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Conformational Changes of Cytochromes P-450$_{cam}$ and P-450$_{lin}$ Induced by High Pressure†

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ABSTRACT: Absorbance and fluorescence spectra of bacterial cytochrome P-450$_{cam}$ and cytochrome P-450$_{lin}$ have been studied as a function of pressure. These pressure-induced spectral perturbations fall into two categories, which are interpreted as resulting from denaturation domains and are discussed in terms of protein structural dynamics. The results presented herein support a view that these two bacterial cytochromes have large structural differences and suggest a picture in which the gellike cortex of each protein may play an essential role in stability and function.

High pressure has been used to induce conformational changes in several heme proteins (Ogunmola et al., 1977), and this perturbation has been previously applied to the bacterial cytochrome P-450$_{cam}$ (Hui Bon Hoa et al., 1982; Fisher et al., 1985), a monooxygenase that catalyzes the hydroxylation of camphor. Like many other heme proteins, the ferric form of this cytochrome is characterized by a spin state influenced by various parameters (Sligar, 1976; Lange et al., 1979) and also by a transition from the native form to a nonnative form termed cytochrome P-420 (Yu et al., 1974). Both of these processes can be induced by increasing pressure and can be readily "ressolved" in time (Marden et al., 1982, 1986).

The dominant fluorophores in proteins are tryptophan residues attached to well-defined sites along the polypeptide backbone. Fluorescence yield, lifetime, and emission maxima are often very sensitive to the local environment and hence can sometimes serve as a good probe of the structure and dynamics of local polypeptide chain fluctuations. In heme proteins, the fluorescence emission of tryptophan residues overlaps the absorption band of the heme, and there can be efficient nonradioactive energy transfer between these groups. This energy transfer serves to reduce the fluorescence yield and excited-state lifetime and can reveal subtle changes in distance, defined to a first approximation via the theory of Forster (1959).

The three-dimensional structure of P-450$_{cam}$ (Poulos et al., 1985) revealed the presence of five tryptophan residues at amino acid positions 42, 55, 63, 374, and 406. The closest distance to the heme is that of Try-42 (17.5 Å) and the longest that for Try-406 (31.3 Å). The distance of the three other

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ranges between 25 and 28 Å. The occurrence of energy transfer will reduce the fluorescence yield and lifetime of these tryptophan residues, and any conformational change could influence this yield and be used to detect overall changes in the protein structure.

In previous publications (Marden et al., 1982; Fisher et al., 1985), it was shown that increasing pressure first induces a high-spin to low-spin transition of substrate-bound cytochrome P-450$_{cam}$ and at higher pressures a cytochrome P-450 to cytochrome P-420 interconversion. In the present work, we have monitored the fluorescence yield and optical absorption as a function of pressure for P-450$_{cam}$ as well as another bacterial cytochrome P-450 catalyzing the oxidation of linalool and termed cytochrome P-450$_{lin}$ (Ullah et al., 1983). From this approach, we are able to quantitate the P-450 $\leftrightarrow$ P-420 interconversion equilibrium, document substantial differences in the pressure-induced perturbations, in terms of both equilibrium states and dynamic transitions for both cytochromes in their various oxidation and ligand states, and relate these observations to a generalized polymer–gel model previously introduced (Douzou, 1987).

Materials and Methods

Cytochromes P-450. Two bacterial cytochrome P-450 proteins were used for these investigations. The camphor hydroxylating cytochrome P-450 from *Pseudomonas putida* (P-450$_{cam}$) and the linalool hydroxylating cytochrome P-450 from *Pseudomonas incognita* (P-450$_{lin}$) were purified as previously described (Gunsalus et al., 1978; Ullah et al., 1983). All protein samples had a 392-nm to 280-nm absorbance ratio of 1.4 or greater. The stock solutions of cytochrome were stored at −80 °C and contained no measurable P-420. Tris-HCl buffer was used for the pH 7 studies and sodium phosphate buffer for those at pH 6. The pH of phosphate buffer decreases by 0.33 unit per kilobar while Tris buffer increases by 0.02 unit per kilobar (Newman et al., 1973). In order to separate ionic strength from cation effects, the buffer concentration was kept near 10 mM, and the potassium cation concentration (Sigma) varied from 0 to 240 mM. The substrates of camphor and linalool were purchased respectively from Merck and Sigma and were used without further purification.

Substrate-free protein was first obtained by passage of the protein through a Sephadex G-25 fine column equilibrated at 4 °C with the same buffer used for the high-pressure experiments. The protein concentration used for fluorescence studies was 3.2 μM and was saturated with 200 μM substrate, while a more concentrated protein solution was used (10 μM) for the corresponding absorbance experiments. Reduced substrate-bound cytochromes P-450 were obtained by adding dissolved crystals of dithionite in a vacuum-degassed and argon-saturated buffer solution of 4 °C. Excess dithionite was removed from the reduced cytochrome P-450 by quick dialysis against a perfectly degassed argon-saturated buffer using a wet cellulose membrane tubing. Before being transferred to the high-pressure optical cell in an anaerobic box, the reduced solution was saturated with carbon monoxide.

High-Pressure System. The optical pressure system, capable of generating a pressure of 6200 bar, has been described previously (Hui Bon Hoa et al., 1982). The high-pressure bomb is made of margin steel and is surrounded by a copper jacket for temperature control. The bomb plus jacket fits snugly into an SLM 4800 spectrofluorometer or a Cary 219 spectrophotometer sample compartment, thus minimizing any movement relative to the light path. The high-pressure windows are sapphire disks polished optically flat. Pressure is given in units of bar (1 bar = 0.987 atm = 10^5 Pa = 10^5 kg m^{-1} s^{-2}). The high-pressure cuvette is a 0.4-mL quartz cylinder, 2 cm long, 0.5-cm inner diameter, with two flattened sides. A thin Teflon membrane (0.1 mm thick) separates the sample from the pressurizing fluid, typically pentane or heptane (Merck). The high-pressure-generating equipment is composed of a low-pressure (700 bar) oil hydrolic press (Enerpac), a 31-fold intensifier (10 000 bar), and a high-pressure Bourdon-type gauge (6000 bar) coupled in parallel with a Manganin transducer and a Coleraine bridge supply instrument. The high-pressure connections between intensifier, bomb, and gauge are made with stainless-steel flexible tubing (internal diameter 0.2 mm, external diameter 1.6 mm).

Results

It is well appreciated that protein denaturation can be produced by the application of hydrostatic pressure (Weber et al., 1983). Typical pressures required to denature globular proteins range from 3 to 7 kbar. In this respect, one may compare the behavior of solutions under high pressure to those containing urea, hydrogen ions, or any number of chemical additives that may be employed to selectively perturb the conformational structure of proteins. In studies of protein denaturation, one hopes to surmise which factors are important determinants or tertiary structure by following the actual folding transitions. Pressure as a tool for the study of protein folding has many obvious advantages compared to the other protein denaturants. One of the most appealing aspects of pressure investigations is the relative simplicity and continuity with which the thermodynamic nature of experimental results may be established, and the use of kinetic and reversibility determinations. Often, pressure-induced denaturation does not lead to irreversible aggregate and precipitate formation that is often observed with thermal denaturation.

Effects of Pressure on Camphor-Bound Cytochrome P-450$_{cam}$. Figure 1A shows the effect of hydrostatic pressure on the Soret absorbance spectra of cytochrome P-450$_{cam}$ in the presence of Tris buffer (10 mM, pH 7.2) and 200 μM camphor, T = 4 °C. At 1 bar, camphor-bound cytochrome P-450 shows mixed spin states, and the equilibrium coefficient defined as the ratio of high- and low-spin forms, K_e = [HS]/[LS], depends on experimental conditions, most notably the potassium ion concentration (Sligar, 1976). High-spin species show a maximal absorbance at 392 nm and low-spin species at 417 nm. Increase of pressure to 800 bar shifts the spectra to the low-spin form with an isosbestic point at λ = 404 nm representative of the presence of two species in equilibrium. The reaction volume of this spin change ranges from 20 to 80 mL/mol depending on experimental conditions (Hui Bon Hoa et al., 1982). This reaction has been identified as a pressure-induced dissociation of substrate from the active site (Fisher et al., 1985; Marden et al., 1987). Further increase of pressure has a second effect, namely, a decrease in intensity of the maximum with a shift toward longer wavelength. At 2500 bar, the spectra are characterized by maxima at 367 and 425 nm with a shoulder at 456 nm. These spectra correspond to the inactivated form P-420 as is demonstrated by isolating the pressure-treated sample and forming the ferrous carbon monoxide adduct.

Figure 1B shows the effect of pressure on a preformed ferrous carbon monoxide adduct. At 1 bar, the spectral Soret maximum at 446 nm is clearly indicative of a thiolate axial ligand to the heme of cytochrome P-450$_{cam}$ (Sato et al., 1978; Poulos et al., 1985). As pressure is increased, the intensity of the maximum decreases, and a new optical band appears at 424 nm which is characterized by a new isosbestic point

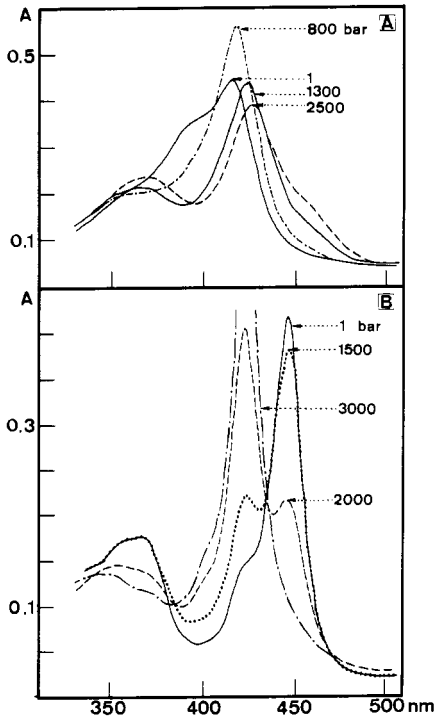

FIGURE 1: Soret absorbance spectrum of camphor-bound cytochrome P-450 as a function of hydrostatic pressure. (A) Ferric cytochrome P-450 in 10 mM Tris buffer, pH 7.2, and 200 μM camphor, 4 °C; protein concentration is 10.4 μM in a high-pressure cuvette with a 0.5-cm optical path. (B) Ferrous CO–cytochrome P-450 10.4 μM in 10 mM Tris buffer, pH 7.2, and 200 μM camphor, 4 °C.

at λ = 434 nm. At 3000 bar, the total absorbance spectrum is composed of two bands, characterized by Soret maxima at λ = 424 and 367 nm.

Figure 2A,B illustrates the effect of pressure on the tryptophan fluorescence emission spectra of camphor-bound cytochrome P-450$_{cam}$ under the same conditions as described in a previous paper. The fluorescence emission spectra illustrated in Figure 2 are characteristic of tryptophan residues located in a relatively hydrophobic environment such as found in the interior of proteins (335-nm emission maximum) as compared to the fluorescence of free DL-tryptophan in aqueous solution (maximum near 355 nm). This 18-nm blue-shift from free tryptophan emission and the corresponding alteration in quantum yield can be used to follow the structure of P-450$_{cam}$ under pressure. As the applied pressure increases, the intensity of fluorescence initially decreases reversibly until an inactivation pressured defined as P_i. Further increase of pressure enhances the fluorescence intensity about 2-fold, which is accompanied by a 5-nm red-shift in emission. This range of pressure-induced fluorescence changes is irreversible and was shown to correspond to a transition from P-450 to P-420. The fluorescence of a stable P-420 species as a function of pressure is reversible and decreases as the pressure increases. However, the pressure-induced P-450 to P-420 transition is completely reversible in the presence of sulfhydryl compounds (Hui Bon Hoa et al., 1988).

Figure 3 illustrates the pressure dependence of fluorescence yield for different states of camphor-bound cytochrome P-450. The ordinate of this graph normalizes the pressure dependence of the relative fluorescence intensity (or yield) to that at 1 bar. For both the oxidized (solid line) and reduced carbon monoxide (dashed line) forms of cytochrome P-450, the relative fluorescence yield decreases reversibly as the pressure increases. This decrease is around 2–10% depending on the experimental conditions and the oxidation or ligand states of protein.

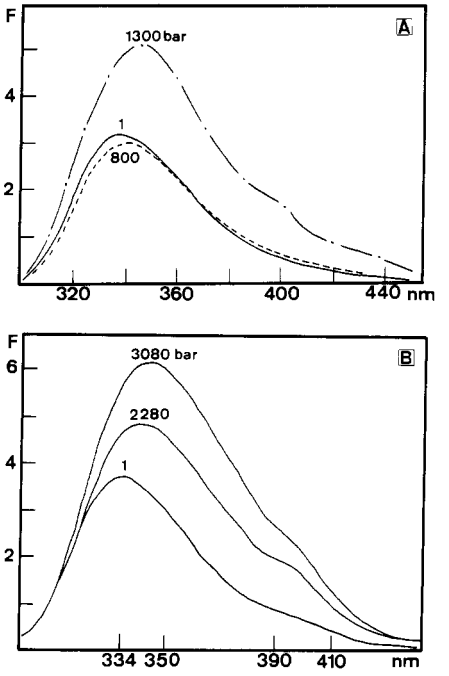

FIGURE 2: Tryptophan fluorescence spectrum of camphor-bound cytochrome P-450 as a function of hydrostatic pressure. (A) Ferric cytochrome P-450 in 10 mM Tris buffer, pH 7.2, and 200 μM camphor, 4 °C; protein concentration was 3.2 μM in a high-pressure quartz cuvette with a 0.5-cm optical path. The excitation wavelength was 295 nm. (B) Ferrous CO–cytochrome P-450 in 10 mM Tris buffer, pH 7.2, and 200 μM camphor, 4 °C, with the same protein concentration as in (A). Dithionite was used to reduce the protein and subsequently removed by dialysis under CO-saturated buffer solution at 4 °C as described under Materials and Methods. F represents the fluorescence intensity in arbitrary units.

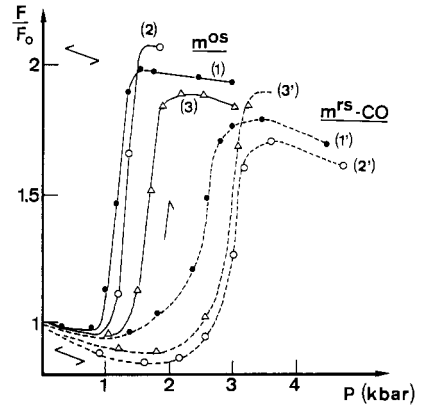

FIGURE 3: Relative tryptophan fluorescence of camphor-bound (200 μM) cytochrome P-450 as a function of hydrostatic pressure. F_0 is the fluorescence intensity at an emission maximum at 334 nm and a pressure of 1 bar. The excitation wavelength was 295 nm. Solid lines characterize ferric substrate-bound cytochrome P-450 and dashed lines ferrous CO–cytochrome P-450 (same buffer conditions as in Figure 2). (●—●, ●---●) 0 mM KCl; (○—○, ○---○) 100 mM KCl; (△—△, △---△) 100 mM KCl + 1 mM camphor.

Previous work on the tryptophan fluorescence of myoglobin has shown (Marden et al., 1986) that, to a first approximation, if the protein is incompressible, then the fluorescence enhancement follows that expected for solvent contraction (V_0/V) with a typical increase in yield of 3% per kilobar. For equal compressibility of protein and solvent, differential effects will not be observable, and there should be no observed change in fluorescence. If the protein is more compressible than the solvent, then the fluorescence intensity decreases versus pressure as in our case, but there is no easy or precise determination as to structural origins due to the complexity of

the situation induced by the multiplicity of tryptophans, possible movement of the tryptophan transition dipole relative to that of heme, dynamic motions of the protein, and changes in the microdielectric constant.

Above an inactivation pressure P_i around 1 kbar for the oxidized forms and 2.3 kbar for the reduced carbon monoxide adducts of cytochrome P-450, the relative fluorescence yield increases above the 2-fold value in a very narrow range of pressure. This apparent "phase transition" is irreversible in the absence of sulfhydryl compounds and corresponds to the appearance of a stable inactive P-420 form. These increases in fluorescence yield are smaller by a factor of 4 than those observed by treating the unfolding protein with detergent. Hence, the structure of the P-420 state induced by high hydrostatic pressure probably is not completely unfolded. However, when the protein is pressurized in the reduced carbon monoxide forms, the increases in fluorescence yield are much smaller. In addition, after release of pressure to 1 bar, these solutions show a small amount of turbidity which could decrease the observed intensity of the tryptophan emission by trivial scattering effects. The presence of turbidity in the solution is indicative of some precipitation of the reduced carbon monoxide forms of cytochrome P-450$_{cam}$ at very high pressure.

Several physicochemical compounds can be used to modulate the P-450 ↔ P-420 transition induced by pressure. It is shown in Figure 3 that potassium cations (curve 2) have a weak effect, while high concentration of substrate (curve 3) has an effect on the fluorescence changes as a function of pressure. High concentrations of the substrate camphor (curve 3), which are high enough to cause the saturation concentration of a second substrate binding site (Marden et al., 1987), shift the stability of the native complex toward more higher pressure. In the case of the reduced carbon monoxide form, potassium cations (curve 2′) and a high concentration of substrate (curve 3′) shift identically the stability of the native liganded structure (curve 1′) to higher pressure. Finally, a decrease of pH (data not shown) from 7.2 to 5 has no effect on the oxidized substrate-bound complex but decreases the stability of the P-450–carbon monoxide complex by about 1 kbar.

Effects of Pressure on Linalool-Bound Cytochrome P-450$_{lin}$. The cytochrome P-450$_{lin}$ system is active in the hydroxylation of linalool by *Pseudomonas incognita* and has been studied by numerous techniques (Bhattacharyya et al., 1984; Jung et al., 1988). Interestingly, P-450$_{lin}$ displays a striking difference in physical–chemical behavior. The linalool cytochrome P-450 presents identical absorption spectra with those of camphor-bound cytochrome P-450$_{cam}$, in the oxidized, reduced, and CO complex forms, in relative intensities and peak positions for both the substrate-bound and free forms (Jung et al., 1988). The linalool-bound cytochrome P-450 complex also exists as a mixture of ferric spin states under normal conditions in temperature and pressure. However, the pressure dependence of the spin changes is quite different from that of the camphor system. The reaction volume is about 17 mL/mol and is independent of KCl and linalool concentrations (Marden et al., 1987).

Figure 4 shows the comparative stability of the two cytochromes P-450 in the oxidized, substrate-free, and substrate-bound forms. All oxidized forms of cytochrome P-450$_{lin}$ (solid lines) are stable up to 2.2 kbar. Above this critical pressure, the measured absorbance spectra of the sample increase due to light scattering. At the end of the high-pressure experiment, the P-420 spectra present a continually increasing base line. This process is irreversible and presumably is due

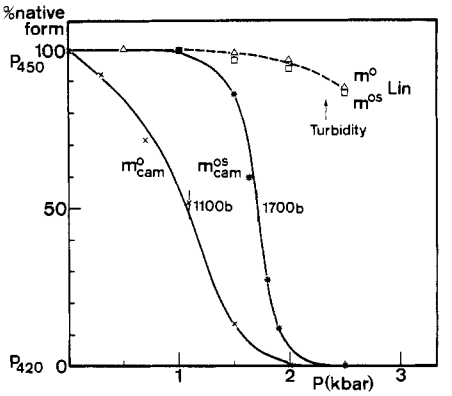

FIGURE 4: Inactivation profiles of cytochrome P-450 as a function of hydrostatic pressure. The percentage of native cytochrome P-450 and inactive cytochrome P-420 was determined by using the absorbance at 404 nm (isosbestic point of the spin transition); ϵ_{404nm}(P-450) = 77 mM^{-1} cm^{-1}, ϵ_{404nm}(P-420) = 53 mM^{-1} cm^{-1} at 4 °C. Solid lines characterize cytochrome P-450$_{cam}$: camphor-free (×) protein in 10 mM Tris buffer, pH 7.2; camphor-bound (200 μM) protein (*) in the presence of 240 mM KCl. Dashed lines characterize cytochrome P-450$_{lin}$: (△) linalool-free protein in 10 mM Tris buffer, pH 7.2; (□) linalool-bound (200 μM) protein in the presence or absence of KCl. The vertical line at the midpoint transition indicates the pressure $P_{1/2}$ (half percent of inactivated cytochrome P-420). The arrow indicates the appearance of turbidity in the solution.

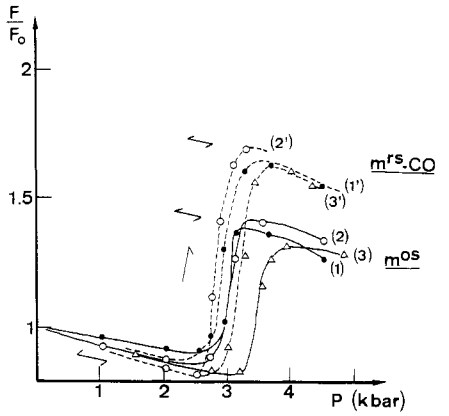

FIGURE 5: Relative tryptophan fluorescence of linalool-bound (200 μM) cytochrome P-450 as a function of hydrostatic pressure. F_0 is the fluorescence intensity at an emission of 336 nm and at 1 bar. The excitation wavelength was 295 nm. Solid lines characterize ferric substrate-bound cytochrome P-450 and dashed lines ferrous CO–cytochrome P-450 in the same buffer conditions as in Figure 2. (●—●, ●---●) 0 mM KCl; (○—○, ○---○) 100 mM KCl; (△—△, △---△) 100 mM KCl + 4 mM linalool.

to irreversible unfolding and denaturation. On the other hand, titration curves of P-450$_{cam}$ in the substrate-free and substrate-bound states (dashed lines) are stable, and the P-420 form is reversible in the presence of sulfhydryl compounds such as cysteine. Last, the oxidized states of P-450$_{cam}$ are much more pressure sensitive than the corresponding states of P-450$_{lin}$.

Figure 5 shows the fluorescence yield versus pressure of different states of linalool-bound cytochrome P-450. Excitation and emission spectra of the linalool system also display the typical characteristics of tryptophan residues of proteins, having an emission maximum blue-shifted 16 nm relative to that of the solvated amino acid. The quantum yield at 20 °C with 295-nm excitation is 10% that of DL-tryptophan. The relative fluorescence intensity (or yield) is plotted as a function of pressure in Figure 5, where several observations are immediately evident. First the fluorescence yield first decreases by 10–18% between 1 bar and 3 kbar whereas at higher pressure this yield increases steeply (for the substrate-bound

oxidized and ferrous–CO states) but is smaller than that of the P-450$_{cam}$ system. Absorbance measurements clearly show a precipitation of the P-420 state, presumably as the result of a total unfolding. A semilogarithmic plot of the relative absorbance change at λ = 500 nm as a function of time is linear, leading to a time constant τ = 12 min. The corresponding rate constant of unfolding at 3 kbar and 4 °C is 31.9 10^{-4} s^{-1} for the linalool-bound cytochrome P-450. Curves 2 and 2′ show that the presence effect is not influenced by potassium cations, and curves 3 and 3′ show that an increase in substrate linalool concentration slightly increases the stability of the protein complex. Observations not shown in this paper indicate a large effect on pH on such stability, since a decrease in pH from 7.2 to 6.0 decreases the stability of the oxidized and reduced CO substrate-bound states by 1 kbar.

Discussion

Evidently, pressure has a profound effect on the stability and structure of the two bacterial cytochromes P-450 investigated herein. Figures 3 and 5 clearly show that there exists two distinct pressure regions for both cytochromes P-450$_{cam}$ and P-450$_{lin}$. One is a low-pressure range characterized by a decrease in tryptophan fluorescence yield of a few percent per kilobar which is directly due to an increase of energy transfer from excited tryptophans to the heme group. This is hypothesized to be the result of protein–solvent compressibility. A second high-pressure range exists which is much more dramatic. Here both proteins show a large increase in fluorescence coupled with their conversion into the inactive cytochrome P-420 spectral form. The lack of protein aggregation and precipitation, together with the observations of others (Satake et al., 1976), suggests that this conversion to P-420 (particularly for the camphor system) occurs without loss of tertiary structure. The large increase in fluorescence yield, however, does suggest significant rearrangements of the tryptophan residues with respect to the heme prosthetic group.

As shown in Figures 3 and 5, the range of low pressure in which there is a decrease in fluorescence yield is quite different for cytochromes P-450$_{cam}$ and P-450$_{lin}$. The large increase in fluorescence yield observed at higher pressure is also larger for cytochrome P-450$_{cam}$. Together with the observed precipitation of the cytochrome P-450$_{lin}$ under these conditions, it is apparent that these two bacterial cytochromes have very different physical–chemical behavior.

The low-pressure transition region will be discussed first. Here, an increase in pressure affects a decrease in fluorescence yield (Figure 2) and also a change in the absorption spectrum that is identified as a high-spin to low-spin transition (Figure 1). Surprisingly, this transition, when induced by other parameters such as KCl and substrate concentration, is not accompanied by a decrease in tryptophan fluorescence yield (data not shown). Thus, this pressure–induced decrease in yield, perhaps due to an increase in energy transfer from excited tryptophan residues to the heme group, seems to be an independent process associated with the compression of the solvent–protein system. This relatively small decrease in fluorescence yield suggests small changes in the average microscopic dielectric constants, spectral overlap integrals, intramolecular distance(s), and/or orientation(s) of the tryptophan residues that are able to transfer for excitation energy to the heme group.

Previous work (Fisher et al., 1985; Marden et al., 1987) has shown that application of high hydrostatic pressure acts to drive the camphor from the substrate binding pocket of P-450$_{cam}$. Substrate binding is known from the X-ray structure to displace a hydrogen-bound array of water molecules at the active site (Poulos et al., 1985, 1986). In addition, there are substantial changes in the thermal motions of the protein structure. These observations indicate a certain degree of flexibility in and near the substrate binding pocket. Such a conclusion is also in agreement with pressure jump experiments (Douzou et al., 1987) showing that pressure-induced spin relaxation kinetics strongly increase with the substrate concentration until saturation conditions are attained. This observation suggests a two-step substrate binding process: a fast association step followed by a slow conformational relaxation.

Cytochrome P-450$_{lin}$ shows a much greater stability under increasing pressure with either the substrate-free or the substrate-bound forms converted into cytochrome P-420 in the same high-pressure range (Figures 4 and 5). The formation of cytochrome P-420$_{lin}$ is accompanied by unfolding and precipitation of the protein as detected by both absorption and fluorescence measurements. Lastly, in the low-pressure region where cytochrome P-450$_{lin}$ is still "stable", the rates of the spin transition are faster than those observed on cytochrome P-450$_{cam}$ (data not shown).

These observations, together with those of differential scanning calometry (Jung et al., 1987), suggest a lack of flexibility of cytochrome P-450$_{lin}$ compared to cytochrome P-450$_{cam}$, and this property explains its high resistance to deformation by pressure. Cytochrome P-450$_{cam}$, on the contrary, appears to offer much less resistance to deformation when free of substrate, and its limited resistance when substrate bound may be due to the fact that pressure drives the camphor from the pocket. The two structures, with and without substrate, show very few differences in the positions of the amino acid side chains (Poulos et al., 1985, 1986), but substrate-free cytochrome appears to be much more flexible and is then converted into a stable cytochrome P-420 form, while this form is unstable when induced on cytochrome P-450$_{lin}$.

These essential differences between the two cytochromes are absent when the complex substrate-bound cytochrome P-450$_{cam}$ is reduced and bound with carbon monoxide. The pressure inactivation profile is then similar to that of the analogous complex of cytochrome P-450$_{lin}$ (Figure 3 vs Figure 5), and the pressure dependence of this protein does not vary significantly irrespective of redox or ligation state.

In a previous work (Douzou et al., 1988), we assumed that cytochrome P-450$_{cam}$ involves a cortex gel responding to specific stimuli and external parameters through changes in physical–chemical properties influencing the dynamics of the whole molecule. Present results are compatible with this assumption but indicate that the cortex of cytochrome P-450$_{lin}$ is genuinely insensitive to stimuli and external parameters, an observation suggesting that the physical state of its hydrated cortex is different from that of cytochrome P-450$_{cam}$. The latter seems to adopt this state when combined to the substrate, reduced, and bound to carbon monoxide. More detailed experiments are needed to determine the type of changes occurring in the cortex of cytochrome P-450$_{cam}$ and the actual state of this cortex in the two cytochromes free from substrate.

These results permit one to suggest that cytochrome P-450$_{cam}$ is elastic and can be deformed to a stable cytochrome P-420, whereas P-450$_{lin}$ resists compression until a critical point wherein protein precipitation occurs (unfolding).

Registry No. Cytochrome P-450, 9035-51-2.

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Secondary Structure Prediction of 52 Membrane-Bound Cytochromes P450 Shows a Strong Structural Similarity to $P450_{cam}$†

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ABSTRACT: The secondary structure of 52 aligned cytochrome P450 sequences, all of which are membrane bound, is predicted and collectively compared with the crystal structure of the soluble cytochrome $P450_{cam}$. Ten of 13 helical regions, 6 of 7 β-pair regions, and β-structure corresponding to a known β-bulge near the active site of $P450_{cam}$ are predicted to exist in the membrane-bound P450s. Three turns associated with β-structure in the soluble enzyme are also predicted for the membrane-bound forms. A strong structural similarity is evident between membrane P450s and the soluble $P450_{cam}$. Consequently, a multitransmembrane structure involving much of P450 seems highly unlikely. A structure with two N-terminal transmembrane segments is compatible with these observations.

Cytochrome P450 designates a diverse class of *b*-type cytochromes that activate molecular oxygen. The activated oxygen then reacts in situ with many lipophilic substrates (Ortiz de Montellano, 1986; Black & Coon, 1987). There is considerable interest in the structure and function of these enzymes. Since 1982, over 100 P450 sequences have been published, representing at least 53 unique P450 proteins and many variants (Nebert et al., 1987, 1989). It is estimated that a single mammalian species may have from 50 to 200 cytochrome P450 genes (Nebert et al., 1987; Nelson & Strobel, 1987; Marx, 1985). These genes are organized in multigene familes on many different chromosomes (Nebert et al., 1987, 1989). Some P450 genes are polymorphic and are implicated in disease (Guengerich et al., 1987).

Sequence similarity around a 100% conserved cysteine suggests a common ancestral gene for all P450s. However, bacterial P450s are soluble, while eukaryotic P450s are membrane bound. Recently, the crystal structure of cytochrome $P450_{cam}$ from *Pseudomonas putida* was determined (Poulos et al., 1987). These authors proposed that mem-

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