of the amino acid compared to the shifts observed for the protons on 1^{4+} and the minimal shifts of the nonaromatic protons of the amino acids strongly suggest the formation of inclusion complexes, in which the aromatic group of the amino acid is engulfed inside the paraquat-lined cavity of the cyclophane host as illustrated in Scheme I for tyrosine. The quick broadening of the aromatic resonances of the amino acids can also be understood as a result of the strong charge-transfer interactions in these complexes.

Therefore, 1^{4+} constitutes the first example of a new class of receptors, based on charge-transfer interactions between aromatic rings, capable of binding amino acids possessing electron-rich aromatic moieties. One can envision 1^{4+} as a building block for highly selective amino acid receptors. We are currently investigating the feasibility of using 1^{4+} in the topographical analysis of the solution-phase conformations of proteins. Because of the solubility of its tetrachloride in water, 1^{4+} is expected to act as a molecular probe to detect and, perhaps, quantify water-exposed tryptophan and tyrosine residues.

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Aromatic Hole Superexchange through Position 82 of Cytochrome *c* Is Not Required for Intracomplex Electron Transfer to Zinc Cytochrome *c* Peroxidase

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Nature has placed an evolutionarily conserved phenylalanine at position 82 of Cc,[1] and its possible role in electron transfer has long been suspected. We previously reported[2] results obtained by using a suite of position 82 mutants of yeast iso-1 Cc that indicated that the rate constant, k_b, for the thermal $Fe^{2+}P \rightarrow (ZnP)^+$ ET reaction within [ZnCcP,Cc] complexes is large when residue 82 is aromatic and small when it is aliphatic, although it was left undetermined whether the difference reflected conformational effects[3,4] or the abolition of hole superexchange pathways.[5] Measurements with a new apparatus now show that superexchange through position 82 does *not* significantly enhance this reaction. For every Cc variant, the charge-separated intermediate, $[(ZnP)^+CcP,Fe^{2+}Cc]$ (I), converts back to the ground state, $[(ZnP)CcP,Fe^{3+}Cc]$ (A), with multiphasic kinetics[6] that include components with *both* large and small values of k_b, *independent* of whether position 82 of yeast Cc has an aromatic or aliphatic residue.

Luminescence decay and transient absorption measurements[7] on the triplet excited state, $[^3(ZnP)CcP,Fe^{3+}Cc]$ (A*), of complexes prepared with position-82 mutants of yeast iso-1 Cc, where a Cys 102 → Thr modification has been introduced for stability,[8] show that in the presence of excess $Fe^{3+}Cc$ the triplet state decays exponentially, rate constant k_p. In agreement with the initial study of position-82 mutants,[2] intracomplex quenching of the $^3(ZnP)CcP$ by the ferriheme of $Fe^{3+}Cc$ contributes to the triplet-state decay with a quenching rate constant[9a] that varies with cytochrome, from ~170 to ~30 s^{-1} (Table I) under the conditions employed here.

The time evolution of I was monitored for each of the [ZnCcP,Cc] complexes by following the absorbance change at the λ = 549 nm $^3(ZnP)/ZnP$ isosbestic. In our initial studies, for Cc having an aliphatic residue at position 82 we observed I to appear with rate constant k_p and decay more slowly, with an ET rate constant $k_b < k_p$, whereas for variants with an aromatic residue, I appeared rapidly, with $k_b > k_p$, and decayed with k_p. However, the small absorbances associated with the transient were then[2] at the limits of instrumental detection. Moreover, much of the data was collected at 0 °C because the initial suite of mutants[2a] did not contain the stabilizing Thr 102 modification,[8a] and this further diminishes the signals. Extensive measurements at 20 °C with greatly improved S/N[7] (Figure 1) now show that all the Cc variants display multiphasic kinetics for I, as reported for the complexes with *Candida krusei* and horse Cc.[6] Thus, the rapid rise of the intermediate is confirmed for those Cc with an aromatic residue 82 (e.g., Phe; Figure 1, upper left) but an additional slow decay has been detected (Figure 1, upper right), whereas the slow decay for the others is confirmed (e.g., Leu; Figure 1, middle right) but a rapid rise now has been found (Figure 1, middle left).

Excellent self-consistent fits to the observed kinetics of I are obtained with the triphasic function employed earlier,[6] eq 1. Here

$$\Delta A(t) = \beta \sum f_i \frac{(e^{-k_p t} - e^{-k_i t})}{(k_i - k_p)} + \Delta A_f e^{-k_p t} \qquad (1)$$

i = 1-3; k_i is the I → A rate constant (k_b) for phase i, and f_i is the weight of phase i;[9b] the term proportional to ΔA_f accommodates departures from a triplet, ground-state isosbestic. The wavelength dependence of the transient signal confirms that it

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(1) Abbreviations: ZnCcP, zinc-substituted cytochrome *c* peroxidase; Cc, cytochrome *c*; ET, electron transfer; WT, wild type.

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(9) (a) The quenching rate constant is defined as $k_q = k_p - k_d$ where k_d is the decay rate constant for the $[^3ZnCcP,Fe^{2+}Cc]$ complex. As noted elsewhere (ref 6), in general the quenching has contributions both from electron transfer (rate constant k_t) and from energy transfer, with the proportions differing with the cytochrome. (b) The prefactor has the form $\beta = k_t \Delta\epsilon(I-A)A^*(0)$, where the first factor is the $A^* \rightarrow I$ ET rate constant, the second is the difference in extinction coefficients, $\epsilon(I) - \epsilon(A)$, and the third is the concentration of A^* immediately after photolysis. The actual fits used f_2 and f_3 as fitting parameters and obtained f_1 from the relation $\sum f_i = 1$. Determination of the values for the k_i, f_2, and f_3 involved jointly fitting data obtained on short and long time scales, with k_1 being determined by the former, k_2 and k_3, by the latter (Figure 1); k_p could be determined independently from the decay of A^*. A distribution of slowly decaying phases could also fit the data; we have provisionally chosen to use the triphasic scheme for simplicity and clarity.

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Table I. Kinetic Parameters for Electron Transfer within [ZnCcP,Cc] Complexes[a]

residue 82	k_p (k_q)[b]	k_1	f_1	k_2	f_2	k_3	f_3
Phe(WT)	265 (150)	4900	0.84	27	0.04	3	0.12
Tyr	285 (170)	4300	0.92	21	0.06	4	0.02
Ser	260 (145)	2500	0.72	38	0.12	9	0.16
Leu	190 (75)	2100	0.88	17	0.08	3	0.04
Ile	145 (30)	1900	0.89	19	0.05	4	0.05
Gly	155 (40)	1800	0.77	55	0.13	15	0.10

[a] All Cc variants are yeast iso-1 Cc with an additional Cys 102 → Thr substitution for stability (ref 8a). Kinetic parameters were obtained by nonlinear least-squares fits to eq 1 (ref 9); rate constants have the units reciprocal seconds. Estimated uncertainties are as follows: k_p, ±5 s^{-1}; k_1, ±500 s^{-1}; k_2, ±10 s^{-1}; k_3, ±2 s^{-1}; f_2, f_3, ±40%. Conditions: [ZnCcP] ~ 5 μM; [Fe^{3+}Cc] ~ 12 μM; 1 mM KP_i, pH 7.0, 20 °C. [b] Derived parameters: $k_q = k_p - k_d$ (see ref 9a) where k_d = 115 s^{-1} is the triplet decay rate constant for the complex [(^{3}ZnP)CcP,Fe^{2+}Cc].

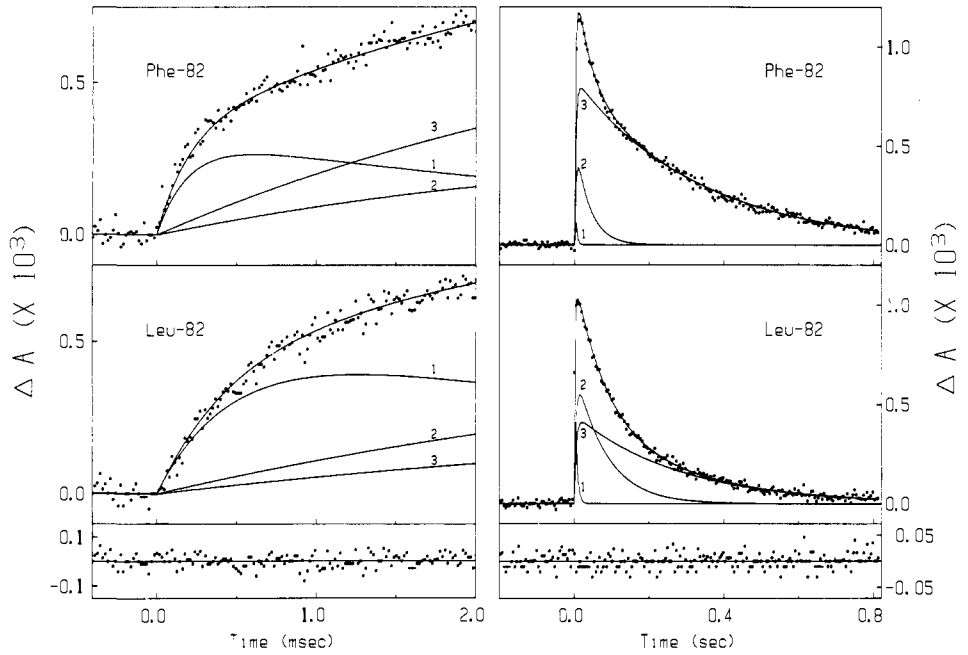

Figure 1. Transient absorbance difference of the electron-transfer intermediate I formed upon flash photolysis of complexes of ZnCcP with Cc(Phe82;WT) (upper) and Cc(Leu82) (middle). Solid lines through the data are resultants of joint fits of the short-time (left) and long-time (right) data to eq 1. Decompositions of the full fits into the individual kinetic phases (i = 1-3) also are presented. The lower panels are the residuals to the fit for Cc(Leu82); they are typical of those for all the complexes of Table I. Kinetic fit parameters are listed in Table I. Conditions: [ZnCcP] ~ 5 μM; [Cc] ~ 12 μM; 1 mM KP_i; pH 7; 20 °C. Short-time traces averaged 400 transients (amplifier response time ~ 1 μs); long-time traces averaged 50 transients (~10 μs response time).

is associated with I. Figure 1 displays both the full curves for $\Delta A(t)$ and the time courses for the individual phases. The kinetic fit parameters for each complex are given in Table I; in each case, phase 1 dominates ($f_1 \gtrsim 0.7$), with $2 \times 10^3 \lesssim k_1 \lesssim 5 \times 10^3$ s^{-1}. As can be seen for the Phe and Leu variants in Figure 1, right, differences in the relative fractions of the slow phases (2 and 3) cause substantial differences in the appearance of the overall decay envelopes. The slow I → A phases (i = 2, 3) do not reflect the second-order recombination of Fe^{2+}Cc and (ZnP)$^+$CcP formed by dissociation of I, for the long-time traces cannot be fit to a second-order equation and do not change appreciably with the amount of excess Fe^{3+}Cc in solution, the one caution being that it is hard to rule out *all* second-order contamination of the slowest phase 3. Instead, as previously discussed, we infer the kinetics embodied in eq 1 to imply that the charge-transfer intermediate I exists in three (or more) bound forms that exhibit very different ET reactivities.

Complexes with all of the yeast iso-1 Cc variants exhibit qualitatively similar multiphasic kinetics with the rate constants for the fastest and slowest phases of the I → A process differing by 10^2–10^3 at 20 °C (Table I). Because the three k_i change minimally upon alteration of residue 82, none of them can depend significantly on aromatic superexchange through this position. In particular, the large value of k_1 relative to k_2 and k_3 cannot reflect the occurrence of hole superexchange through this residue in one form of the complex but not in others, and the same is almost certainly true for complexes with the Cc from horse and C. krusei, which showed similar behavior.[6] This absence of superexchange enhancement in first-order rate constants for the intracomplex $Fe^{2+}P \rightarrow (ZnP)^+$ (I → A) ET reaction is reflected both in the steady-state ET kinetics[10a] of Cc and FeCcP and in NMR-detected electron exchange between[10b] Cc and cytochrome b_5.

The differences among the kinetic phases described here likely reflect changes in the structure and/or dynamics at the protein–protein interface,[11] although they also could represent the influence of a conformational change of one of the partners[4c] or even the involvement of a second bound Cc.[12] Such explanations are consistent with the result that the fractions of the phases, f_i, vary among the Cc mutants (Table I) and that binding of Cc to CcP[13] and cytochrome oxidase[8d] is sensitive to changes in residue 82. Experiments directed toward understanding these differences in the ET reactivity of the [ZnCcP,Cc] complex are in progress.

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