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Thermodynamic Properties of Oxidation–Reduction Reactions of Bacterial, Microsomal, and Mitochondrial Cytochromes P-450: An Entropy–Enthalpy Compensation Effect†

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ABSTRACT: An optically transparent thin-layer electrode cell with a very small volume was used for determination of the formal reduction potentials of bacterial, microsomal, and mitochondrial cytochromes P-450. At an extrapolated zero concentration of dye, the bacterial cytochrome from *Pseudomonas putida* catalyzing the hydroxylation of camphor and the adrenal mitochondrial cytochrome catalyzing the cholesterol side-chain cleavage reaction had formal reduction potentials of −168 and −285 mV (pH 7.5 and 25 °C), respectively. The oxidation–reduction potentials for the rabbit liver microsomal cytochrome P-450 induced by 3-methylcholanthrene and the mitochondrial cytochrome for steroid 11β-hydroxylation were found as −360 and −286 mV, respectively. Potential measurements at different temperatures allowed documentation of the standard thermodynamic parameters for cytochrome P-450 reduction for the first time. All cytochromes tested were found to have a relatively large negative entropy change upon reduction. The extent of these changes is comparable to that observed for the ferric–ferrous couple of cytochrome *c*. An entropy–enthalpy compensation effect was observed among the four cytochromes P-450 examined although the correlation is weaker than that observed with cytochrome *c* isolated from various sources.

Cytochrome P-450[1] is distributed widely in the biosphere, being found in mammals, plants, insects, bacteria, and yeast, and serves as a terminal oxidase of a variety of monooxygenase reactions (White & Coon, 1980). The various cytochromes P-450 display considerable homology at both the amino acid and nucleotide primary sequence levels (Morohashi et al., 1984). In order to investigate the possible functional similarities among bacterial, microsomal, and mitochondrial cytochromes in light of their common features of electron-transfer reactions resulting in the ferric–ferrous reduction of the heme iron, we have measured their formal oxidation–reduction potentials at different temperatures, thus determining the standard thermodynamic parameters for this redox couple. Clearly observed is an entropy–enthalpy compensation effect among the various cytochromes P-450 tested.

Materials and Methods

Materials. BV and IC were obtained from K and K Corp. BV was recrystallized twice from cold methanol by the addition of ether. IC was recrystallized twice from water by the addition of 2-propanol. Glucose oxidase (type VII) and catalase thymol free) were purchased from Sigma.

Methods. P-450$_{scc}$ and P-450$_{11\beta}$ were prepared from bovine adrenal cortex mitochondria. Purification of P-450$_{scc}$ was performed by the method described previously (Hsu, 1984). The specific content of final preparations was about 8

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[1] Abbreviations: BV, benzylviologen; DOC, deoxycorticosterone; DTT, dithiothreitol; $E^{\circ\prime}$, formal reduction potential vs. normal hydrogen electrode; EDTA, ethylenediaminetetraacetate; IC, indigo carmine; *n*, the electron equivalent per reaction; NHE, normal hydrogen electrode; SCE, saturated calomel electrode; P-450, cytochrome P-450; P-450$_{cam}$, cytochrome P-450 catalyzing camphor 5-exo-hydroxylation; P-450$_{LM_4}$, cytochrome P-450 induced by 3-methylcholanthrene or β-naphthoflavone and purified from liver microsomes of rabbits; P-450$_{11\beta}$, cytochrome P-450 catalyzing steroid 11β-hydroxylation; P-450$_{scc}$, cytochrome P-450 catalyzing cholesterol side-chain cleavage reaction; *r*, correlation coefficient.

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nmol/mg of protein. Purification of P-450$_{11\beta}$ was carried out as follows. The sonicated mitochondrial particles (4.2 g of protein) frozen in liquid nitrogen were thawed and solubilized in 330 mL of 50 mM potassium phosphate buffer (pH 7.25) containing 1.27% sodium cholate (protein:sodium cholate 1:1), 0.1 mM DTT, 0.1 mM EDTA, and 50 μM DOC. The subsequent operations were performed at 0–4 °C. The mixtures were gently stirred for 1 h and then centrifuged at 105000g for 60 min. The procedures, including ammonium sulfate fractionation, aluminum Cγ treatment, and dialysis of the solution, were the same as the method for Suhara et al. (1978). The dialyzate was centrifuged at 12000g for 30 min; the precipitate was solubilized in 100 mL of 50 mM potassium phosphate buffer (pH 7.25) containing 0.7% sodium cholate, 0.2 M KCl, 0.1 mM DTT, 0.1 mM EDTA, and 50 μM DOC and then applied to an ω-aminooctyl-Sepharose 4B column (2 × 19 cm) previously equilibrated with the same buffer. The P-450 was adsorbed on the upper portion of the column. After the column was washed with 120 mL of the same buffer, the absorbed band was separated into two portions. Most of the P-450 band, having a dark red color, descended to the middle portion of the column. As is typically observed, a relatively small amount of the P-450 band, having a clear red color found in the top of the column, did not elute in the washing step. After the upper band was cut out, P-450$_{11\beta}$ was eluted with 50 mM potassium phosphate buffer (pH 7.5) containing 1% sodium cholate, 0.5% Triton X-100, 0.5 M KCl, 0.1 mM DTT, 0.1 mM EDTA, and 50 μM DOC. The oxidized spectrum of this fraction had a Soret peak in the 390-nm region, indicating a form of high-spin P-450. The clear red band cut out was overlaid to another ω-aminooctyl-Sepharose 4B column and was eluted with the same buffer. The oxidized spectrum of this fraction exhibited a Soret peak at 417 nm, indicating a low-spin cytochrome. Difference spectrum with 17α-hydroxyprogesterone demonstrated that this P-450 was P-450$_{C21}$, thus being successfully separated from the high-spin P-450$_{11\beta}$. The specific content of purified P-450$_{11\beta}$ was in the range of 11.7–17.6 nmol/mg of protein.

P-450$_{cam}$ and P-450$_{LM4}$ were prepared by the reported methods from *Pseudomonas putida* (Yu et al., 1974) and rabbit liver microsomes (Haugen & Coon, 1976), respectively. The specific contents of heme for bacterial and microsomal P-450's were 14.1 and 13.1 nmol/mg of protein, respectively.

Their optical properties of these proteins are summarized as follows. P-450$_{cam}$, P-450$_{LM_4}$, P-450$_{scc}$, and P-450$_{11\beta}$ had their oxidized maxima at 391, 394, 393, and 393 nm, respectively, with their reduced Soret maxima at 408, 411, 410, and 410 nm, respectively. Differential extinction coefficients between oxidized and reduced forms were 25.3 at 408 nm (Gunsalus et al. 1973), 10.8 at 411 nm (Haugen & Coon, 1976), 6.6 at 410 nm, and 14.1 mM^{-1} cm^{-1}, respectively. The temperature for these determinations was 22 °C in all cases. All cytochromes used were found to be enzymatically active in their respective hydroxylation reactions.

The optical-transparent thin-layer electrode cell with a small volume was prepared as described previously (Huang & Kimura, 1983). The spectropotentiostatic experiments were performed with a three-electrode system on an Amel Model 551 potentiostat/galvanostat equipped with an Amel Model 566 function generator. The reduction potential ($E°'$) was calculated from the standard Nernst equation. Entropy for the complete cell reaction adjusted to the NHE scale, $\Delta S°$, was determined from (Taniguchi et al., 1980)

$$\Delta S° = F(dE°'/dT) - 15.6 \text{ eu}$$

Standard free-energy change $\Delta G°$ for the cell reaction was calculated from the $E°'$ value at 25 °C, and the standard enthalpy change $\Delta H°$ was determined from $\Delta G°$ and $\Delta S°$, as described previously (Huang & Kimura, 1984). The extinction coefficients for the dyes used in this study are as follows: 10.1 and 22.2 mM^{-1} cm^{-1} for BV at 595 nm and for IC at 600 nm, respectively. ω-Aminooctyl-Sepharose 4B was prepared by the method of Cuatrecasas and Anfinsen (1971). P-450 and protein were determined by the methods of Omura and Sato (1964) and Lowry et al. (1951), respectively.

Results

Stability Test. In order to make sure P-450's remained completely active during measurements of oxidation–reduction potential, we first documented the stability of these cytochromes under various conditions. In the presence of substrate, P-450$_{cam}$ was completely stable for up to 120 min at 35 °C. P-450$_{scc}$ and P-450$_{LM_4}$ were also quite stable up to 120 min at 35 °C. Contrary to these cytochromes, P-450$_{11\beta}$ was found to be very unstable even in the presence of DOC. P-450$_{11\beta}$ (0.05 mg of protein/mL) was easily precipitated at 25 °C within 60 min due to its denaturation, and 70% of this cytochrome was lost at 10 °C after two cycles of electrochemical reduction and reoxidation or after 6 h at this temperature. This result demanded that P-450$_{11\beta}$ experiments must be completed within 120 min at low temperature the oxidation–reduction cycle should not be repeated more than once. We have applied various criteria to demonstrate the intactness of proteins during and following reduction and reoxidation. The formation of cytochrome P-420, the loss of CO binding capacity, and the reversibility of the oxidation–reduction reaction were tested for each individual cytochrome. The n value determined serves as an initial estimation of stability. According to the Nernst equation, the n value must be 1.000 for a one-electron-transfer reaction. Only data with n values between 0.900 and 1.100 were used.

In the absence of substrate, all oxidized cytochromes except P-450$_{LM_4}$ were unstable during the period of our experiments. P-450$_{LM_4}$ was a high-spin form[2] as isolated, and the high-spin form was stable up to 120 min at 35 °C without addition of substrate. The reduced forms of P-450$_{cam}$, P-450$_{scc}$, and P-450$_{11\beta}$ were found to be unstable in the absence of substrate according to the above-mentioned criteria. Additions of stabilizers such as glycerol and ethylene glycol were unable to stabilize these cytochromes, and hence, no redox potentials were determined for these low-spin forms.

Selection of Wavelength for Potential Determination. We have observed that all cytochromes used in this study required the addition of appropriate dye for efficient electrochemical reduction. Electrons generated on the surface of the electrode were not capable of reducing the heme protein. Spectrophotometric determination of oxidation–reduction state was carried out in the presence of dye, with BV used for P-450$_{LM_4}$, P-450$_{scc}$, and P-450$_{11\beta}$ and IC for P-450$_{cam}$. The oxidized spectrum of BV has no absorption maximum between 350 and 700 nm, whereas the reduced spectrum displayed a broad maximum at 595 nm. At high concentrations of BV, the maximum was shifted to 540 nm due to dimerization of the molecules. The reduced pigment did not have any absorbance between 410 and 430 nm. The potential determinations for P-450$_{LM_4}$, P-450$_{scc}$, and P-450$_{11\beta}$ were carried out, therefore, at 417, 420, and 420 nm, respectively. The oxidized and

[2] Since P-450$_{cam}$, P-450$_{LM_4}$, P-450$_{scc}$, and P-450$_{11\beta}$ had respective extinction coefficients of 87, 96, 98, and 90 mM^{-1} cm^{-1} at high-spin maxima (22 °C), it appears that a thermal equilibrium of mixed-spin states was reached at the temperature used.

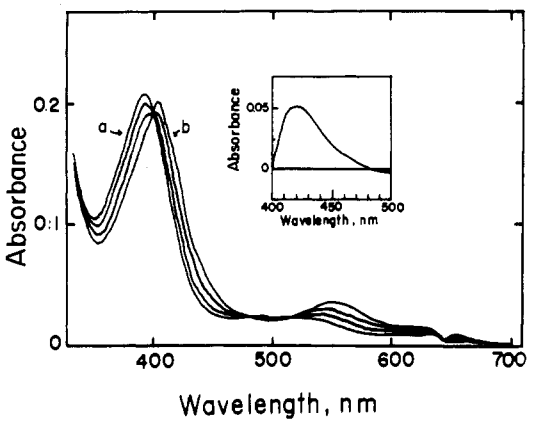

FIGURE 1: Oxidized and electrochemically reduced spectra of P-450$_{11\beta}$. Reaction mixtures consisted of 80 μM P-450$_{11\beta}$, 1 mM DOC, and 40 μM BV in 0.15 mL of 35 mM potassium phosphate buffer (pH 7.5) containing 20% glycerol, 0.35 M KCl, 0.7% sodium cholate, 0.35% Triton X-100, 40 mM glucose, 0.3 mg/mL glucose oxidase, and 0.1 mg/mL catalase. All manipulations were carried out at 10 °C. After the oxidized spectrum (a) was recorded at 0 mV vs. SCE, reduction was carried out at −700 mV vs. SCE for 30 min, and then the reduced spectrum (b) was recorded. Curves in between indicate intermediate states. The cell path length was 0.3 mm. The inset shows the difference spectrum between the reduced and oxidized forms. The absorption maximum was found at 418 nm. The reduced spectrum of BV displayed maxima at 595, 400, and 371 nm.

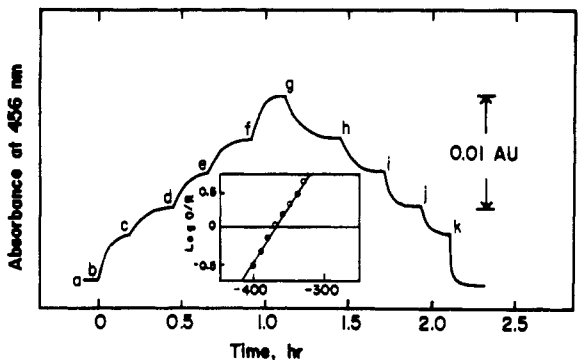

FIGURE 2: Reversible reduction and reoxidation of P-450$_{cam}$. Reaction mixtures consisted of 76 μM P-450$_{cam}$, 76 μM IC, and 152 μM camphor in 0.1 M potassium phosphate buffer (pH 7.5) containing 0.1 M KCl, 0.1 M glucose, 0.3 mg/mL glucose oxidase, and 0.1 mg/mL catalase. Reduction and reoxidation were carried out at 25 °C. a–k show applied potentials of 0, −340, −360, −380, −400, −650, −400, −380, −360, −340, and 0 mV vs. SCE, respectively. The inset shows the Nernst plots yielding $E^{\circ\prime}$ = −126 mV vs. NHE, n = 0.982, and r = 0.9987 at 25 °C. Open circles represent data from separate experiments.

reduced spectra of IC displayed an isosbestic point at 456 nm, where only a small difference in absorbance between oxidized and reduced P-450$_{cam}$ was also detectable. The potential determinations for P-450$_{cam}$ were, therefore, carried out at 456 nm.

Figure 1 shows typical spectra of oxidized and reduced P-450$_{11\beta}$ in the presence of BV. The reduced maximum was found at 404 nm, which was shifted toward lower wavelength by 6 nm due to the effect of reduced BV present in the sample. As shown in the inset of Figure 1, the difference spectrum between oxidized and reduced forms had a maximum at near 420 nm.

Reversibility of the Oxidation–Reduction Reaction. In order to assure that the oxidation–reduction reaction is strictly reversible, we have carried out stepwise reduction and reoxidation of P-450 by applying various potentials. Figure 2 shows a typical example for P-450$_{cam}$. From this titration, $E^{\circ\prime}$ of −126 mV vs. NHE and an n value of 0.982 were obtained at

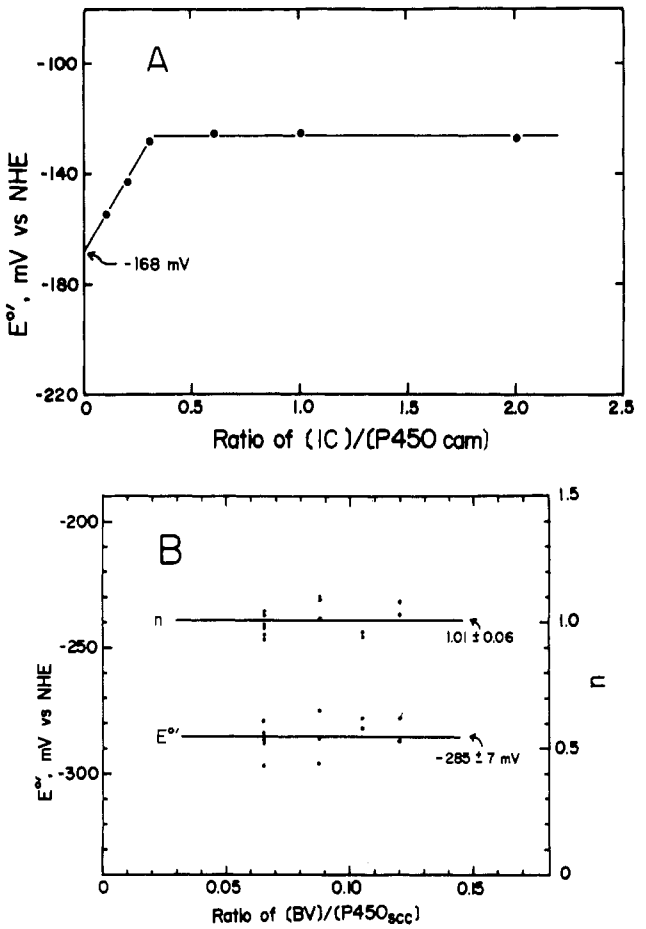

FIGURE 3: Effect of concentration of dye on $E^{\circ\prime}$ of P-450$_{cam}$ (A) and P-450$_{scc}$ (B). Reaction mixtures (A) contained 60–75 μM P-450$_{cam}$ and various concentrations of IC, as described in Figure 2. Electrochemical reduction was carried out at 25 °C. When $E^{\circ\prime}$ was extrapolated to zero concentration of IC, the value of −168 mV vs. NHE was obtained. Reaction mixtures (B) contained 40 μM P-450$_{scc}$, 80 μM cholesterol, and various concentrations of BV, in 0.1 M potassium phosphate buffer (pH 7.4) containing 20% glycerol, 0.1 M KCl, 0.1 M glucose, 0.3 mg/mL glucose oxidase, and 0.1 mg/mL catalase. Electrochemical reduction was carried out at 25 °C. $E^{\circ\prime}$ and n are calculated from all points obtained as the mean ± SD, since all values are within experimental errors.

the indicated dye concentration. Similar results were obtained for P-450$_{LM_4}$, P-450$_{scc}$, and P-450$_{11\beta}$. The values for $E^{\circ\prime}$ were calculated as −357, −279, and −285 mV vs. NHE for P-450$_{LM4}$, P-450$_{scc}$, and P-450$_{11\beta}$, respectively, with corresponding n values of 1.100, 0.967, and 0.903. These results clearly indicate that all P-450's tested undergo a simple one-electron transferring reaction under these experimental conditions. No P-420 formation was detected on the sample after each redox titration experiment.

Effect of Dye Concentration. Many dyes are capable of nonspecific binding to proteins, which could drastically affect the dye potential. To eliminate this possibility, we have measured the potential at various concentrations of dye and extrapolated the value to zero concentration. Figure 3 indicates the effects of dye concentration on the potentials of P-450$_{cam}$ and P-450$_{scc}$. The redox potential of P-450$_{cam}$ was independent of dye concentration from ratio of IC to P-450$_{cam}$ between 0.4 and 2.0. Below a dye to protein ratio of 0.4, the potential decreased linearly. At the zero concentration, an $E^{\circ\prime}$ of −168 mV vs. NHE is obtained. This value is close to a value reported previously (Sligar et al., 1979). The P-450$_{scc}$ potential was independent of BV concentration from a ratio of 0.065 to 0.12, suggesting that BV does not affect the potential of

Table I: Thermodynamic Parameters of P-450's at 25 °C[a]

P-450	$\Delta G°$ (kcal/mol)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (eu)	$E°'$ (mV vs. NHE)
P-450$_{cam}$	2.75	−13.6	−54.7	−128 ± 1[b]
P-450$_{11\beta}$	6.92	−4.08	−36.6	−286 ± 5[c]
P-450$_{scc}$	6.50	0.36	−20.6	−285 ± 7[d]
P-450$_{LM_4}$	8.39	−0.13	−28.6	−360 ± 2[e]

[a] $\Delta G°$, $\Delta H°$, and $\Delta S°$ values were calculated as described under Materials and Methods. The value of P-450$_{11\beta}$ was calculated by extrapolation to 25 °C, where the cytochrome is unstable and the determination was experimentally impossible. [b] Data from Figure 4A. [c] Data at the ratio of BV to P-450$_{11\beta}$ as 1:4. [d] Data from Figure 3B. [e] Data from Figure 4C.

P-450$_{scc}$ at a low concentration. The effect of BV concentration on the potential of P-450$_{11\beta}$ was then examined. At low concentrations of BV [BV/P450 < 1/4 (mol/mol)], the observed redox potential was dependent on the dye concentration. At an extrapolated zero concentration of dye, a value −281 mV vs. NHE was calculated for P-450$_{11\beta}$.

Effect of Temperature and an Entropy–Enthalpy Compensation. In order to obtain thermodynamic parameters of the oxidation–reduction reactions, we measured the redox potentials at a range of different temperatures wherein the specific P-450 species is stable. Curves A–C of Figure 4 show these profiles of P-450$_{cam}$, P-450$_{LM_4}$, and P-450$_{scc}$, respectively. P-450$_{cam}$ displayed a distinct break point at 30 °C. In Figure 4A, the solid lines were obtained by best fitting the data points into two linear lines giving optimum correlation coefficients. P-450$_{LM_4}$ and P-450$_{scc}$ showed a lesser dependence on temperature, being similar to that of P-450$_{cam}$ below 30 °C. Since P-450$_{11\beta}$ was only stable below 10 °C, we have determined the parameters at both 10 and 2 °C, and hence, the accuracy of these parameters may be less than those of other cytochromes P-450 tested, as summarized in Table I. When $\Delta H°$ was plotted against $\Delta S°$, a linear relationship was obtained with a correlation coefficient of 0.976, suggesting a compensation effect of entropy $\Delta S°$ with enthalpy $\Delta H°$ (Figure 5).

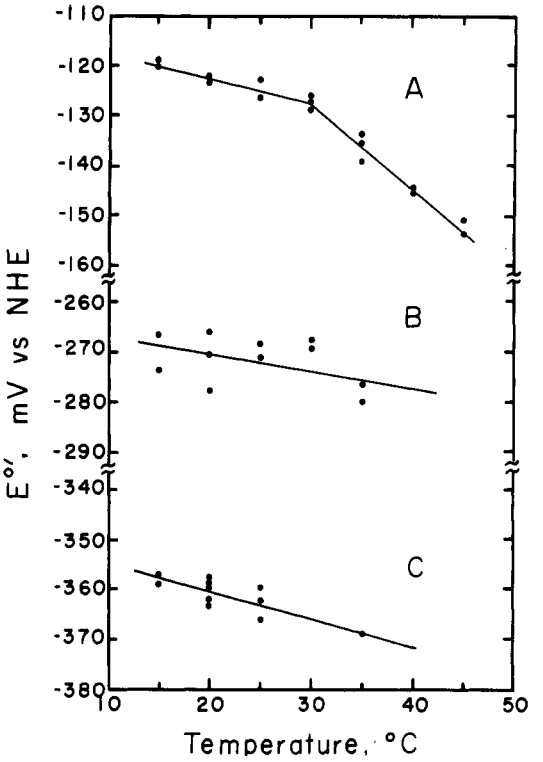

FIGURE 4: Temperature effect on the $E°'$ of P-450$_{cam}$ (A), P-450$_{scc}$ (B), and P-450$_{LM_4}$ (C). The reaction mixtures (A) contained 50 μM P-450$_{cam}$, 50 μM IC, and 50 μM camphor as described in Figure 2, electrochemical reduction was carried out at various temperatures as indicated in the figure, and calculated values of $E°'$ are shown. The lines were drawn on the basis of the best fit on data points: below 30 °C, r was 0.920; above 30 °C, r was 0.982. Reaction mixtures (B) contained 40 μM P-450$_{scc}$, 4 μM BV, and 80 μM cholesterol, as described in Figure 3B. Electrochemical reduction was carried out at various temperatures as indicated in the figure, and calculated values of $E°'$ are shown. Reaction mixtures (C) contained 30 μM P-450$_{LM_4}$ and 3 μM BV in 0.1 M potassium phosphate buffer (pH 7.4) containing 20% glycerol, 0.2 M KCl, 0.1 M glucose, 0.3 mg/mL glucose oxidase, and 0.1 mg/mL catalase. Electrochemical reduction was carried out at various temperatures as indicated in the figure, and calculated values of $E°'$ are shown.

Discussion

The redox potentials for variety of P-450's have been reported in the literature by the number of different methods including enzymatic reduction, dithionite reduction, and photochemical reduction (Table II). In this paper, we have carried out the measurements using an optically transparent thin-layer electrode, which provides the crucial reversibility and stoichiometry determinations. The benefits of this method are discussed in our previous paper (Huang & Kimura, 1983).

Among various cytochromes P-450, reported values of $E°'$ range from −170 to −400 mV vs. NHE, indicating that these cytochromes can express a wide range of potentials.

Among the reported redox titrations, a stoichiometry of n = 2 for P-450$_{scc}$ was observed in a titrations of ferric P-450$_{scc}$ with sodium dithionite (Lambeth & Pember, 1983). These authors obtained the value of 1, when P-450$_{scc}$ was titrated with NADPH in the presence of adrenodoxin reductase and

Table II: Reduction Potentials and n Values of Purified P-450's

P-450's	$E°'$ (mV vs. NHE)	n	method	ref
P-450$_{cam}$ (*Pseudomonas*)	−170 ± 5	1	dithionite reduction	Peterson (1971), Sligar (1976), Sligar et al. (1979)
	−168 (pH 7.4) (+camphor)	0.93 ± 0.03	electrochemical reduction, extrapolation at 0 or [dye]	this work
P-450$_{scc}$ (bovine adrenal)	−297 ± 4 (pH 7.4)	1.0 ± 0.2	enzyme reduction and EPR	Light & Orme-Johnson (1981)
		2	dithionite reduction	Lambeth & Pember (1983)
	−284 (pH 7.0)	1	enzyme reduction	Lambeth & Pember (1983)
	−285 ± 7 (pH 7.4) (+cholesterol)	1.01 ± 0.06	electrochemical reduction	this work
P-450$_{11\beta}$ (bovine adrenal)	−286 ± 5 (pH 7.5) (+DOC)	0.98 ± 0.02	electrochemical reduction	this work
P-450$_{LM_2}$ (rabbit liver)		1.02	dithionite reduction	Peterson et al. (1977)
P-450$_{LM_4}$ (rabbit liver)		1	dithionite reduction	Peterson et al. (1977)
	−360 ± 2 (pH 7.4)	1.00 ± 0.00	electrochemical reduction	this work
P-450$_{PB\text{-}B}$ (rat liver)	−319 ± 4	*b*	photochemical reduction	Guengerich (1983)
P-450$_{\beta NF\text{-}B}$ (rat liver)	−299	*b*	photochemical reduction	Guengerich (1983)

[a] The list only includes cytochromes P-450 related to those used in this study and selected for the highly pure ones. The membrane-bound forms are not included. P-450$_{PB\text{-}B}$ and P-450$_{\beta NF\text{-}B}$ are purified from rat liver and homologues to rabbit P-450$_{LM_2}$ and P-450$_{LM_4}$, respectively. [b] The value of n was reported as 0.85–1.22.

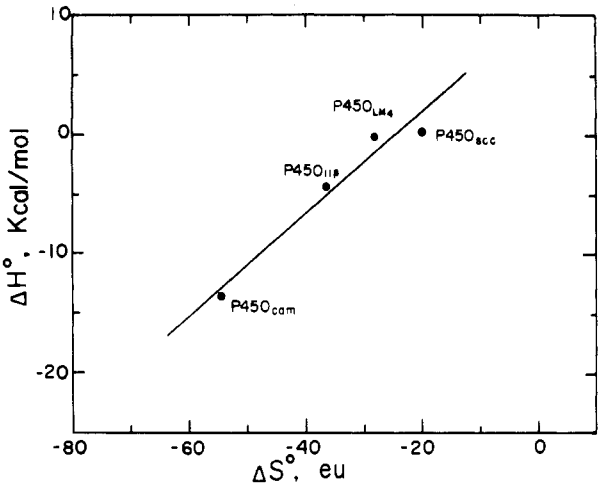

FIGURE 5: $\Delta H°$–$\Delta S°$ diagrams for reduction reaction of various cytochrome P-450's. The $\Delta H°$ and $\Delta S°$ values of various P-450's calculated from Table I were plotted as $\Delta H°$–$\Delta S°$ diagrams. The linearity with a correlation coefficient of 0.976 gave a slope of 433 K. The value for P-450_{cam} in the low-temperature region was used to obtain the best correction. When that in the high-temperature region was used, the correlation coefficient of 0.571 was calculated.

adrenodoxin. Our present studies using an electrochemical method revealed that all cytochromes had a redox stoichiometry of unity, which experimentally varied from 0.900 to 1.100. We did not examine the effect of medium ionic strength extensively, since for electrochemical measurements the presence of salts such as KCl as indispensible. Particularly, P-$450_{11\beta}$ was found stable only in the presence of a high concentration of KCl. Except for P-$450_{11\beta}$, the measurements were carried out in the presence of 0.1 M KCl for other cytochromes P-450.

For the first time, we could obtain the reduction potential of P-$450_{11\beta}$ and the values of $\Delta G°$, $\Delta H°$, and $\Delta S°$ from temperature-dependent effects of four different P-450 systems. Large negative values of $\Delta S°$ for the cytochromes P-450 (−20.6 to −54.7 eu) were seen for these proteins upon reduction. Cytochromes *c* from various sources also exhibit large negative values of $\Delta S°$, ranging from 0 to −40 eu (Haung & Kimura, 1984). Therefore, the distribution (34 eu) of $\Delta S°$ values of the cytochromes P-450 examined in this study appears to be similar to that (40 eu) of the cytochromes *c*. It is difficult to explain the large negative value of $\Delta H°$ observed particularly for P-450_{cam}, although the possibility of a large enhancement of π-back-bonding in the ferrous state of this cytochrome cannot be excluded. When $\Delta H°$ values were plotted against $\Delta S°$ values, a linear relationship among P-450's was observed with a correlation coefficient of 0.976. Similar analysis for various cytochromes *c* also gave a linear relationship with a correlation coefficient of 0.986 (Huang & Kimura, 1984), leading us to conclude an entropy–enthalpy compensation effect among cytochromes *c*. For the P-450 systems, the observed correlation is less rigorous than that in the cytochromes *c*.

When we calculated the compensation factor (β) or isokinetic temperature from the slope, the value of 433 K was obtained. The β value for cytochromes *c* was 264 K. For most entropy–enthalpy compensation processes in chemistry, the β values lie in a relatively narrow range between 250 and 315 K (Lumry & Rajender, 1970). Our value of 433 K appears to be very high, although previous literature reference do not include biological oxidation–reduction reactions. The molecular mechanisms for this correlation are currently under active investigation.

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

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