at −40 °C in THF),[10] was reacted with benzaldehyde in THF at −78 °C (strictly salt-free conditions). Immediate examination by ^{31}P NMR at −60 °C revealed two sharp singlets at −70.2 and −71.0 ppm, attributed to cis and trans oxaphosphetanes **2b** and **3b**, respectively, in a 47:53 ratio, as well as a minor singlet (ca. 12%) at 40.8 ppm for tributylphosphine oxide.[11] At −40 °C the singlet for **3b** increased at the expense of the one for **2b** with a $t_{1/2}$ of ca. 130 min, while the phosphine oxide singlet remained constant. At −10 °C the phosphine oxide peak grew at the expense of the oxaphosphetane peaks (present at this temperature in a 2:98 cis/trans ratio) with a $t_{1/2}$ of ca. 380 min. Thus, in this example the equilibration could be studied separately, allowing both processes to be understood by inspection of the kinetic data. Importantly, "stereochemical drift" here is largely divorced from oxaphosphetane decomposition to alkenes and phosphine oxide; it is primarily connected with oxaphosphetane equilibration.[12] GLC analysis of the completed reaction showed alkenes **4** and **5** in an 8:92 ratio. The high stereoselectivity for *E* alkene in this type of Wittig reaction (one involving a trialkyl ylide) arises from *two* sources (not just one[1c]): (1) direct production of a greater proportion of trans oxaphosphetane in the carbon–carbon bond-forming process and (2) equilibration of oxaphosphetanes with a strong preference for the trans isomer (cf. **1b** with **1a**).

The reaction of ylide **1b** with hexanal at −60 °C showed oxaphosphetanes by ^{31}P NMR in a cis/trans ratio of 14:86 (δ −69.3 and −72.8; singlet at 32.8 ppm for 30% of total phosphorus, tentatively ascribed to a phosphonium salt from enolate formation) that did not appear to change on standing at −30 °C; the final *Z*/*E* alkene ratio was 10:90 (GLC of derived epoxides). However, in the same type of experiment with pivaldehyde (no acidic protons available) we found an initial cis/trans oxaphosphetane ratio of ca. 30:70 (δ −70.1 and −74.0; no 32-ppm singlet), which altered on warming to give eventually an ca. 1:99 cis/trans ratio (at −15 °C); the final *Z*/*E* alkene ratio was 4:96 (GLC of epoxides).

Our results indicate that carbon–carbon bond formation in the Wittig reaction of a "salt-free" trialkyl nonstabilized ylide with aromatic or aliphatic aldehydes can take place *originally* with negligible stereoselectivity (trans/cis ratio of 1.15:1 for benzaldehyde; ca. 2:1 for pivaldehyde), which is not reflected in the *E*-rich alkene product mixture. By contrast, corresponding reactions for a "salt-free" or a lithium-influenced triphenyl nonstabilized ylide occur *originally* with high cis stereoselectivity (≥4:1), although the Li-salt, benzaldehyde reaction displays some "stereochemical drift".[2] The three examples of "stereochemical drift" in the Wittig reaction, especially the dramatic results with **1b** and benzaldehyde or pivaldehyde, raise a cautionary note regarding the use of the alkene isomer ratios for determining initial stereochemistry of carbon-carbon bond formation, not to mention the application of such information to mechanistic ideas.[13]

Registry No. **1a**, 3728-50-5; **1b**, 43216-19-9; **2a**, 89121-74-4; **2b**, 94372-03-9; **3a**, 89121-77-7; **3b**, 94372-04-0; **4**, 7642-18-4; **5**, 16002-93-0; LiHMDS, 4039-32-1; butyl(triphenyl)phosphonium bromide, 1779-51-7; benzaldehyde, 100-52-7; tetrabutylphosphonium bromide, 3115-68-2; *cis*-*P*,*P*,*P*-tributyl-3-pentyl-4-propyloxaphosphetane, 94372-05-1; *trans*-*P*,*P*,*P*-tributyl-3-pentyl-4-propyloxaphosphetane, 94372-06-2; (*Z*)-4-decene, 19398-88-0; (*E*)-4-decene, 19398-89-1; *cis*-*P*,*P*,*P*-tributyl-3-*tert*-butyl-4-propyloxaphosphetane, 94404-12-3; *trans*-*P*,*P*,*P*-tributyl-3-*tert*-butyl-4-propyloxaphosphetane, 94404-13-4; (*Z*)-2,2-dimethyl-3-heptene, 94372-07-3; (*E*)-2,2-dimethyl-3-heptene, 19550-75-5; *erythro*-tributyl(1-(phenylhydroxymethyl)butyl)phosphonium bromide, 94372-08-4; *threo*-tributyl(1-(phenylhydroxymethyl)butyl)phosphonium bromide, 94372-09-5; butyl lithium, 109-72-8; tributylphosphine oxide, 814-29-9; hexanal, 66-25-1; pivaldehyde, 630-19-3.

Supplementary Material Available: Differential rate equations, their derivation, and some mechanistic discussion (2 pages). Ordering information is given on any current masthead page.

(10) Schmidbaur, H.; Tronich, W. *Chem. Ber.* **1968**, *101*, 595.

(11) A similar reaction conducted at −60 °C was quenched (cold) with HBr gas, and the β-hydroxytributylphosphonium salts were isolated as a tan syrup. Proton NMR (360 MHz) indicated a mixture of erythro and threo salts in a ratio of 47:53, via integration of the benzylic resonances: δ 5.23 (d of d, threo, J = 6.3, 14.6 Hz), 5.57 (d, erythro, J = 1.5, 8.1 Hz). The isomer assignment is based on NMR analogy with the corresponding β-hydroxytriphenylphosphonium salts (threo at δ 5.27, J = 19.6 and 6 Hz; erythro at δ 5.38, J = ca. 7 Hz), the threo diastereomer of which was confirmed by X-ray analysis.[2]

(12) (a) Detailed rate data for these two processes will be reported in a full paper. (b) A crossover experiment on this reaction was successful, suggesting that equilibration of **2b** and **3b** is related to reaction reversal.[4]

(13) For example, the steric arguments advanced in ref 1c are drawn into question by our current results.

Species Specificity of Long-Range Electron Transfer within the Complex between Zinc-Substituted Cytochrome *c* Peroxidase and Cytochrome *c*

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Recently,[1] we have used hybrid hemoglobins to show that long-range electron transfer[2,3] between centers held at fixed and known distances can be studied by substituting zinc protoporphyrin for heme in one of the protein partners that form an electron-transfer complex. This approach now has been applied to an archetypical physiological electron-transfer reaction, that between yeast cytochrome *c* peroxidase (CCP) and cytochrome *c* (cyt *c*),[4,5] by employing the complex between zinc-substituted CCP (ZnCCP)[6] and native cyt *c*. In this complex the spatial structure of each partner is well-known from single-crystal X-ray diffraction experiments,[7] and modeling studies by Poulos and Kraut[8] led them to propose a structure for the complex in which the two heme planes are nearly parallel, at an edge-to-edge distance of 17–18 Å.

As in the case of the hybrid hemoglobins, reaction within the [ZnCCP/cyt c^+] complex is initiated by flash photoexcitation, which forms the slowly decaying zinc protoporphyrin triplet state (^{3}ZnP) of ZnCCP.[9] The ^{3}ZnP ($E_0' \approx +0.64$)[10] can decay back

(1) McGourty, J. L.; Blough, N. V.; Hoffman, B. M. *J. Am. Chem. Soc.* **1983**, *105*, 4470–4472. (b) Peterson-Kennedy, S. E.; McGourty, J. L.; Hoffman, B. M. *J. Am. Chem. Soc.* **1984**, *106*, 5010–5012.

(2) (a) DeVault, D. *Q. Rev. Biophys.* **1980**, *13*, 387–564. (b) Chance, B., et al., Eds. "Tunnelling in Biological Systems"; Academic Press: New York, 1979. (c) See also: Sutin, N. *Acc. Chem. Res.* **1982**, *15*, 275–283.

(3) Other recent and related work in this area includes: (a) Winkler, J. R.; Nocera, D. G.; Yocom, K. M.; Bordignon, E.; Gray, H. B. *J. Am. Chem. Soc.* **1982**, *104*, 5798–5800. (b) Isied, S. S.; Worosila, G.; Atherton, S. J. *J. Am. Chem. Soc.* **1982**, *104*, 7659–7661. (c) Kostic, N. M.; Margalit, R.; Che, C.-M.; Gray, H. B. *J. Am. Chem. Soc.* **1983**, *105*, 7765–7767. (d) Miller, J. R.; Calcaterra, L. T.; Closs, G. L. *J. Am. Chem. Soc.* **1984**, *106*, 3047–3049. The photoexcitation approach also is being employed: (e) Simolo, K. P.; McLendon, G. L.; Mauk, M. R.; Mauk, A. G. *J. Am. Chem. Soc.* **1984**, *106*, 5012–5013. (f) Horie, T.; Maniara, G.; Vanderkooi, J. M. *FEBS Lett.* **1984**, *177*, 287–290.

(4) Abbreviations: cytochrome *c* peroxidase, CCP; cytochrome *c* and, where valence is significant, the ferrous form, cyt *c*; ferricytochrome *c*, cyt c^+; zinc protoporphyrin, ZnP; ferroheme, $Fe^{II}P$; ferriheme, $Fe^{III}P$.

(5) Yonetani, T. "The Enzymes"; Boyer, P. D., Ed.; Academic Press: New York, 1966; Vol. XIII, pp 345–361.

(6) ZnCCP was prepared by a procedure modified from that reported for the preparation and heme reconsitution of the apoperoxidase (Yonetani, T. *J. Biol. Chem.* **1967**, *242*, 5008–5013) and will be discussed in detail elsewhere.

(7) (a) Crystal structure of tuna cyt *c*: Swanson, R.; Trus, B. L.; Mandel, N.; Mandel, G.; Kallai, O. B.; Dickerson, R. E. *J. Biol. Chem.* **1977**, *252*, 759–775. Crystal structure of yeast CCP: (b) Poulos, T. L.; Freer, S. T.; Alden, R. A.; Edwards, S. L.; Skoglund, U.; Takio, K.; Eriksson, B.; Xuong, N. H.; Yonetani, T.; Kraut, J. *J. Biol. Chem.* **1980**, *255*, 575–580. (c) Finzel, B. C.; Poulos, T. L.; Kraut, J. *J. Biol. Chem.* **1984**, *259*, 13027–13036.

(8) (a) Poulos, T. L.; Kraut, J. *J. Biol. Chem.* **1980**, *255*, 10322–10330. (b) Poulos, T. L.; Finzel, B. C. *Protein Peptide Rev.*, in press.

(9) (a) The kinetics for ^{3}ZnP decay were monitored at 430 nm on a computer-interfaced flash photolysis apparatus.[9b] Samples were prepared in 0.01 M potassium phosphate pH 7.0 buffers, conditions that facilitate formation of a 1:1 complex between CCP and cyt *c*.[9c] (b) Stanford, M. A.; Hoffman, B. M. *J. Am. Chem. Soc.* **1981**, *103*, 4104–4114. (c) Kang, C. H.; Ferguson-Miller, S.; Margoliash, E. *J. Biol. Chem.* **1977**, *252*, 919–926.

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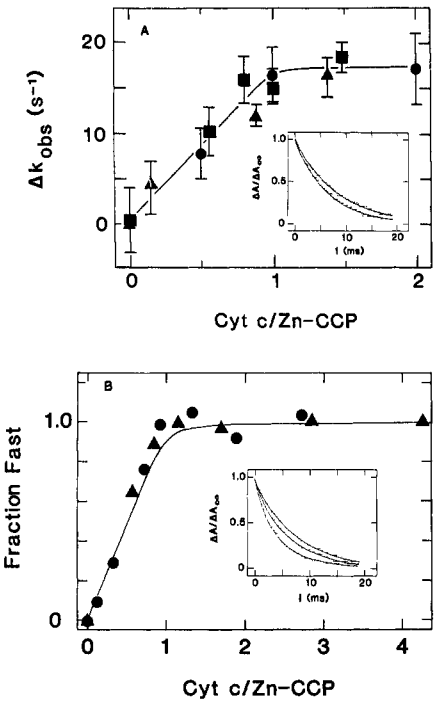

Figure 1. (A) Change (Δk_{obsd}) in the observed rate constant (k_{obsd}) for the first-order decay of ^{3}ZnP upon titration of ZnCCP with horse cyt c^+. Data are from three independent sets of measurements. The curve represents protein binding assuming a composite rate as described in text and a single cyt c^+ binding site having $K_A = 10^8$ M^{-1}. Inset: The ^{3}ZnP decay traces for the uncomplexed ZnCCP and fully complexed ZnCCP; solid lines are theoretical, employing $k_{obsd} = 126$ and 144 s^{-1}, respectively. For conditions, see ref 9a. (B) Proportion of the rapid component in a description of the ^{3}ZnCCP decay curve by a two exponential function (free ^{3}ZnCCP decay, $k_D = 126$ s^{-1}; complex, $k_p = 264$ s^{-1}; see text) during titration of ZnCCP with yeast cyt c^+. Data are from two independent sets of experiments. The curve represents protein binding assuming a single cyt c binding site having $K_A = 10^8$ M^{-1}. Inset: Experimental and theoretical progress curves for ^{3}ZnP decay as the fraction of [yeast cyt c^+/ZnCCP] complex assumes the values 0, 0.32, and 1.0. For conditions, see ref 9a.

to the ground state and can reduce the cytochrome c ferriheme ($E_0' \approx +0.26$V)[11] by long-range electron transfer:[4]

$$^3\text{ZnP} + \text{Fe}^{\text{III}}\text{P} \xrightarrow{k_t} (\text{ZnP})^+ + \text{Fe}^{\text{II}}\text{P} \qquad \Delta E_0' \approx 0.90 \text{ V} \qquad (1a)$$

As a consequence ^{3}ZnP within the [ZnCCP/cyt c^+] complex decays with a rate constant, k_P, which is the sum of the intrinsic triplet decay rate constant, k_D, and of that for electron transfer: $k_P = k_D + k_t$. The intermediates formed by (a) regenerate the ground state is a reaction directly analogous to the physiological process,[5] namely, electron transfer from $Fe^{II}P$ to the thermalized cation radical ZnP^+.[10] We also have now observed this reaction, which occurs at a rate $k_h \gg k_t$:

$$(\text{ZnP}^+) + \text{Fe}^{\text{II}}\text{P} \xrightarrow{k_h} \text{ZnP} + \text{Fe}^{\text{III}}\text{P} \qquad \Delta E_0' \approx 0.85 \text{ V} \qquad (1b)$$

The triplet decay curves of ^{3}ZnCCP and of a [^{3}ZnCCP/cyt c] complex, in which electron transfer has been blocked by prior reduction of the cytochrome c heme, are exponential, with the same decay rate, $k_D = 126 \pm 2$ s^{-1}, in each case. Upon titration of ZnCCP with horse cyt c^+, the ^{3}ZnCCP decay remains first order, but the rate increases from k_D, linearly with cytochrome c, until a 1:1 ratio is reached, and then remains constant at a plateau value characteristic of the complex itself: $k_P = 143 \pm 3$ s^{-1} (Figure 1A). Because k_D and k_P are similar in magnitude, for solutions with substoichiometric cyt c^+, the ^{3}ZnP progress curves exhibit a composite rate, $k_{obsd} = (1 - f)k_D + fk_P$, where f is the fraction of [ZnCCP/cyt c^+] complex; Figure 1A in fact presents $\Delta k_{obsd} = k_{obsd} - k_D = (k_P - k_D)f$. The shape of the titration indicates that ZnCCP and the cytochrome c form a strong 1:1 complex ($K_A \geq 10^8$ M^{-1}), consistent with previous studies.[5,8,9c] The enhanced triplet decay within the complex represents long-range electron transfer according to eq a, with the plateau rate being $k_P = k_D + k_t$. From the measured k_P and k_D, one obtains the electron-transfer rate within the [ZnCCP/horse cyt c^+] complex: $k_t = 17 \pm 3$ s^{-1} at 20 °C.

First-order analysis of the ^{3}ZnP decay curves upon addition of yeast[12a,13] cyt c^+ to ZnCCP gives a titration curve like that of Figure 1A. However, the plateau rate characteristic of the complex itself is much greater for the yeast cyt c^+: $k_P = 264 \pm 11$ s^{-1}. Careful examination of the substoichiometric progress curves idicates that they can be analyzed as the sum of a slow phase, free ZnCCP, and a rapid phase, the complex. Fixing the rate constants for slow and rapid components at $k_D = 126$ s^{-1} and $k_P = 264$ s^{-1}, the fraction of peroxidase exhibiting the fast phase grows linearly with the addition of cyt c^+, reaching unity at equimolar concentrations (Figure 1B). The sharp break at 1:1 stoichiometry again corresponds to complex formation with $K_A \geq 10^8$ M^{-1}. From k_P and k_D one obtains a much higher rate for electron transfer within the [ZnCCP/yeast cyt c^+] complex: $k_t = 138 \pm 12$ s^{-1} at 20 °C.

The kinetic difference spectra obtained from the complex between the two yeast proteins[12b] disclose a small absorbance that decays with rate k_P and matches that of the electron-transfer intermediate [$(ZnCCP)^+$/cyt c].[14] Preliminary steady-state kinetic analysis of this transient, performed at wavelengths corresponding to the (^{3}ZnCCP/ZnCCP) isosbestics, indicates that $k_t/k_h \sim 1/100$, and thus, $\sim 10^4$ s^{-1}. This represents the first direct measure of the rate for the thermal process, (b), an exact analogue of the physiological electron-transfer reaction. Note particularly the large difference in 20 °C rates for two processes, (a) and (b), which are comparably exoergic.

Although yeast and horse cytochromes c have identical reduction potentials,[10] form comparably strong 1:1 complexes with the yeast ZnCCP, and in all other ways are highly similar,[13] electron transfer from the ^{3}ZnP of the peroxidase to the ferriheme of the cytochrome occurs roughly 10-fold faster with the evolutionarily homologous yeast cytochrome. This demonstrates the fine degree of species specificity involved in physiological electron transfer and must reflect subtle structural differences between the two complexes. Studies in progress with a wide variety of natural and chemically modified cytochromes should permit a correlation of electron-transfer rate with protein structure.

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Registry No. Cyt c, 9007-43-6.

(10) (a) Voltages are relative to NHE. $E_0[(ZnP)^+/^3ZnP] \approx E_0[(ZnP)^+/ZnP] - E_T = +1.20$ V $- 1.84$ V[7b] $= -0.64$ V. $E_0[(ZnP)^+/ZnP]$ was measured by titration with potassium hexachloroiridate (to be published). (b) Stanford, M. A.; Hoffman, B. M. *J. Am. Chem. Soc.* **1981**, *72*, 4104–4114.

(11) Potentials (vs. NHE) are for uncomplexed cytochromes: (a) E_0 for horse heart cytochrome $c = +0.25$ V (Henderson, R. W.; Rawlinson, W. A. *Biochem. J.* **1956**, *62*, 21–26). (b) E_0 for yeast cytochrome $c = +0.26$ V (Henderson, R. W.; Rawlinson, W. A. "Haematin Enzymes"; Falk, Lemberg, Morton, Eds.; Academic Press: New York, 1961; p 370).

(12) (a) Yeast iso-2 cyt c[7] was used in these studies because a surface sulfhydryl group on the right side of the protein renders the yeast iso-1 protein susceptible to disulfide dimerization. However, preliminary measurements indicate that k_t is essentially the same for iso-2 and for iso-1 that has been stabilized by carboxymethylation of cysteine 103. (b) These measurements employed yeast iso-1 cyt c.

(13) (a) Margoliash, E.; Schejter, A. *Adv. Protein Chem.* **1966**, *21*, 113. (b) Brautigan, D. L.; Feinberg, B. A.; Hoffman, B. M.; Margoliash, E.; Peisach, J.; Blumberg, W. E. *J. Biol. Chem.* **1977**, *252*, 574–582. (c) Margoliash, E. "Hemes and Hemoproteins"; Chance, Estabrook, Yonetani, Eds.; Academic Press: New York, 1966; pp 371–390.

(14) The low value of k_t/k_h means that the intermediate is in low concentration, but the high photostability of this system has permitted us to identify small contributions to the kinetic difference spectrum.