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Journal of Pharmaceutical and Biomedical Analysis 15 (1997) 1215–1222

JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS

# Time-resolved study of effect of chlorpromazine on mobility of cytochrome *P*-450 and phospholipids in the inner membrane of adrenocortical mitochondria[1]

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Received 6 August 1996; accepted 25 September 1996

## Abstract

The effects of chlorpromazine on the mobility of cytochrome *P*-450 and the fluidity of lipid membranes have been investigated in bovine adrenocortical submitochondrial particles (SMP). Rotational diffusion of the cytochrome was measured by observing the decay of absorption anisotropy, $r_a(t)$, after photolysis of the heme·CO complex by a vertically polarized laser flash. Analysis of $r_a(t)$ was based on a 'rotation-about-membrane-normal' model. The anisotropy decayed within 2 ms to a time independent value $r_3$. The presence of chlorpromazine decreased the mobile population of cytochrome *P*-450 from 28 to 23%. The rotational relaxation time $\phi_a$ of the mobile population ($\sim 1100$ μs) was, however, not significantly changed by chlorpromazine. The lipid fluidity was examined by observing time-resolved fluorescence anisotropy, $r_f(t)$, of 1,6-diphenyl 1,3,5-hexatriene (DPH). The anisotropy $r_f(t)$ decayed within 70 ns to a time independent value $r_\infty$. The motion of DPH was analyzed based on a 'wobbling-in-cone' model. The presence of chlorpromazine decreased the cone angle from 42° to 39°, while the rotational relaxation time $\phi_f$ ($\sim 2$ ns) was not significantly changed by the presence of chlorpromazine. These results demonstrate that chlorpromazine decreased the mobility of not only lipids but also membrane proteins. © 1997 Elsevier Science B.V.

*Keywords:* Chlorpromazine; Cytochrome *P*-450; Protein rotation; Fluorescence; Lipid fluidity

*Abbreviations:* ADR: NADPH-adrenodoxin reductase; ADX: adrenodoxin; CPZ: chlorpromazine; Cytochrome P450scc: cytochrome P450 having cholesterol side-chain cleavage activity (P45011A1); Cytochrome P45011*β*: cytochrome P450 having 11 *β*-hydroxylation activity (P45011B1); DPH: 1,6-diphenyl 1,3,5-hexatrien; SMP: submitochondrial particle.

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[1] Presented at the Seventh International Symposium on Pharmaceutical and Biomedical Analysis, August, 1996, Osaka, Japan.

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*PII* S0731-7085(96)01980-2

## 1. Introduction

Tranquilizers and local anaesthetics are amphiphilic drugs and surface-active compounds. The pharmacological effects of these drugs were often interpreted as being due to their interactions with phospholipids in the membranes of e.g. nerve cells. Consistent with this idea, these drugs have been observed to inhibit various biochemical processes that depend on phospholipids such as the phospholipid-dependent activity of protein kinase C [1–3] and adenylate cyclase [4]. Chlorpromazine, a tranquilizer, was intensively investigated for interactions with the lipid bilayer, however, as yet an inadequate amount of evidence has been accumulated for interactions with membrane proteins, except for studies in erythrocytes [5]. Spin label studies suggested that chlorpromazine interacted preferentially with erythrocyte membrane proteins rather than membrane lipids [6].

Here we investigate interactions of chlorpromazine with adrenocortical mitochondria which have been extensively analyzed for dynamic properties of membrane proteins as well as lipids. Adrenocortical mitochondria play a crucial role for steroid hormone synthesis which is performed by two types of cytochrome *P*-450s which are *P*-450scc and *P*-45011 $\beta$. Both cytochromes are integral membrane proteins. Cytochrome *P*-450scc carries out side chain cleavage of cholesterol resulting in pregnenolone production [7]. Cytochrome *P*-45011 $\beta$ catalyzes 11 $\beta$-hydroxylation of various steroids [8]. For these catalyses, both cytochromes require electrons which are transferred from NADPH to the *P*-450s via NADPH-adrenodoxin reductase (ADR) and adrenodoxin (ADX) [9].

Rotational mobility of the cytochrome is sensitive to changes in the membrane. Rotational mobility was, for example, significantly dependent on the presence and absence of redox partners such as ADX and ADR [10].

Here we report the influence of chlorpromazine on the rotational mobility of not only lipids but also cytochrome *P*-450 in order to obtain a comprehensive and quantitative view of the effect of tranquilizers on biological membranes. Time-resolved measurements provide quantitative information about motions of proteins and lipids, as compared with conventional time-averaged studies such as steady-state fluorescence and spin label measurements which are more qualitative.

## 2. Experimental procedures

### 2.1. Preparations of SMP and proteins

SMP were prepared from freshly prepared mitochondria from the zona fasciculata-reticularis of bovine adrenal cortex as previously described [10,11]. Briefly, after pressing mitochondria once through a French Pressure Cell at 16 000 p.s.i., unbroken mitochondria were removed by centrifugation at $27\,000 \times g$ for 10 min. The SMP were sedimented by centrifugation at $100\,000 \times g$ for 60 min. The SMP were resuspended in 50 mM Hepes buffer (pH 7.4) containing, 320 mM mannitol, 1 mM EDTA and 0.1 mM dithiothreitol, and were stored at − 80°C. The content of cytochrome *P*-450 in SMP was around 1.3 nmol *P*-450 per mg protein. ADX and ADR were purified from bovine adrenal glands according to the methods of Suhara et al. [12] and Montelius et al. [13], respectively.

### 2.2. Chemicals

Chlorpromazine was purchased from Wako Pure Chemicals (Japan). DPH was from Nakarai Chemicals (Japan). BCA protein assay reagent was from Pierce Chemical Company (USA). Resorufin 3 $\beta$-hydroxy-22,23-bisnor-5-cholenyl ether (cholesterol-resorufin) was from Molecular Probes (USA). Other chemicals were of the highest grade commercially available.

### 2.3. Cholesterol side chain cleavage activity of cytochrome P-450scc

The cholesterol side-chain cleavage activity of cytochrome *P*-450scc in SMP was measured with a Hitachi F-3000 spectrofluorometer using cholesterol-resorufin as a substrate according to Marrone et al. [14]. Cholesterol-resorufin dissolved in

DMSO was incorporated into SMP by incubation for 40 min at 37°C. The cleavage reaction was initiated by the addition of 500 μM NADPH (final concentration) to SMP suspensions containing 0.25 μM ADR, 5 μM ADX, 10 μM cholesterol-resorufin, 1.0 μM cytochrome *P*-450 (about 0.5 μM *P*-450scc) in 50 mM Hepes buffer pH 7.4, at 37°C. Upon conversion of cholesterol-resorufin to pregnenolone and resorufin by *P*-450scc, the fluorescence at 590 nm was linearly increased due to the production of resorufin anion, using 530 nm for excitation. The pregnenolone production activity was calculated to be 64 pmol $nmol^{-1}$ *P*-450scc $h^{-1}$.

### 2.4. Rotational diffusion measurements of cytochrome P-450 and analysis

SMP were incubated with chlorpromazine for 30 min at room temperature with the concentration ratio of 40 nmol $mg^{-1}$ for chlorpromazine to protein. For rotational diffusion measurements, 60% (w/w) sucrose was dissolved in SMP suspensions (50 mM Hepes buffer, pH 7.4, 1 mM EDTA, 0.1 mM dithiothreitol) in order to reduce light scattering and SMP tumbling. The final concentration of chlorpromazine was 118 μM. The sample was bubbled with CO for 5 s and then reduced with a few grains of dithionite. The sample cuvette was then sealed by a rubber cap to keep CO concentration constant.

The time-resolved flash photolysis anisotropy measurements was performed as described elsewhere [15–17]. The sample (4 μM in heme) was photolyzed by a vertically polarized flash at 532 nm from a Nd/YAG laser (DCR-2, Spectra Physics) and absorbance changes were measured at 450 nm. The signals were analyzed by calculating the absorption anisotropy, $r_a(t)$, and the total absorbance change, $A(t)$, given by

$$r_a(t) = [A_V(t) - A_H(t) \cdot S_a]/A(t) \quad (1)$$

$$A(t) = A_V(t) + 2A_H(t) \cdot S_a \quad (2)$$

where $A_V(t)$ and $A_H(t)$ are, respectively, absorption changes for vertical and horizontal polarization at time $t$ after the laser flash. A slight unbalance of the two photomultipliers was corrected using $S_a = A_{HV}/A_{HH}$ which is the ratio of time averaged absorption changes of vertical and horizontal components obtained with horizontal flash excitation. In each experiment, 16 384 signals were averaged using a Toyo Technica 2805 transient memory.

Analysis of $r_a(t)$ is based on a model of the axial rotation of cytochrome *P*-450 about the membrane normal [18–20]. When there is a single rotating species of cytochrome *P*-450 with rotational relaxation time $\phi_{\parallel}$, $r_a(t)$ is given by

$$\begin{aligned} r_a(t)/r_a(0) &= 3\sin^2\theta_N \cos^2\theta_N \exp(-t/\phi_{\parallel}) \\ &\quad + (3/4)\sin^4\theta_N \exp(-4t/\phi_{\parallel}) \\ &\quad + (1/4)(3\cos^2\theta_N - 1)^2 \end{aligned} \quad (3)$$

where $\theta_N$ is the tilt angle of the heme plane from the membrane plane. Multiple rotating species of cytochrome *P*-450 with different $\phi_{\parallel}$'s are considered by analyzing the data by

$$r_a(t) = r_1 \exp(-t/\phi_a) + r_2 \exp(-4t/\phi_a) + r_3 \quad (4)$$

where $\phi_a$ is the average rotational relaxation time over multiple rotating species of *P*-450 and $r_1$, $r_2$, $r_3$ are constants. Curve fitting of the data based on Eq. (4) was accomplished by a PDP-11/73 minicomputer. It should be noted that in Eqs. (3) and (4), $r_a(t)/r_a(0)$ does not depend on the intensity of the photoselecting flash and $r_a(0)$ only depends on the laser flash intensity [18]. The population of mobile *P*-450, $p_m$%, was calculated with Eq. (5) on the basis of the experimentally determined minimal anisotropy, $[r_3/r_a(0)]_{min} = (1/4)(3\cos^2\theta_N - 1)^2 = 0$, when all *P*-450s were rotating in SMP in the presence of 500 mM KCl [10]:

$$\begin{aligned} p_m(\%) &= 100 \times \{1 - r_3/r_a(0)\}/\{1 - [r_3/r_a(0)]_{min}\} \\ &= 100 \times \{1 - r_3/r_a(0)\} \end{aligned} \quad (5)$$

### 2.5. Time-resolved fluorescence anisotropy decay

Fluorescent labeling was performed by adding 5 μl of 1 mM DPH in tetrahydrofuran to 5 ml of SMP suspensions (0.63 mg protein $ml^{-1}$), and the suspensions were incubated for 90 min at room temperature. SMP were then incubated with

chlorpromazine for 30 min at room temperature with the concentration ratio of 80 nmol $mg^{-1}$, 50 μM in absolute concentration, for chlorpromazine to protein.

The nanosecond pulse fluorometer used for fluorescence decay measurements was detailed elsewhere [21,22]. Briefly the sample suspensions were excited by a vertically polarized flash of about 1-ns duration and the time course of fluorescence decays were measured by a single photon counting techniques. The excitation wavelength for DPH was 360 nm and all the fluorescence above 460 nm was collected through cut-off filters (SC46, Y46). The signals were analyzed by calculating the total fluorescence intensity $I_T(t)$ and the fluorescence anisotropy $r_f(t)$, given by

$$I_T(t) = I_V(t) + 2I_H(t) \tag{6}$$

$$r_f(t) = [I_V(t) - I_H(t)]/I_T(t) \tag{7}$$

where $I_V(t)$ and $I_H(t)$, respectively, represent the intensities of vertically and horizontally polarized components at time $t$ after the flash. A slight unbalance of two photomultipliers was corrected by changing the polarization of analyzer for each of two photomultipliers at every $10^6$ flashes. The quantities $I_T(t)$ and $r_f(t)$ are related to $I_T^\delta(t)$ and $r_f^\delta(t)$, the response to an impulsive excitation expressed as $\delta(t)$ by the equations:

$$I_T(t) = \int_0^t g(t')I_T^\delta(t-t')\,dt' \tag{8}$$

$$r_f(t)I_T(t) = \int_0^t g(t')r_f^\delta(t-t')I_T^\delta(t-t')\,dt' \tag{9}$$

where $g(t)$ is the response function of the apparatus [21].

Analysis of $r_f(t)$ is based on a model of the wobbling of DPH in a cone of half angle $\theta_C$ [23,24]. The decay curve was analyzed with

$$r_f^\delta(t) = (r_0 - r_\infty)\exp(-t/\phi_f) + r_\infty \tag{10}$$

$$r_\infty/r_0 = [\cos\theta_C(1+\cos\theta_C)/2]^2 \tag{11}$$

where $\phi_f$ is the average rotational relaxation time.

The fluorescence lifetime $\tau_i$ was determined by curve fitting procedures, by assuming that $I_T^\delta(t)$ was expressed as sums of exponential functions:

$$I_T^\delta(t) = \sum_{i=1}^{n} I_i \exp(-t/\tau_i) \tag{12}$$

The average lifetime is defined as

$$\langle\tau\rangle = \sum_{i=1}^{n} \alpha_i\tau_i \tag{13}$$

where

$$\alpha_i = I_i \Big/ \sum_{j=1}^{n} I_j \; (i = 1, \ldots, n) \tag{14}$$

is an exponential fraction. Curve fitting of the data based on Eqs. (8)–(10) was accomplished by a NEC PC-9801 personal computer.

In some experiments, the steady-state anisotropy $r^s$ was measured for quick investigation of the lipid fluidity using a Hitachi F-3000 spectrofluorometer equipped with a set of polarizer and analyzer. Steady-state anisotropy $r^s$ is related to the time-resolved anisotropy and intensity by

$$r^s = \int_0^\infty r_f^\delta(t)I_T^\delta(t)\,dt \Big/ \int_0^\infty I_T^\delta(t)\,dt \tag{15}$$

### *2.6. Other methods*

Cytochrome *P*-450 was measured spectrophotometrically with a Shimazu MPS-2000 spectrophotometer according to Omura and Sato [25]. Protein concentration was determined with the BCA protein assay using bovine serum albumin as standard.

## 3. Results and discussions

### *3.1. Effect of chlorpromazine on rotational diffusion of cytochrome P-450 in SMP*

Rotational diffusion measurements were performed at 20°C. In all samples examined, the absorption anisotropy $r_a(t)$ decayed within 2 ms to a time-independent value $r_3$ (Fig. 1). The $r_a(t)$ curves were analyzed by Eq. (4) on the basis of the rotation about the normal axis of the membrane plane. Decay parameters are summarized in Table 1. Coexistence of mobile and immobile populations for cytochrome *P*-450 was present in SMP. The rotational relaxation time $\phi_a$ (inversely

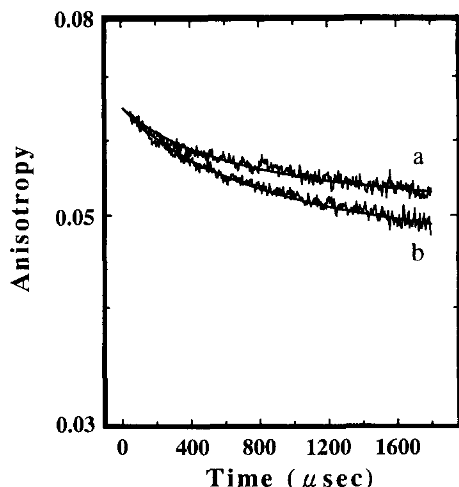


Fig. 1. Effect of chlorpromazine on the time-dependent absorption anisotropy of cytochrome $P$-450 in submitochondrial particles. Samples (4 $\mu$M in heme) were photolyzed by a vertically polarized laser flash at 532 nm, and $r_a(t)$ was recorded at 450 nm as described under Experimental Procedures. Measurements were performed in 60% sucrose solution at 20°C ($\sim$0.6 poise). Curve $a$, $r_a(t)$ in the presence of chlorpromazine; curve $b$, $r_a(t)$ in the absence of chlorpromazine. The zigzag lines are experimental data and the solid curves were obtained by fitting the data to Eq. (4). The initial anisotropy of curve $b$ is slightly normalized to the same $r_a(0)$ of curve a to facilitate comparison. This is justified by the fact that although $r_a(0)$ depends on the laser flash intensity, the normalized anisotropy $r_a(t)/r_a(0)$ is not affected by the different flash intensity [18].

proportional to the rate of rotation) for the mobile population was around 1100 μs. The presence of chlorpromazine decreased the mobile population from 28 to 23%, while $\phi_a$ was not changed within the experimental error (Table 1).

This 5% decrease in the mobile population by chlorpromazine is significant but not very large as compared with, for example, the 19% mobilization of $P$-450 induced by the presence ADX and 6% immobilization of $P$-450 by the further addition of ADR to this system of SMP plus ADX [10]. It should be noted that SMP do not contain either ADX or ADR, because these peripheral proteins were removed during the French Press procedures to prepare vesicles of the inner mitochondrial membranes. Since interactions of $P$-450 with ADX and ADR are essential for the electron transfer activity, these numbers of 6–19% for mobilization/immobilization should be a significant change.

The 77% of cytochrome $P$-450 which was immobile has a rotational relaxation time $\phi$ larger than 10 ms in the presence of chlorpromazine. The immobile molecules of $P$-450 probably form microaggregates, because theoretically the population rotating very slowly with a large $\phi$ should have a large size. The rotational relaxation time $\phi_{\parallel}$ in Eq. (3) is expressed as $\phi_{\parallel} = 4\pi a^2 h\eta/kT$ and hence $\phi_{\parallel} \propto a^2$ where $a$ is the radius of the cross section of a cylindrical protein immersed in the membrane, $h$ the length immersed in the membrane, $\eta$ the membrane viscosity, $k$ the Boltzmann constant, $T$ the absolute temperature. We can estimate an approximate radius $a_1$ of the immobile protein aggregates having $\phi_1 \geq 10$ ms to be $a_1/a_2 \geq [10 \times 10^3$ μs/40 μs] deduced from a $\phi_2$ value of 40 μs for monomeric P–450 [26]. Therefore, the immobile $P$-450s are microaggregates having the diameter of more than 16-fold of that of the monomer. However, considering the rela-

Table 1
Decay parameters of the time-dependent absorption anisotropy of the cytochrome $P$-450. *CO complex in submitochondrial particles analyzed by Eq. (4)

| Samples | $\phi_a$ ($\mu$s) | $r_3/r(0)$ | Mobile $P$-450(%)[a] | Cpz/protein[b] (nmol mg$^{-1}$) |
|---|---|---|---|---|
| −Cpz | 1104 (57)[c] | 0.72 (0.01) | 28 (1) | 0 |
| +Cpz | 1052 (54) | 0.77 (0.02) | 23 (2) | 40 |

All measurements were performed in 60% sucrose at 20°C.
[a]The percentage of mobile cytochrome $P$-450 was calculated from Eq. (5).
[b]Cpz is chlorpromazine.
[c]Numbers in parentheses are standard deviation from 10 experiments.

Table 2
Effect of chlorpromazine on decay parameters of the time-dependent fluorescence anisotropy of DPH in submitochondrial particles analyzed by Eq. (4)

| Samples | $\phi_f$ (ns) | $r_\infty/r(0)$ | $\theta c$[a] (degree) | Cpz/protein[b] (nmol mg$^{-1}$) |
|---|---|---|---|---|
| −Cpz | 2.4 (0.1)[c] | 0.42 (0.01) | 42 (1) | 0 |
| +Cpz | 1.4 (0.1) | 0.48 (0.01) | 39 (1) | 80 |

All measurements were performed at 20°C without sucrose.
[a]The cone angle was calculated from Eq. (11).
[b]Cpz is chlorpromazine.
[c]Numbers in parentheses are standard deviation from several experiments.

tively small size of SMP with the circumference of around 314 nm (assuming 100 nm in diameter) and the size of monomeric *P*-450 of 4 nm in diameter, even smaller oligomers of 10–16 fold of monomeric diameter would be already immobile due to steric hindrance.

### *3.2. Influence of chlorpromazine on DPH wobbling in SMP*

Chlorpromazine decreased the membrane fluidity. By increasing the amount of chlorpromazine from 20 to 160 nmol mg$^{-1}$ protein, corresponding to an increase in the absolute concentration from 25 to 200 μM, the steady-state anisotropy $r^s$ of DPH was increased in the inner membrane of mitochondria. This effect of membrane rigidification was saturated at 80 nmol mg$^{-1}$ protein and no further increase in $r^s$ was observed for higher concentrations of chlorpromazine.

The lipid motion was quantitatively examined by observing the time-resolved fluorescence anisotropy, $r_f(t)$, of DPH. The anisotropy decayed with a rotational relaxation time $\phi_f$ of around 2 ns to a time independent value $r_\infty$ within the time scale of 70 ns. The motion of DPH, having a structure similar to that of a lipid acylchain, is well illustrated with wobbling motion in a cone of half angle $\theta_C$ [21,22,27,28]. The angular range $\theta_C$ of wobbling for DPH was decreased from 42 to 39° by the presence of chlorpromazine, while $\phi_f$ value was slightly decreased from 2.4 to 1.4 ns (see Fig. 2). The 7% decrease in the angular range $2\theta_C$ for wobbling of DPH may be significant but is not considerably large. The results imply that chlorpromazine rigidified the lipid bilayer to a certain extent, however this is far from a hard 'packing' of the bilayer. The present analysis using $\theta_C$ and $\phi_f$ is much more precise than the term 'fluidity' which is obtained by the steady-state

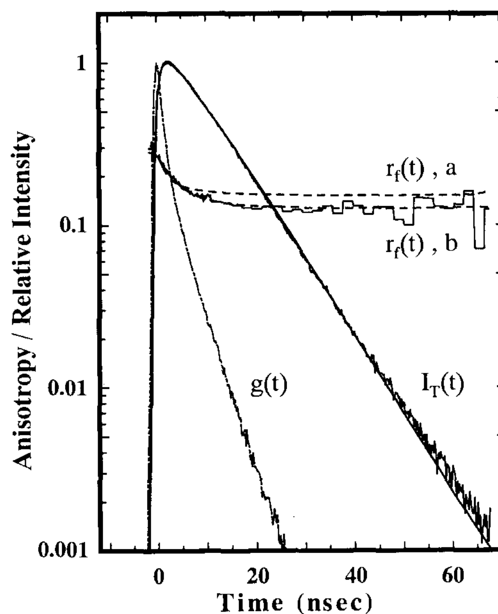


Fig. 2. Effect of chlorpromazine on the time-dependent anisotropy of DPH in submitochondrial particles. Measurements were performed under the conditions as described in Experimental Procedures. Curve *a*, $r_f(t)$ in the presence of chlorpromazine; curve *b*, $r_f(t)$ in the absence of chlorpromazine. $r_f(t)$: The zigzag line is experimental data, and the dashed lines were obtained by fitting the data to Eq. (10). $I_T(t)$: Zigzag line is experimental data in the absence of chlorpromazine, and the solid line is the best fit curve by a double-exponential approximation of Eq. (12) ($\alpha_1 = 0.412$, $\tau_1 = 2.6$ ns, $\alpha_2 = 0.588$, $\tau_2 = 9.1$ ns). $g(t)$ (chain line) is the apparatus response function.

anisotropy $r^s$ or by spin label measurements that show time-averaged numbers.

We used concentrations of 50 and 118 μM for chlorpromazine, respectively, for DPH motion experiments and *P*-450 rotation experiments. Both concentrations are below the critical micelle concentration of 300 μM for chlorpromazine in isotonic solution [29]. There are reports implying that chlorpromazine decreases the erythrocyte membrane fluidity at a high concentration around 10–50 mM but increases the fluidity at a low concentration around 0.1–4 mM [6]. This fluidity increase at 0.1–4 mM chlorpromazine may be due to difference in characteristics between erythrocytes and mitochondria where we observed a fluidity decrease at 25–200 μM chlorpromazine. It should be noted that in *E. coli* cell membranes we also observed a decrease in the lipid fluidity by increasing the concentration of chlorpromazine from 10 to 100 μM, and no further fluidity decrease was observed from 100 up to 250 μM [30].

### 3.3. Rebinding kinetics of CO to P-450 and fluorescence lifetime of DPH

The total absorption decay $A(t)$ of cytochrome *P*-450 was close to monophasic in SMP under the same conditions as those of rotational diffusion measurements. $A(t)$ was, therefore, analyzed by a monoexponential approximation. The lifetime of photodissociated reduced *P*-450, obtained from $A(t)$, was $\tau = 5.5$ ms. The presence of chlorpromazine did not change this number significantly. These results suggest that chlorpromazine does not affect the active site heme of cytochrome *P*-450.

The total fluorescence intensity $I_T^{\delta}(t)$ was analyzed by a double exponential approximation. The averaged fluorescence lifetime $\langle\tau\rangle$ of DPH was only slightly decreased from 6.4 ns to 5.0 ns by the presence of chlorpromazine.

### 3.4. Comparison with other investigations

Erythrocyte membranes were studied exceptionally extensively, since chlorpromazine may be transported to the brain and/or other organs by means of erythrocytes. The investigations with fatty acid spin labels showed two signals which are the strongly immobilized component and the weakly immobilized one in chlorpromazine-treated erythrocytes [6,31,32]. The strongly immobilized component was assigned to spin labels attached to membrane proteins and the weakly immobilized one to spin labels in the lipid bilayer.

Yamaguchi et al. [6] suggested that chlorpromazine induces packing of lipid domains, resulting in preferable binding of fatty acid spin labels to membrane proteins. Chlorpromazine was indicated to induce holes in erythrocyte membrane [33]. However, these results did not demonstrate the direct effect of chlorpromazine on the dynamic state of membrane proteins.

Besides the direct effect on membranes, which kind of other intracellular responses are induced by chlorpromazine? Sekimizu and co-workers [34] reported that chlorpromazine induced heat shock proteins and inhibited accurate transcription from promoters in nuclear extracts in *E. coli.* They observed a reasonable correlation between the induction of heat shock proteins and the lipid fluidity decrease in *E. coli* cell membranes, suggesting the participation of phospholipids in the system [30].

Investigations of direct interactions between chlorpromazine and membrane bound protein would much contribute to further quantitative understanding of the mechanisms of chlorpromazine effect on cell functions.

## 4. Conclusions

Chlorpromazine was first demonstrated to affect the mobility of membrane protein with time-resolved measurements. The mobility of both cytochrome *P*-450 and lipids was decreased by the presence of chlorpromazine in the inner membrane of mitochondria. These quantitative results are useful to understand characteristics of interactions of chlorpromazine with biological membranes.

## Acknowledgements

This work is supported by the grants from the Ministry of Education, Science and Culture in Japan.

## References


[1] D. Papahadjoupoulos, Biochim. Biophys. Acta, 265 (1972) 169–186.

[2] T. Mori, Y. Takai, R. Minakuchi, B. Yu and Y. Nishizuka, J. Biol. Chem., 255 (1980) 8378–8380.

[3] K. Sekimizu and A. Kornberg, J. Biol. Chem., 263 (1988) 7131–7135.

[4] R. Salesse, J. Garnier, F. Leterrier, D. Daveloose and J. Viret, Biochemistry, 21 (1982) 1581–1586.

[5] R. Salesse and J. Garnier, Biochim. Biophys. Acta, 554 (1979) 102–103.

[6] T. Yamaguchi, S. Watanabe and E. Kimoto, Biochim. Biophys. Acta, 820 (1985) 157–164.

[7] S. Takemori, H. Sato, T. Gomi, K. Suhara and M. Katagiri, Biochem. Biophys. Res. Commun., 67 (1975) 1151–1157.

[8] K. Suhara, T. Gomi, H. Sato, E. Inagaki, S. Takemori and M. Katagiri, Arch. Biochem. Biophys., 190 (1978) 290–299.

[9] T. Omura, E. Sanders, R.W. Estabrook, D.Y. Cooper and O. Rosenthal, Arch. Biochem. Biophys., 117 (1966) 660–673.

[10] Y. Ohta, K. Yanagibashi, T. Hara, M. Kawamura and S. Kawato, J. Biochem., 109 (1991) 594–599.

[11] M. Muller, J.J.R. Krebs, R.J. Cherry and S. Kawato, J. Biol. Chem., 257 (1982) 1117–1120.

[12] K. Suhara, S. Takemori and M. Katagiri, Biochim. Biophys. Acta, 263 (1972) 272–278.

[13] J. Montelius, S. Carlsson, J. Lindstedt, D. Galaris, B. Hoejeberg and J. Rydstroem, J. Steroid Biochem., 11 (1979) 1551–1559.

[14] B.L. Marrone, D.J. Simpson, T.M. Yoshida, C.J. Unkefer, T.W. Whaley and T.N. Buican, Endocrinology, 128 (1991) 2654–2656.

[15] R.J. Cherry, Methods Enzymol., 54 (1978) 47–61.

[16] S. Kawato, F. Mitani, T. Iizuka and Y. Ishimura, J. Biochem., 104 (1988) 188–191.

[17] Y. Ohta, F. Mitani, Y. Ishimura, K. Yanagibashi, M. Kawamura and S. Kawato, J. Biochem., 107 (1990) 97–104.

[18] S. Kawato and K. Kinoshita Jr., Biophys. J., 36 (1981) 277–296.

[19] S. Kawato, J. Gut, R.J. Cherry, K.H. Winterhalter and C. Richter, J. Biol. Chem., 257 (1982) 7023–7029.

[20] S. Komura, Y. Ohta and S. Kawato, J. Phys. Soc. Japan, 59 (1990) 2584–2595.

[21] S. Kawato, K. Kinoshita Jr. and A. Ikegami, Biochemistry, 16 (1977) 2319–2324.

[22] K. Kinoshita Jr., R. Kataoka, K. Yoshiaki, O. Gotoh and A. Ikegami, Biochemistry, 20 (1981) 4270–4277.

[23] K. Kinoshita Jr., S. Kawato and A. Ikegami, Biophys. J., 20 (1977) 289–305.

[24] S. Kawato, E. Sigel, E. Carafori and R.J. Cherry, J. Biol. Chem., 256 (1981) 7518–7527.

[25] T. Omura and R. Sato, J. Biol. Chem., 239 (1964) 2370–2378.

[26] J. Gut, C. Richter, R.J. Cherry, K.H. Winterhalter and S. Kawato, J. Biol. Chem., 257 (1982) 7030–7036.

[27] K. Kinoshita Jr., S. Kawato, A. Ikegami, S. Yoshida and Y. Orii, Biochim. Biophys. Acta, 647 (1981) 7–17.

[28] S. Suzuki, S. Kawato, T. Kouyama, K. Kinoshita Jr., A. Ikegami and M. Kawakita, Biochemistry, 28 (1989) 7734–7740.

[29] T. Ogiso, M. Kurobe, H. Masuda and Y. Kato, Chem. Pharm. Bull. (Tokyo), 25 (1977) 1078–1088.

[30] K. Tanji, Y. Ohta, S. Kawato, T. Mizushima, S. Natori and K. Sekimizu, J. Pharm. Pharmacol., 44 (1992) 1036–1037.

[31] D.E. Holmes and L.H. Piette, J. Pharmacol. Exp. Ther., 173 (1970) 78–84.

[32] G. Benga, M. Ionescu, O. Popescu and V.I. Pop, Mol. Pharmacol., 23 (1983) 771–778.

[33] M.R. Lieber, Y. Lange, R.S. Weinstein and T.L. Steck, J. Biol. Chem., 259 (1984) 9225–9234.

[34] K. Tanji, T. Mizushima, S. Natori and K. Sekimizu, Biochim. Biophys. Acta, 1129 (1992) 172–176.