Time-resolved Fourier-transform infrared studies of the cytochrome P-450$_{cam}$ carbonmonoxide complex bound with (1R)-camphor and (1S)-camphor substrate

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Abstract The CO-binding reaction of cytochrome P-450$_{cam}$ bound with (1R)-camphor and (1S)-camphor are compared in the temperature region of 210–260 K using time-resolved Fourier-transform infrared spectroscopy with the CO stretch vibration as spectroscopic probe. For (1S)-camphor as substrate the association of CO is slowed down by a factor of 2, while the dissociation is accelerated by a factor of 3. The CO complex for the (1S)-camphor-bound P-450 is less stabilized ($\Delta G = -22$ kJ/mol) compared to the natural substrate (1R)-camphor ($\Delta G = -30$ kJ/mol). The data are interpreted by a smaller change of the mobility of the (1S)-camphor due to CO binding as compared to (1R)-camphor, which would indicate a higher mobility of (1S)-camphor already in the CO free reduced form of P-450$_{cam}$. The higher mobility of (1S)-camphor in the heme pocket might explain the increased uncoupling rate (hydrogen peroxide formation) of 11% [Maryniak et al. (1993) Tetrahedron 49, 9373–9384] during the P-450$_{cam}$ catalyzed hydroxylation compared to 3% for the conversion of (1R)-camphor.

Key words: Cytochrome P-450$_{cam}$; Flash photolysis; Carbon monoxide; Substrate interaction; Infrared; Thermodynamic parameter

1. Introduction

Cytochromes P-450 are a superfamily of heme protein enzymes that catalyse the oxygenation of many organic substrates in biosynthetic and degradative pathways. Cytochrome P-450$_{cam}$ from *Pseudomonas putida* catalyses the regio- and stereospecific hydroxylation of its natural substrate (1R)-camphor at the 5-exo position, but also many other substrates of camphor, norbornane, adamantane or cyclohexane type [1–7] with varying regio- and stereospecificity. P-450$_{cam}$ is one of the best characterized monooxygenases, for which the crystal structure is known [8].

P-450$_{cam}$ also hydroxylates (1S)-camphor at the 5-exo position with high regiospecificity, but the yield of 5-exo-hydroxy-camphor is decreased, while the formation of hydrogen peroxide in the uncoupling reaction is increased (11% for (1S)-camphor vs. 3% for (1R)-camphor) [9].

Both enantiomers have the same physico-chemical properties like mass, size, dipole moment, polarizability and solubility in non-chiral solvents. Hence, there should be the same conditions for substrate entry and product exit during the catalytic process, and the differences in the ratio of hydroxylation and uncoupling between (1R)- and (1S)-camphor should be due to different steric interactions of the substrates with the heme dioxygen ligand and the protein in the active site of P-450$_{cam}$. For a deeper understanding of this phenomenon we performed flash photolysis studies on P-450$_{cam}$-CO bound with (1R)-camphor and (1S)-camphor, respectively, using time-resolved Fourier-transform infrared spectroscopy with the CO stretch mode as spectroscopic probe. Our laboratory has recently shown that the CO stretch mode is very sensitive towards the heme pocket structure and substrate binding [10] and that substate equilibria can be analyzed [11–13]. In the present paper we report first data obtained for the recombination process in the temperature region of 210–260 K and the time window of 0.1–2 s. We will show that (1S)-camphor is less rigidly bound in the heme pocket than (1R)-camphor which might explain the increased contribution of the uncoupling reaction to the overall dioxygen consumption by P-450$_{cam}$.

2. Materials and Methods

Cytochrome P-450$_{cam}$ was expressed in *E. coli* strain TB1 and purified as described [10]. The natural (1R)-camphor substrate was removed by dialysis against 50 mM Tris-HCl buffer (pH 7.4) containing 20% glycerol by mass and by chromatography (Sephadex G25 (medium) column equilibrated with the same buffer). The solution was concentrated by ultrafiltration (Centricon 30) to 1 mM cytochrome P-450 and finally dialysed against 0.1 M potassium phosphate buffer (pH 7) containing 63% glycerol by mass. This stock solution was used for the sample preparation.

The stock solution was diluted with 0.1 M potassium phosphate buffer (pH 7) containing 63% glycerol by mass to 0.5 mM cytochrome P-450. (1R)-Camphor (Aldrich) or (1S)-camphor (Merck) was added as ethanolic solution in 35-fold excess to ensure complete substrate binding, and the solution was reduced with 50-fold excess of fresh prepared anaerobic sodium dithionite. The carbon monoxide complex was formed by equilibrating the solution in a gas mixture of CO and N_2 for 1 h. The CO concentration of the solution was determined using Henry's law [14] and was between 0.1 mM and 0.4 mM.

Immediately after preparation, the sample was filled into a sealed CaF_2 cell with 100 μm path-length and the P-450–CO concentration was checked by UV-VIS spectroscopy. The cell was mounted in a closed-cycle helium cryostat (Cryogenics Modell 22), which enabled temperature control from room temperature down to 20 K. The CO complex was photolyzed by a dye laser (UDL105 from LTB with coumarin 2 laser dye, 446 nm, 70 μJ per pulse) that was pumped by a nitrogen laser (MSG800 from LTB, 500 ps pulse-width). IR spectra were recorded with a Bruker IFS66 single channel Fourier-transform IR spectrometer equiped for time-resolved spectroscopy. The principle of rapid scanning FTIR spectroscopy was described elsewhere [15,16].

Flash photolysis experiments were carried out at 210 K to 260 K. In this temperature range, the sample is still liquid due to the high glycerol concentration. The glass transition temperature of the solvent is between 190 K and 180 K. However, the main reason for the tem-

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Abbreviations: P-450$_{cam}$, cytochrome P-450 from *Pseudomonas putida* (CYP101); P-450$_{cam}$(1R), (1R)-camphor bound form of cytochrome P-450$_{cam}$; P-450$_{cam}$(1S), (1S)-camphor bound form of cytochrome P-450$_{cam}$; Mb, myoglobin; P-450$_{nor}$, cytochrome P-450 from *Fusarium oxysporum*; FTIR, Fourier-transform infrared.

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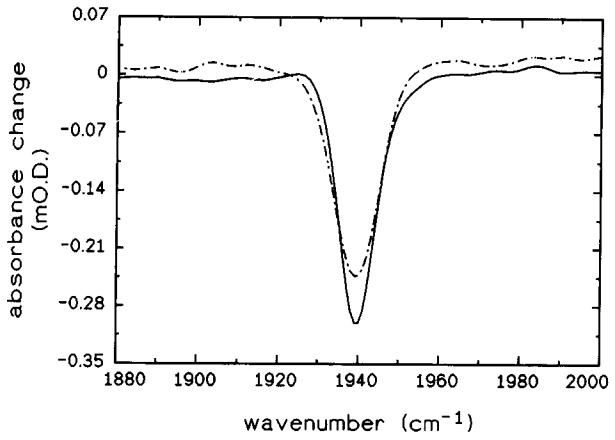

Fig. 1. IR absorbance difference spectra of P-450$_{cam}$(1R)–CO complex (dashed line) and P-450$_{cam}$(1S)–CO complex (solid line) 44 ms after laser irradiation at 240 K. The absorbance minima are at 1939.4 cm^{-1} (P-450$_{cam}$(1R)–CO) and 1939.6 cm^{-1} (P-450$_{cam}$(1S)–CO). 1024 photolysis cycles were averaged and spectra were recorded at 4 cm^{-1} resolution.

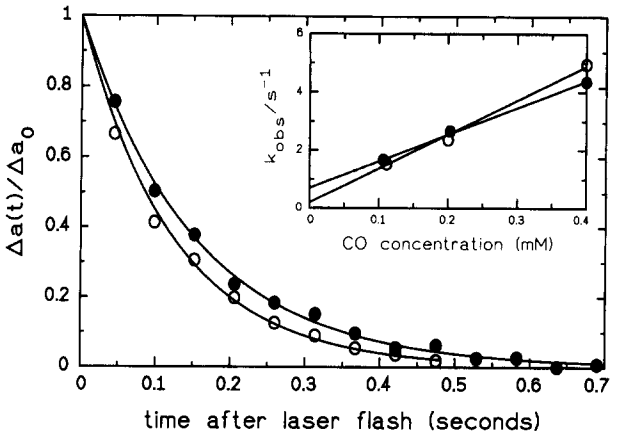

Fig. 2. Rebinding of CO to P-450$_{cam}$ after photodissociation at 240 K. The plot shows the time-course of the relative band area $\Delta a(t)/\Delta a_0$ for the CO stretch band of P-450$_{cam}$–CO from the absorbance difference spectra. The CO concentration is 0.4 mM and Δa_0 was obtained from the fit of Eqn. 1 (○, P-450$_{cam}$(1R); ●, P-450$_{cam}$(1S)). Inset: plot of the observed rate constant k_{obs} obtained from the fit of Eqn. 1 vs. CO concentration.

perature choice was to slow down the rebinding reaction so that it fits into the time scale of the FTIR spectrometer. At each temperature of interest, a reference interferogram of the dark P-450–CO was recorded first. Subsequently a laser flash was triggered by the spectrometer to dissociate a small amount of the sample and 24 consecutive interferograms were recorded, each from one mirror sweep of the interferometer. When the sample has relaxed to equilibrium, the time course was repeated. 640 to 1024 photolysis cycles were averaged to increase the signal-to-noise ratio. The time required for one mirror sweep of the interferometer was 55 ms at 4 cm^{-1} resolution. Interferogram data were stored and Fourier-transformed with a zero-filling factor of 4 and the Blackman-Harries 4-point apodization function after the experiment. From the resulting single channel spectra of the dark sample I_D, and of the photolyzed sample at time t after the flash $I(t)$, absorbance difference spectra were calculated as $\Delta Abs(t) = \log(I_D/I(t))$ (see Fig. 1).

The area of the CO stretching band in the absorbance difference spectra is proportional to the decrease of P-450–CO molecules due to flash photolysis. Absorbance difference spectra were baseline corrected by a cubic function, which was determined by a least-square fit of the spectral range from 1890 cm^{-1} to 1918 cm^{-1}, and from 1960 cm^{-1} to 1980 cm^{-1} on each side of the peak. The area of the CO stretching band $\Delta a(t)$ was computed in the range from 1918 cm^{-1} to 1960 cm^{-1} by using a standard integration method from the spectrometer software. The spectra obtained in this way correspond to an absorbance difference spectrum between the absolute spectrum of the CO bound form in the dark (so-called dark-spectrum) and the absolute spectrum of the photolyzed sample (so-called light-spectrum). For the dark-spectrum of (1R)-camphor-bound P-450 we have shown elsewhere [12] that the infrared band is asymmetric which results from an overlap of the subconformer bands. Similar behavior is observed also for (1S)-camphor-bound P-450$_{cam}$–CO, however, with different shape (Schulze, H., private communication). In the light- over dark-spectra in the temperature region of our experiments, however, this subconformer equilibrium is not clearly detected because we had to reduce the spectral resolution to keep the time for the interferometer mirror sweeps small. Therefore, we used the total area $\Delta a(t)$ of the CO stretch bands for all analyses.

3. Results

A typical plot of $\Delta a(t)$ vs. t is shown in Fig. 2. For all temperatures in the investigated range, the observed rebinding kinetics can be fitted to a single exponential function

$$\Delta a(t) = \Delta a_0 \cdot \exp(-k_{obs} \cdot t) \quad (1)$$

where k_{obs} is the observed rebinding rate constant and Δa_0 corresponds to the initial number of photolyzed P-450–CO molecules. Both are obtained from the fit procedure. Therefore, a simple one-step model for CO rebinding from solvent is sufficient to describe our experimental data.

$$\text{P450} + \text{CO} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \text{P450-CO} \quad (2)$$

If the concentration of free P-450$_{cam}$ is small compared to the concentration of CO in the solution, then the bimolecular rebinding process can be described by the pseudo-first-order kinetic law Eqn. 1, and k_{obs} is related to k_{on} and k_{off} by $k_{obs} = k_{on} \cdot c(CO) + k_{off}$ [17]. This requirement is given in our experiments because only a small amount of 0.3% of the

Table 1
Kinetic parameters for CO binding to P-450$_{cam}$ with (1R)-camphor and (1S)-camphor

Compound	T (K)	c(CO) (mM)	k_{obs} (s^{-1})	k_{on} ($10^4 \times M^{-1} \cdot s^{-1}$)	$k_{\infty,on}$ ($10^8 \times M^{-1} \cdot s^{-1}$)	$E_{a,on}$ (kJ/mol)	k_{off} (s^{-1})	$k_{\infty,off}$ ($10^3 \times M^{-1} \cdot s^{-1}$)	$E_{a,off}$ (kJ/mol)
P-450$_{cam}$(1S)[a]	240.7	0.4	6.6 ± 0.3	1.4 ± 0.3	1.15	18.1 ± 2	1.0 ± 0.8	82.1	22.5 ± 2
P-450$_{cam}$(1R)[a]	240.8	0.4	8.1 ± 0.2	2.2 ± 0.3	19.5	23.0 ± 2	0.3 ± 0.4	0.25	13.5 ± 2
P-450$_{cam}$(1R)[b]	293	–	–	6.8 10[e]	–	–	3.03	–	–
P-450$_{cam}$[b,c]	293	–	–	732 850[e]	–	–	129	–	–
Mb[d]	298	–	–	38–73	–	–	0.02	–	–

[a]This work; [b]values from [35]; [c]substrate-free P-450$_{cam}$; [d]sperm whale myoglobin, see references in [35]; [e]values from [36].

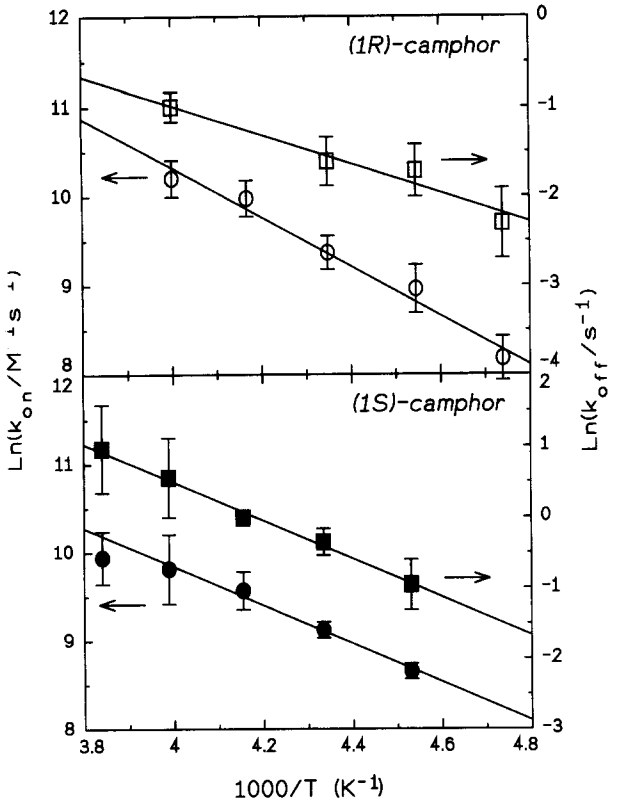

Fig. 3. Arrhenuis plot of $\ln(k_{on})$ and $\ln(k_{off})$ versus $1/T$ for CO rebinding. Upper panel: (1R)-camphor-bound P-450$_{cam}$ (○, *on* process; □, *off* process). Lower Panel: (1S)-camphor-bound P-450$_{cam}$ (●, *on* process; ■, *off* process).

whole P-450–CO is flashed off. Thus, the CO concentration is 30 times larger than the concentration of CO-free reduced P-450 which is regarded as constant. We determined k_{on} and k_{off} from the plot of k_{obs} vs. CO concentration from the slope and the y-intercept of the line, respectively (see inset of Fig. 2 and Table 1). The plot of $\ln(k_{on})$ and $\ln(k_{off})$ vs. $1/T$ gave a straight line (Fig. 3), so the temperature dependence of k_{on} and k_{off} can be described by an Arrhenius equation $k = k_\infty \cdot \exp(-E_a/RT)$, where k_∞ is the frequency factor and E_a is the activation energy. It is now straightforward to calculate thermodynamic parameters for the CO binding according to the transition state theory [18]. The activation enthalpy is calculated from the activation energy as $\Delta H^{\neq} = E_a$, and the activation entropy from the frequency factor as $k_\infty = (k_B T/h)\exp(\Delta S^{\neq}/R)$, where k_B is the Boltzmann constant and h the Planck constant. Values for the free enthalpy $\Delta G^{\neq}$ were obtained from $\Delta G^{\neq} = \Delta H^{\neq} - T\Delta S^{\neq}$. Finally, values for ΔH, ΔS and ΔG are computed from the differences of the respective activation values of the *on* and *off* process. All data are collected in Tables 1 and 2 and calculated for a temperature of 25°C to allow comparison with the literature values of the CO binding to myoglobin and cytochromes P-450.

The values for k_{on} and k_{off} are in the same range for both enantiomers. k_{on} is lower for (1S)-camphor by a factor of 2 compared to (1R)-camphor, while the behavior is inverse for k_{off}. At 25°C the *on* process for both substrates is controlled by the enthalpic and the entropic terms to an almost similar extent. The *off* process, however, is dominated by the entropic term. This holds also for the whole temperature region studied. In spite of this qualitative agreement, distinct quantitative differences exist between the enantiomer complexes. For the *on* process the activation barrier is lower and the activation entropy is stronger negative for (1S)-camphor as compared to (1R)-camphor-bound P-450. For the *off* process, however, both enantiomers show inverse behavior. The compensation behavior of $\Delta H^{\neq}$ and $\Delta S^{\neq}$ leads to the conclusion that only the free enthalpy $\Delta G^{\neq}$ for the *off* process is remarkably affected by the nature of the substrate. For the CO binding equilibrium the enthalpy is negative and the contributing entropy for the stabilization of the CO complex is less pronounced for the (1S)-enantiomer. In contrast, the entropic term is dominating the stability of the CO complex for the (1R)-camphor complex while the enthalpy is positive. The stability of the CO complex is decreased by 8 kJ/mol when (1S)-camphor binds to P-450$_{cam}$ instead of its natural substrate, (1R)-camphor.

4. Discussion

Several recent papers have demonstrated, that the mobility of the substrate bound in the heme pocket of cytochrome P-450 is an important factor influencing the ratio of stereospecific substrate hydroxylation and uncoupling hydrogen peroxide formation [19,20]. Furthermore, crystal structure analysis of various substrate analogue P-450$_{cam}$ complexes [21] and studies with P-450$_{cam}$ mutants [22–26] indicated that there seems to be an interrelation between mobility of the substrate, water flux into the heme pocket and flexibility and polarity in the I-helix around Thr-252. In spite of accumulating indications for the importance of the heme pocket dynamics for the relation of hydroxylation and uncoupling little is known about the structural parameters which finally determine the dynamics. For a deeper understanding of these parameters, we used the flash photolysis technique combined with time-resolved Fourier-transform infrared spectroscopy to analyse the heme pocket dynamics in P-450$_{cam}$. The differences in

Table 2
Thermodynamic parameters for CO binding to P-450$_{cam}$, P-450$_{nor}$, ferroprotoporphyrin IX and myoglobin at 25°C

Compound	Glycerol (wt%)	Method	$\Delta H^{\neq}_{on}$ (kJ/mol)	$\Delta H^{\neq}_{off}$	$\Delta S^{\neq}_{on}$ (J/(K•mol))	$\Delta S^{\neq}_{off}$	$\Delta G^{\neq}_{on}$ (kJ/mol)	$\Delta G^{\neq}_{off}$	ΔH (kJ/mol)	ΔS (J/(K•mol))	ΔG (kJ/mol)
P-450$_{cam}$(1S)[a]	63	IR	18.1±2	22.5±2	−90.6±6	−150.8±8	45.1±3	67.4±3	−4.4±2	60.2±10	−22.3±3
P-450$_{cam}$(1R)[a]	63	IR	23.0±2	13.5±2	−67.1±9	−199.0±9	43.0±3	72.9±3	9.5±3	131.9±13	−29.9±4
P-450$_{cam}$(1R)[b]	0	VIS	31.8±1	14.6±2	−43.6±4	−186.8±7	44.8±2	70.3±3	17.2±3	143.2±8	−25.5±3
P-450$_{cam}$[b,c]	0	VIS	61.9±4	5.8±10	98.7±15	−186.0±33	32.5±7	61.3±14	56.1±11	284.7±36	−28.8±16
P-450$_{nor}$[d]	0	VIS	15.9	34.7	−79.6	−111.8	39.6	68.1	−18.8	32.2	−28.5
Fe-Protoporphyrin IX[e]	100	VIS	51.0	–	145.2	–	–	–	–	–	–
Mb[f]	0	VIS	19.6	64.4	−72.9	−54.5	41.4	80.7	−44.8	−18.4	−39.3

[a]This work; [b]values from [35]; [c]substrate-free P-450$_{cam}$; [d]values from [34]; [e]values from [37]; [f]sperm whale myoglobin, see references in [35].

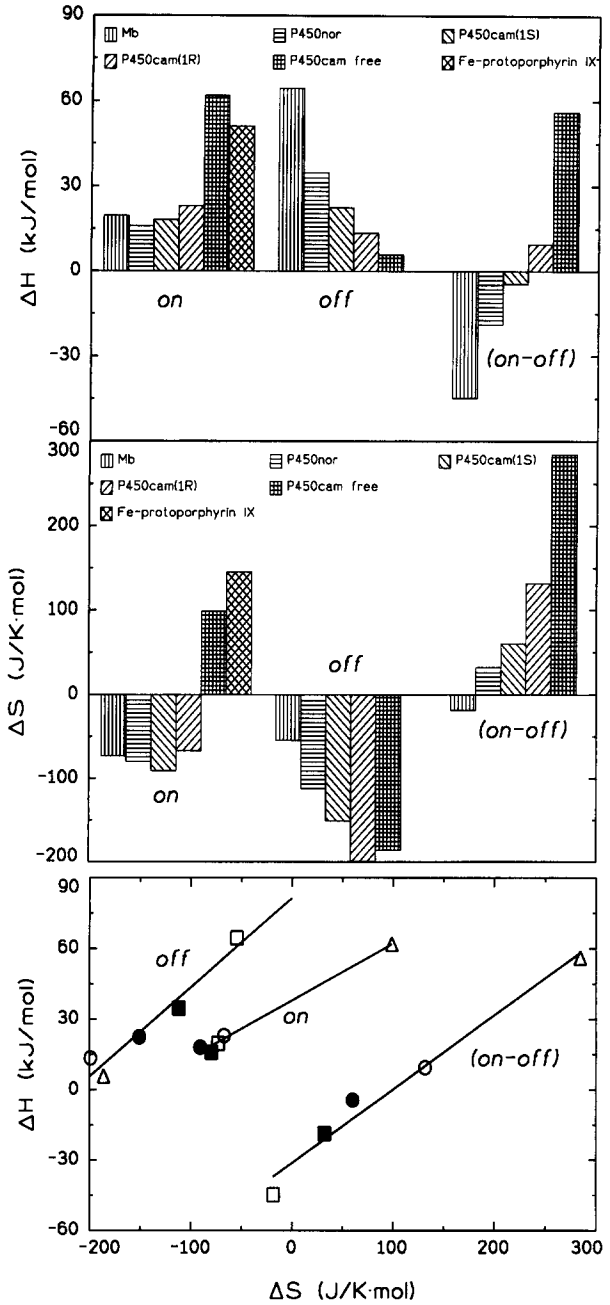

Fig. 4. Thermodynamic parameters for CO binding to various hemoproteins from Table 2. The *upper panel* shows the enthalpy ΔH and the *middle panel* the entropy ΔS for the association (*on* process) and dissociation (*off* process) of carbon monoxide to the heme group, and the equilibrium reaction (*on–off* process). Lower panel: ΔH is plotted versus ΔS for the different processes to visualize the compensation relation between both (○, P-450$_{cam}$(1R); ●, P-450$_{cam}$(1S); △, substrate free P-450$_{cam}$; ■, P-450$_{nor}$; □, sperm whale myoglobin).

the rebinding kinetics of the photodissociated CO ligand observed for P-450$_{cam}$ bound with (1R)-camphor and (1S)-camphor can be assigned to result only from sterical differences because the chemical properties are the same for both enantiomers. The natural substrate (1R)-camphor binds in the heme pocket by forming a hydrogen bond between Tyr-96 and the camphor keto group [8]. Additionally, and equally important, van der Waals contacts between the camphor methyl groups to Val-295 and Val-247 guide the substrate in the correct geometry for optimal stereo- and regiospecific insertion of an oxygen atom into the 5-exo carbon–hydrogen bond of camphor [27]. Light absorption by the heme leads to a break of the iron CO bond and the kinetics of the rebinding is determined by the dynamics of the heme pocket structure and substrate mobility. Extensive studies were performed on myoglobin by the groups of Frauenfelder and Gibson [28–31] showing that this rebinding process is very complex and consists of at least three main steps — the recombination directly from the heme pocket (geminate recombination), the rebinding from the protein matrix and the rebinding from the solvent. The relative contribution of the different steps to the whole rebinding kinetics depend on the temperature. In the temperature range from 210 K to 260 K, used in the present study, the rebinding from the solvent is the most important step. This is clearly indicated from the dependence of the rate constant k_{obs} from the CO concentration, as well as from other flash photolysis studies using optical spectroscopy, which show that only 2.5% of the dissociated CO rebinds from the geminate position [32] for P-450$_{cam}$ bound with (1R)-camphor.

The formation of the ternary carbon monoxide–substrate–P-450 complex induces several changes in the spatial positions of the substrate and protein near the active site, as was shown by comparison of the crystal structures of the oxidized (1R)-camphor–P-450 complex vs. the reduced (1R)-camphor–P-450–CO complex [8,20]. Although the structure of the oxidized (1R)-camphor–P-450 complex is not identical to the reduced complex, that is created during the flash photolysis experiment, the steric changes that arise from the CO binding should be comparable. Briefly, the CO causes the (1R)-camphor substrate to move 0.8 Å away from the CO, and the temperature factor of the substrate increases, indicating a higher mobility of the substrate in the presence of CO than in its absence. This loosening of protein–substrate interactions is necessary for CO binding. The CO ligand points toward the distal I-helix, which forms a groove between the residues Gly-248 and Thr-252. This groove is slightly enlarged to allow the CO oxygen to fit inside, and this gives rise to movement of the backbone [33]. The increasing mobility of (1R)-camphor on ligand binding, as indicated by the increasing temperature factors has to be considered as an endothermic process, because hydrogen and van der Waals bonds break, and finally this results in a positive contribution to ΔH. An increasing mobility implicates that the molecule has more degrees of freedom and so there is a positive contribution to ΔS, too. The quantity of the contribution to ΔH and ΔS should therefore depend only on the difference in the mobility of the substrate between the CO–ligand-free and the CO–ligand-bound forms of P-450. The enthalpy difference for the CO binding reaction between (1R)-camphor and (1S)-camphor substrate is 13.9 kJ/mol, and the entropy difference is 71.7 J/(K·mol). This difference in the thermodynamic parameters is only due to the different steric interactions for the CO molecule in the heme pocket induced by the enantiomeric substrates. It can be explained by the different mobility of (1S)-camphor and (1R)-camphor. Obviously, the increase of the mobility of the substrate by CO binding to the reduced form of P-450 is not so strongly affected in the case of (1S)-camphor because it is already more loosely bound even in the oxidized as well as in the reduced state of P-450. Hence, there is not such a large positive contribution to ΔH and ΔS from breaking hydrogen

and van der Waals contacts to realise CO and substrate binding. This view is supported by the larger substrate dissociation constant K_d of the enzyme–substrate complex for (1S)-camphor compared to (1R)-camphor and by a faster high-spin to low-spin relaxation after a negative temperature jump (Schulze, H., private communication).

A similar argumentation has been presented by Shiro et al. [34] to explain the increase of ΔH and ΔS in the order of: Mb < P-450$_{nor}$ < P-450$_{cam}$(1R), indicating that less contacts have to be broken in myoglobin and more in P-450$_{cam}$(1R) during CO binding. (1S)-Camphor-bound P-450$_{cam}$ fits in this order, too, as indicated in Fig. 4 (upper and middle panel). ΔH and ΔS follow the order: Mb < P-450$_{nor}$ < P-450$_{cam}$(1S) < P-450$_{cam}$(1R) < P-450$_{cam}$(substrate-free) for the equilibrium reaction.

A detailed look at the *on* and *off* processes in Fig. 4 (upper and middle panel) shows that the studied complexes can be classified in two groups concerning the $\Delta H^{\neq}_{on}$ and $\Delta S^{\neq}_{on}$ values for the *on* process — the first group includes myoglobin, P-450$_{nor}$, P-450$_{cam}$(1S) and P-450$_{cam}$(1R) with almost similar $\Delta H^{\neq}_{on}$ (15.9–23.0 kJ/mol) and $\Delta S^{\neq}_{on}$ (−67.1 to −90.6 J/(K•mol)) and the second group containing substrate-free P-450$_{cam}$ and ferroprotoporphyrin IX with larger $\Delta H^{\neq}_{on}$ (51.0–61.9 kJ/mol) and strongly positive $\Delta S^{\neq}_{on}$ (98.7–145.2 J/(K•mol)). This difference is caused by the strong accessibility of the heme complex for solvent molecules in the latter group. In contrast, the *off* process shows thermodynamic parameters which gradually change in the sequence: Mb > P-450$_{nor}$ > P-450$_{cam}$(1S) > P-450$_{cam}$(1R) > P-450$_{cam}$(substrate-free) for $\Delta H^{\neq}_{off}$ and $\Delta S^{\neq}_{off}$. Obviously, the *off* process reflects the structural difference in the environment of the CO ligand in the heme pocket, which is also reflected in the gradual change of ΔH and ΔS in the equilibrium. The difference in enthalpy and entropy between the CO-bound form and the activated complex is not so strongly pronounced in (1S)-camphor-bound P-450 as compared to (1R)-camphor-bound P-450 which is in agreement with the above conclusion, that less contacts have to be broken during binding or releasing the CO ligand. Interestingly, a plot of ΔH vs. ΔS, as shown in the lower panel of Fig. 4, reveals that each process obeys a compensation relation.

Summarizing the discussed results and comparisons, we conclude that (1S)-camphor is more loosely bound in the heme pocket which might explain the increased uncoupling rate of 11% during the P-450$_{cam}$ catalyzed (1S)-camphor conversion, compared to 3% for the conversion of (1R)-camphor [9]. Because both enantiomers have the same chemical properties and electronic characteristics except their chiral behavior, the observed differences in the recombination kinetics can clearly be assigned to be due to different steric interactions (geometrical arrangement) in the active center of P-450$_{cam}$.

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