, CcO samples dissolved in 0·5% lauryl maltoside, 50 mM sodium phosphate buffer, *p*H 7·4 were used. The concentration of CcO was determined by using $\Delta\varepsilon$ (reduced minus oxidized = 25 mM^{-1} cm^{-1}) at 605 nm [17].

Activity assay

Enzyme activity assay of CcO was performed in 50 mM sodium phosphate buffer, *p*H 7·4 with 1 mM EDTA, 0·1% LM by reported method [17]. The enzyme was incubated for 10 min in different concentrations of CTAB solutions in 50 mM sodium phosphate buffer, *p*H 7·4. In the final assay, the incubated enzyme samples were sufficiently diluted to minimize CTAB concentration in the assay mixture. The electron transfer activity of the enzyme was determined spectrophotometrically by monitoring the decrease in absorbance at 550 nm peak of ferrocytochrome *c*. Ferrocytochrome *c* was prepared by adding sodium ascorbate solution to 3 mM stock solution of cytochrome *c* and then passing the mixture through Sephadex G-25 column equilibrated with 50 mM sodium phosphate, *p*H 7·4.

Steady state fluorescence and optical spectroscopy

Steady state fluorescence measurements were performed using a Shimadzu RF540 spectrofluorophotometer and optical spectra were recorded by Shimadzu UV-2100. The fluorescence spectra were collected using a Schott made WG-320 cut-off filter prior to emission monochromator, to eliminate the scattering contribution due to CTAB addition. Quantum yield for the tryptophan fluorescence of CcO was determined using NATA in water as the reference [14]. The excitation wavelength was at 295 nm and the emission spectra of NATA and enzyme samples were recorded in the range of 300–450 nm keeping all parameters identical for both. Monochromator slit width was 5 nm for both excitation and emission. The absolute quantum yield was calculated using the quantum yield of NATA as 0·13 [18].

Time-resolved fluorescence

Time-resolved tryptophan fluorescence studies were performed using the tunable picosecond dye-laser pulse, from a synchronously pumped cavity-dumped dye (Rhodamine 6G) laser driven by frequency-doubled output (at 532 nm) of the CW mode-locked Nd-YAG laser system described elsewhere [19]. Fluorescence decay profiles were collected using a time-correlated single photon counting set-up coupled to a microchannel plate. Half-width of the instrument response function was typically about 100 ps. The tunable output of the dye laser was frequency-doubled to generate the second harmonic beam at 295 nm and was used to excite the samples. Emission profiles were collected at the magic angle (54·7°) to eliminate any contribution from anisotropy decay. A Schott WG 320 cut-off filter was used to remove the contribution from scattering at wavelengths below 310 nm. CcO samples (10 μM) in 50 mM sodium

phosphate buffer, 0·1% lauryl maltoside, *p*H 7·4 were used for time resolved measurements. To get the fluorescence decay curves, $1 - 3 \times 10^4$ counts were collected in the peak channel (total counts, $5 - 10 \times 10^5$). Each data set was collected in 1024 channels and channel width was 38 ps. Before starting the accumulation of data for the enzyme samples, the apparatus response was checked by measuring the lifetime of NATA as a standard.

The observed fluorescence decay curves were analyzed as a sum of exponentials [20]. To get the amplitudes and lifetimes, an iterative reconvolution was applied using nonlinear least square regression by Marquardt's algorithm for parameter optimization. The goodness of exponential fit was determined from the randomness of the weighted residuals distribution at χ^2 value close to unity.

Analysis of the fluorescence decay profiles was also carried out by maximum entropy method (MEM) using a uniform distribution of the lifetime components in logarithmic time scale [14,21,22]. In MEM, an initial 150 lifetime values ranging from 0·01 to 10 ns equally spaced in a logarithmic scale were used. The final outputs of MEM analysis were displayed as distribution of lifetime components in a logarithmic scale.

Stopped-flow measurements

Millisecond kinetic studies were performed in an SF-61MX multi-mixing stopped-flow spectrometer (Hi-Tech Scientific Co., UK) which was operated in absorption mode. Data acquisition and analysis were performed in a PC486 using the IS1 Rapid Kinetic software package. A variable temperature circulating water-bath was used to maintain the temperature. All the experiments reported here were carried out at 24 ± 2°C.

3. Results and discussion

The change in intrinsic tryptophan fluorescence characteristics of CcO on treatment with increasing concentration of CTAB has been shown in figure 1. Spectrum *a* in figure 1A, corresponds to the native CcO, and on CTAB addition the fluorescence intensity increases very significantly as shown in the spectra $b \sim f$. The emission maxima and percentage change in quantum yield are plotted as function of CTAB concentration as shown in figures 1B and 1C. The changes were found to occur at a fixed molecular ratio of [CTAB] to [CcO], and the change was complete at [CTAB]:[CcO] $\approx$ 2200:1. The emission maximum was shifted [1] by approximately 10 nm red (figure 1B) at saturation concentration of the surfactant. Quantum yield increased very significantly up to 3·4 fold to that of native CcO on addition of CTAB (figure 1C). The change occurred following a single transition, and higher concentration of CTAB (up to a ratio of 15000:1) did not cause any further change in fluorescence quantum yield. The anionic surfactant, SDS also caused similar fluorescence changes in CcO with increasing SDS concentration.

The effect of CTAB on CcO was also monitored by circular dichroism (CD) in the UV (protein region) and near UV-visible (Soret) regions (figure 2). In both the cases, transition was complete when a particular [CTAB]:[CcO] ratio was reached. In the UV region, CD band at around 220 nm was diminished by $\sim$ 40% at high CTAB. The change in the UV region occurred at lesser [CTAB] than that required for similar change in the Soret CD (figure 2B) or tryptophan fluorescence (figure 1C). This indicates that CTAB first opens up the secondary structure of CcO making entry to unfold the heme pocket. This is consistent with the structural disposition of the various

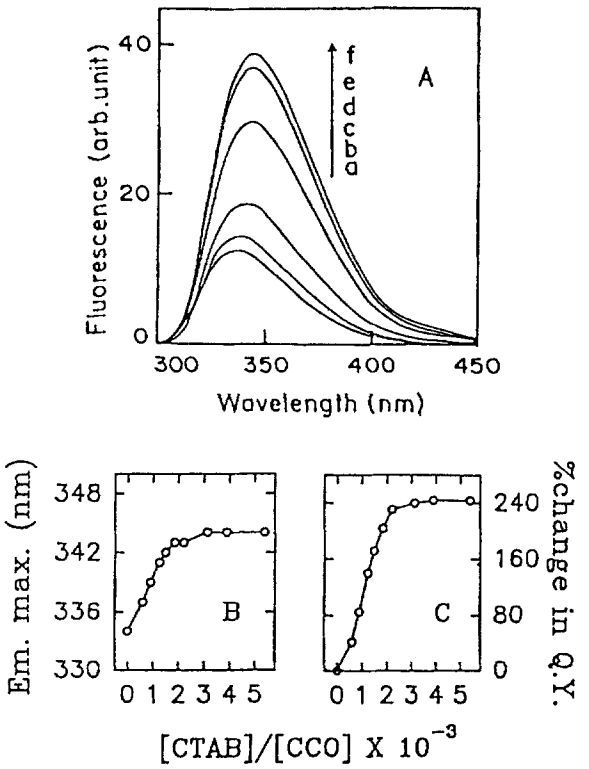

Figure 1. (**A**) Enhancement of tryptophan fluorescence by addition of CTAB to CcO (10 μM, 50 mM sodium phosphate, 0·1% LM, *p*H 7·4). Traces b-f correspond to CTAB concentrations of 4·1, 6·0, 8·2, 10·3 and 12·0 mM respectively, and *a* corresponds to the spectra of the native CcO. (**B**) Plot of the emission maxima and (**C**) Percent changes in quantum yield as a function of [CTAB]:[CcO] ratio.

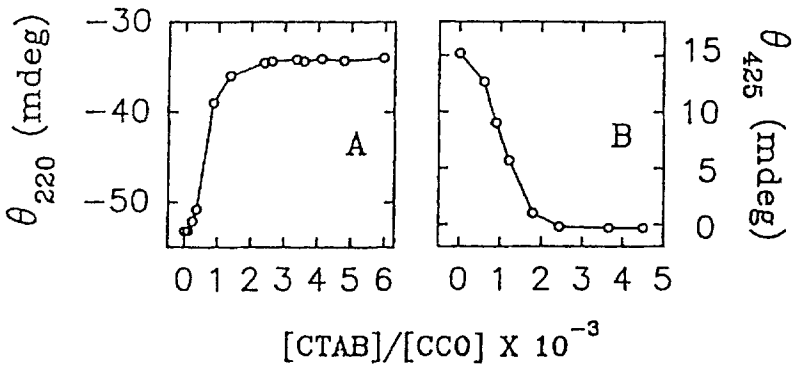

Figure 2. Circular dichroism changes as a function of [CTAB]:[CcO] ratio (**A**) at 220 nm and (**B**) at 425 nm. Sample conditions are same as in figure 1.

subunit of CcO which surrounds the heme-containing subunit I. The major change in tryptophan fluorescence occurs when the heme groups are separated away from the protein matrix, thus eliminating the strong quenching effect of the heme on

tryptophans. The Soret CD band almost completely vanished at the end point of the monophasic transition. The optical activity in the Soret region arises due to the heme *a* and heme a_3 [25]. The presence of the protein environment around the heme gives rise to large ellipticity of the Soret band in hemeproteins [25]. Removal of the heme from hemeproteins and its subsequent encapsulation inside micelles result in an almost isotropic environment around the heme moiety, thus showing a negligibly small ellipticity in the Soret region [15]. However, when the heme is not dissociated from the protein matrix, for example, in SDS induced unfolded cytochrome *c* heme CD band is shifted in position compared to the native protein but does not vanish [26]. Thus in the present case, disappearance of the heme CD band of CcO on addition of CTAB indicates depletion of the heme *a* moiety from the enzyme.

The kinetics of unfolding of CcO (5 μM in 50 mM sodium phosphate, 0·1% LM, *p*H 7·4) by CTAB (40 mM) was followed by stopped-flow absorbance. The unfolding was essentially complete in 20 sec. Transient absorbance difference spectra was generated at different time intervals and they showed a single isosbestic point, indicating a single phase transition (data not shown). Addition of CTAB or SDS causes a blue shift (from 421 nm to 410 nm) in the Soret band absorption maxima of CcO in LM solution, and a band arises at 631 nm characteristic of high spin heme *a* species [15]. The absorption spectra of CTAB treated CcO (or SDS treated CcO) was found to match with that of heme *a* (isolated from CcO by the method of Yanagi *et al* [27]) encapsulated in the micelle [15].

The changes in the activity of CcO as a function of CTAB concentration is shown in figure 3. The activity was measured in terms of the electron transfer rate between CcO and its physiological partner, cytochrome *c*. CcO samples incubated in different concentrations of CTAB were tested for their electron transfer ability by monitoring the 550 nm band of ferrocytochrome *c* for the first 60 seconds. The maximal activity of the native enzyme preparation used here was around 9000 min^{-1} at 25°C, *p*H 7·4. The activity decreased steeply as CcO was incubated in higher concentration of CTAB, and almost disappeared for [CTAB]:[CcO] > 1300:1. This ratio however, was found to be much less than that needed to cause complete disappearance of, for example, heme-CD bands. This indicates that loss of enzyme function occurs at lower concentration of the denaturant (i.e., CTAB) compared to that required for the loss of heme cavity structure.

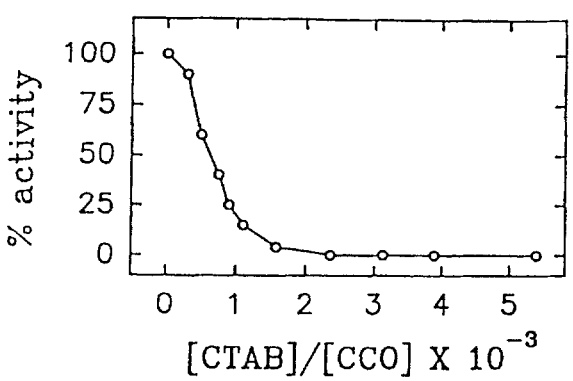

Figure 3. Percent changes in the activity (electron transfer) of cytochrome *c* oxidase as a function of [CTAB]:[CcO] ratio (see Materials and methods).

The specific molecular ratio of CTAB to CcO required for complete denaturation was found to be highly dependent on LM concentration. To understand the effect of LM concentration on CTAB-induced denaturation of CcO, experiments were carried out at different concentrations of LM and it was found that on increasing LM concentration, the [CTAB]:[CcO] ratio required for 50% denaturation (determined from the shift in the Soret band) increased linearly. The intercept of the plot (figure 5) of [CTAB]:[CcO] *vs* [LM] was found to be approximately 350. The intercept value, can be tentatively ascribed to be the number of CTAB molecules bound per CcO unit at equilibrium in the absence of LM, to cause 50% denaturation.

CcO is generally extracted in active form in neutral surfactants such as lauryl maltoside, Triton X, Tween-80 etc. LM is able to both disperse and activate purified CcO better than any other surfactant[28]. Some investigators have found it to be the surfactant of choice for studying rhodopsin[29] and photosynthetic reaction center[30] suggesting that this surfactant may have properties suitable for studies on a variety of intrinsic membrane proteins. The number of bound LM surfactant molecules has also been reported to be as high as ~ 320 per CcO molecule[31].

Presence of LM seems to inhibit denaturation of CcO by CTAB. Higher concentration of CTAB was required to bring about the same extent of denaturation of the enzyme at higher LM concentration (figure 4). The present results indicate that the LM molecules which were bound to the surface of CcO, are possibly replaced by CTAB molecules. The interaction of CTAB with CcO is however expected to be different from that of LM with CcO. In the process of denaturation, CTAB could exert both charged and hydrophobic interaction as was proposed for CTAB-induced unfolding of other hemeproteins[11].

The time-resolved decay of tryptophan fluorescence of CcO in LM solution shows four bands in the lifetime distribution profile[14]. Due to energy transfer to the heme groups, the major component (more than 50%) of lifetimes appears as low as 70 ps. Figure 5 shows the tryptophan fluorescence decay of the (*a*) native and (b) CTAB-treated CcO along with the residuals distribution of exponential analysis. Curve *a* was best fitted to minimum of four exponentials[14] whereas curve *b* could be analyzed as a sum of three exponentials. Residuals distribution for both the cases shows the

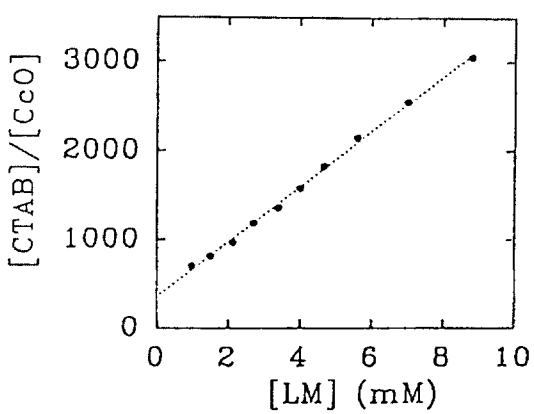

Figure 4. Plot for observed [CTAB]:[CcO] ratio as a function of LM concentration for 50% denaturation of CcO, determined from shift in the Soret band in optical spectra.

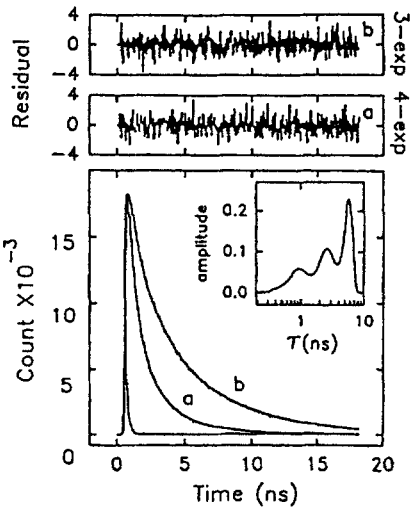

Figure 5. Tryptophan fluorescence decay of (**a**) native and (**b**) CTAB-treated CcO along with the residuals distribution for the four and three-exponential fits respectively. The unmarked trace is the instrument response function. Inset, the amplitude distribution of tryptophan fluorescence lifetimes of CTAB-treated CcO recovered from MEM analysis.

Table 1. Fluorescence decay parameters of CcO treated with CTAB or Gdn.HCl, recovered (**A**) by exponential fitting and (**B**) maximum entropy method (MEM)

A

Protein	$\tau_1(\alpha_1)$**	$\tau_2(\alpha_2)$	$\tau_3(\alpha_3)$	$\tau_4(\alpha_4)$	τ_m
Native CcO*	0·07 (53)	0·49 (23)	1·52 (20)	3·60 (4)	0·602
CTAB-CcO	0·79 (27)	2·67 (37)	5·92 (36)		3·34

B

Protein	τ_1^{M}**	τ_2^{M}	τ_3^{M}	τ_4^{M}
Native CcO*	0·08	0·50	1·56	3·60
CcO in 0·97 M Gdn.HCl	0·294	1·56	4·14	
CcO in 1·78 M Gdn.HCl	0·294	1·79	4·14	
CcO in 4·0 M Gdn.HCl	0·371	1·36	4·34	

*From Das and Mazumdar 1994 (Reference [14])

**τ and α are given in nanosecond and percentage respectively.

***τ^{M} represents peak values (baricenters) of lifetime distributions obtained from MEM analysis.

goodness of the fit (figure 6). Comparison of the traces *a* and *b* shows that the fluorescence lifetimes increased on treatment with CTAB. The shortest lifetime ($\sim$ 70 ps) found for the native CcO disappeared completely in CTAB-CcO and other lifetime components were also increased considerably (table 1). The mean lifetime value (τ_m) increased five-fold in CTAB-CcO compared to native CcO in LM.

We have also analyzed the tryptophan fluorescence decay of CcO by maximum entropy method (MEM) in different unfolding conditions which shows a 'model-free' distribution of lifetimes [14,21,32-34]. MEM analysis of the fluorescence decay for CTAB treated CcO (inset to figure 5) was found to consist of three distributions of lifetimes whose peak values (table 1) matched very well with those obtained from the discrete exponential model. The nature of life-time distribution recovered by MEM clearly indicates that the lifetimes obtained from the exponential model have significant spread around their 'most-probable' values.

A comparison of the denaturation processes by CTAB with that by guanidine hydrochloride (Gdn.HCl) would help in understanding the difference in the mode of action of these two denaturing agents towards CcO. Hill and coworkers [6] observed a biphasic change in fluorescence quantum yield of CcO on Gdn.HCl treatment. We carried out detailed time-resolved fluorescence experiments on Gdn.HCl denaturation of CcO. The time-resolved decays of CcO in presence of Gdn.HCl could not be analyzed using simple discrete exponentials model and the lifetime distributions were obtained by MEM analysis, indicating that addition of Gdn.HCl causes formation of a heterogeneous mixture of species from the enzyme. Figure 6 shows the lifetime distribution profiles of CcO fluorescence recovered by MEM at different concentrations of Gdn.HCl (traces *b*, *c* & *d*). The MEM profile for the native CcO is also plotted in trace *a* (figure 6) for comparison. Lifetime components centered around 80 ps obtained in native CcO disappeared on Gdn.HCl denaturation. At Gdn.HCl concentrations of 1 M or above, three bands were observed which broadened towards longer lifetime side with increasing concentrations of the denaturant, indicating that longer lifetime components increase with denaturation.

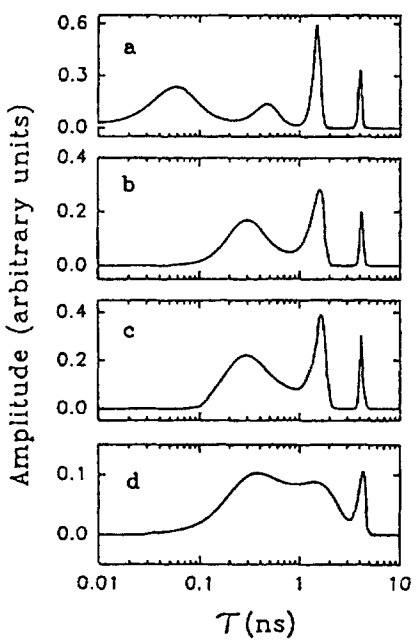

Figure 6. MEM recovered lifetime distributions for tryptophan fluorescence of CcO in absence (**a**) and in presence of (**b**) 0·97 M, (**c**) 1·78 M and (**d**) 4·0 M guanidine hydrochloride. Sample conditions are the same as in figure 1.

The tryptophan fluorescence characteristics of the enzyme thus clearly suggests that the unfolding pathway of CcO induced by Gdn.HCl is different from that induced by CTAB. Addition of large excess of Gdn.HCl was shown [6] to cause a distinct red shift in the Soret band absorption maximum of CcO which is opposite to the observation of blue-shifted spectrum in CTAB-CcO. A comparison of the lifetime patterns in unfolded CcO by CTAB and Gdn.HCl suggests that in CTAB-CcO the heme groups are separated away from the protein matrix so that the longer lifetimes become clear majority. However, in Gdn.HCl-unfolded CcO, the heme groups seem to remain bound to the protein matrix so that the quenching efficiency of heme is still effective on tryptophan fluorescence yield.

Guanidine hydrochloride causes subunit dissociation in CcO and a complex containing heme together with subunits I, II & IV was isolated [6]. The decrease in CD in the UV region (protein region) showed a single transition in case of CTAB induced denaturation of CcO (figure 2A) and even at a high concentration of CTAB around 64% of the signal around 220 nm was retained. Present results indicated that a significant amount of helicity remains intact in case of CTAB-induced unfolding of CcO. Treatment with high concentration of Gdn.HCl, on the other hand retains only small amount of β-sheet structure in the protein [6].

Membrane proteins exhibit a high resistance to denaturation [13,35,36] whereas the water soluble proteins unfold in a cooperative fashion in a narrow range of denaturant concentration. The biphasic response of CcO to the denaturant Gdn.HCl has earlier been [10] described as a combination of the responses observed for water soluble and integral membrane proteins. In the present case, a single sharp transition on addition of CTAB is detected by both CD and fluorescence titration indicating a limited but cooperative unfolding of a part of the CcO structure. This transition was complete at a particular molecular ratio of CTAB to CcO for a given LM concentration. A fixed molecular ratio found for this irreversible unfolding transition indicates that the binding of CTAB to the protein is important to initiate unfolding.

4. Conclusions

Time resolved tryptophan fluorescence studies on CcO in presence of CTAB show distinct effect on the lifetimes of CcO due to depletion of heme *a* from the enzyme. The native enzyme shows a four exponential fluorescence decay while addition of CTAB gives a three exponential fluorescence decay with much longer lifetimes. The fast picosecond lifetime component of CcO vanishes on addition of CTAB. Activity of the enzyme was also found to decrease sharply on addition of CTAB. Presence of lauryl maltoside seems to protect the enzyme from CTAB induced denaturation. Lifetime distribution of fluorescence was found to broaden with increasing concentration of Gdn.HCl while similar effect was not observed with CTAB. Circular dichroism of the heme Soret band vanished on addition of either CTAB or Gdn.HCl but the CD of the protein region suggests conservation of more secondary structure in case of CTAB-induced denaturation of CcO.

The inactivation of CcO and release of heme *a* by CTAB or SDS may involve electrostatic binding of the ionic surfactant to the enzyme. Both cationic and anionic surfactants can bind to the enzyme surface around its heme crevices to cause the release of the heme. Moreover, since the number of anionic amino acids (Glu, Asp) on the surface of CcO is larger than that of cationic amino acid residues (Lys, Arg), the cationic

surfactant (CTAB) is more effective than the anionic surfactant (SDS). The charged head groups of surfactant molecules possibly anchor to the enzyme and their hydrophobic tails may interact with the hydrophobic amino acids to break the structure of the enzyme. The iron-histidine bonds become severely weak because of the change in protein structure in the vicinity of heme centers leading to the release of heme *a* from the enzyme.

Acknowledgments

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Footnote

(1) It is to be noted that the use of WG-320 cut-off filter makes an apparent 4 nm red-shift in the emission maxima by eliminating the intensities below 310 nm. This filter is required in the present case [14,19,23] since increasing CTAB concentration increases scattering in the emission spectra. The native CcO shows fluorescence maximum between 328–330 nm [14,24] this was shifted to 334 nm by using this cut-off filter (figure 1B). Addition of CTAB maximally shifts the emission peak of 334 nm (340 nm without the filter).

Abbreviations

Cetyltrimethyl ammonium bromide, CTAB; sodium dodecyl sulphate SDS; N-acetyl tryptophanamide, NATA; lauryl maltoside, LM; Guanidine hydrochloride, Gdn.HCl; cytochrome *c* oxidase, CcO; Maximum Entropy Method, MEM.

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