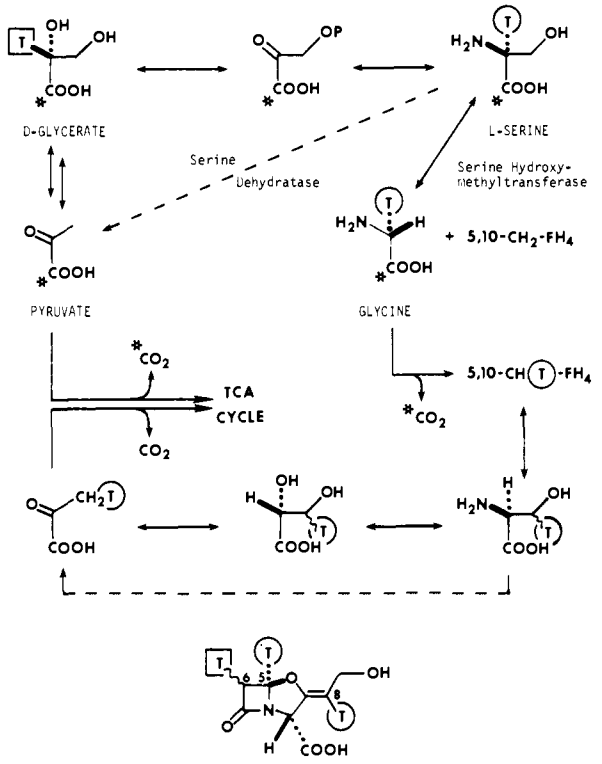
Scheme I



substrates gave comparable levels of ^{14}C incorporation, but only L-serine and D-glycerate gave positive incorporations of tritium. ^{14}C incorporations were somewhat more responsive to increasing amounts of D-glycerate supplied than L-serine (experiments 8 and 9 vs. 11-13), but relative tritium retentions were remarkably similar, albeit low (ca. 10%, cf. experiments 8 and 11), presumably owing to racemization and redox rates competitive with incorporation into clavulanate. The important point, however, is that the direct precursor of the β-lactam carbons would be anticipated to specifically label C-6 of clavulanate with tritium from its α-position, a condition that cannot be met by both D-glycerate and L-serine (see Scheme I). In experimental design, therefore, the low absolute tritium incorporation could be counterbalanced by locating the ^{14}C internal standard at C-1 to be lost when C_3 intermediate (intact incorporation) was metabolized to acetyl-CoA and entered the TCA cycle (secondary incorporation into the C-5 unit).

Careful degradations[14] of **1** (R = PBB), derived from administration of variously labeled substrates, to **2** and **3** allowed determination of the ^{14}C distribution in segments I (C-5-7), II (C-2,3,10), and III (C-8,9) as well as tritium activity at H-5, H-6,[9] and H-8[15] (Table II). A dichotomy emerges in these data that α-tritium from L-serine resides principally at C-8 and C-5 with significantly lesser amounts at C-6 (experiment 16), whereas analogously labeled D-glycerate suffers the opposite fate labeling C-6 most highly with substantially smaller amounts at C-5 and C-8 (experiments 18 and 19). These data imply, beyond the largely intact incorporation of D-glycerate, that L-serine α-tritium label finds its way to the β-carbon of the true C_3 intermediate. Illustrated in Scheme I, this behavior can be readily accounted for by the agency of serine hydroxymethylase[3,16] to afford L-[3-^3H]serine and hence D-[3-^3H]glycerate and, like [1,3-^3H,1,3-^{14}C]glycerol (experiment 21), result in the appearance of tritium activity at C-5 and C-8. To establish firmly this rationale, [2-^3H,1-^{14}C]glycine was examined (experiment 20). Unlike the serine experiment above.[17] high levels of ^3H incorporation were observed and more precise estimation of tritium distribution was possible. As expected, no label was found at C-6 with carbons 5 and 8 bearing all the activity in a ratio of 1:3.

Therefore, of the possible C_3-glycolytic intermediates, it may be concluded that D-glycerate is utilized most directly to become the β-lactam carbons of **1** in union with a C_5 amino acid, ornithine, to generate ultimately clavulanic acid.

Acknowledgment. Financial support of the National Institutes of Health (AI 14937, RR 07041), the A. P. Sloan Foundation, and Merck Laboratories is gratefully acknowledged.

Registry No. 1 (R = H), 58001-44-8; **1** (R = PBB), 60297-60-1; **2**, 14376-86-4; **3**, 63837-22-9; D-glyceric acid, 6000-40-4.

(14) Spillover of ^{14}C disintegrations introduced comparatively larger errors in computing ^3H activities which have been forthrightly recorded in Table II.

(15) Derivation of the C-9 methylene from the carboxylate (C-5) of α-ketoglutarate prohibits tritium activity in the TCA cycle from appearing at C-9 (except by hydride delivery from, e.g., reduced pyridine nucleotide).

(16) Wasserman, H. H.; Sykes, R. J.; Peverada, P.; Shaw, C. K.; Cushley, R. J.; Lipsky, S. R. *J. Am. Chem. Soc.* **1973**, *95*, 6874-6875 and references cited therein.

(17) Considerable loss of serine 2-^3H label occurs in rapid, reversible reaction to 3-phosphohydroxypyruvate.

Detailed Rate Studies on the Wittig Reaction of Nonstabilized Phosphorus Ylides via ^{31}P, ^1H, and ^{13}C NMR Spectroscopy. Insight into Kinetic vs. Thermodynamic Control of Stereochemistry

Bruce E. Maryanoff,* Allen B. Reitz, Martin S. Mutter, Ruth R. Inners, and Harold R. Almond, Jr.

Chemical Research Department, McNeil Pharmaceutical Spring House, Pennsylvania 19477

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Stereochemistry is an interesting, if not important, aspect of the Wittig olefination reaction, but it has generally been viewed only from the isomeric alkene reaction products.[1] However, our observation of both cis and trans oxaphosphetanes, by high-field ^{31}P NMR spectroscopy below -20 °C, has opened a new avenue to stereochemical studies.[2] Indeed, our initial work revealed that alkene Z/E ratios may not correspond with oxaphosphetane cis/trans ratios.[2] For example, condensation of benzaldehyde with ylide **1a** [from the phosphonium bromide and LiHMDS (lithium hexamethyldisilazide)] in tetrahydrofuran (THF) below -30 °C gave a 78:22 mixture of cis/trans oxaphosphetanes **2a**/**3a** but a 60:40 mixture of *Z*-/*E*-*n*-propylstyrenes (Scheme I). Since such "stereochemical drift" has profound significance relative to the validity of using alkene isomer ratios for mechanistic interpretation concerning carbon-carbon bond formation, we pursued the matter further. We report herein the first detailed rate measurements and kinetic analysis on intermediates (oxaphosphetanes) and products (alkenes) in the Wittig reaction. Our results disclose some intimate features of the Wittig reaction heretofore not fully appreciated. In particular, rate studies on the (lithium salt) reaction of **1a** with benzaldehyde pinpoint bias in oxaphosphetane equilibration as the key source of "stereochemical drift". Also, we found that reactions of salt-free ylide **1b** with benzaldehyde or pivaldehyde show dramatic "stereochemical drift", which partly accounts for the unusually high *E* stereoselectivity observed with such trialkylphosphorus ylides compared to the corresponding triphenylphosphorus ylides.[1c,3]

(1) For a variety of examples, see: (a) Schlosser, M. *Top. Stereochem.* **1970**, *5*, 1. (b) Gosney, I.; Rowley, A. G. In "Organophosphorus Reagents in Organic Synthesis"; Cadogan, J. I. G., Ed.; Academic Press: New York, 1979; pp 17-153. (c) Schlosser, M.; Schaub, B. *J. Am. Chem. Soc.* **1982**, *104*, 5821. (d) Vedejs, E.; Meier, G. P.; Snoble, K. A. J. *Ibid.* **1981**, *103*, 2823. (e) McEwen, W. E.; Cooney, J. V. *J. Org. Chem.* **1983**, *48*, 983. (f) Bestmann, H. J. *Pure Appl. Chem.* **1980**, *52*, 771.

(2) Reitz, A. B.; Mutter, M. S.; Maryanoff, B. E. *J. Am. Chem. Soc.* **1984**, *106*, 1873.

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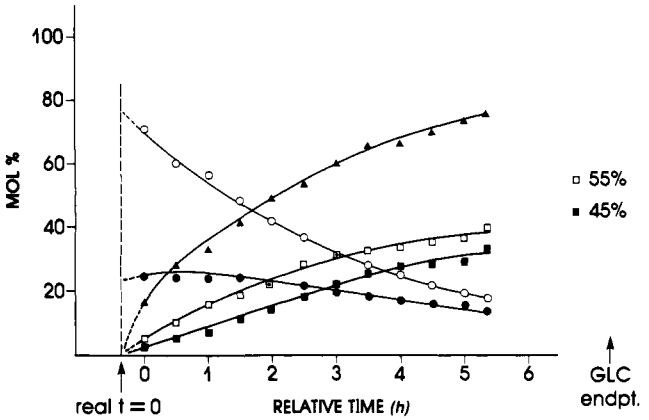
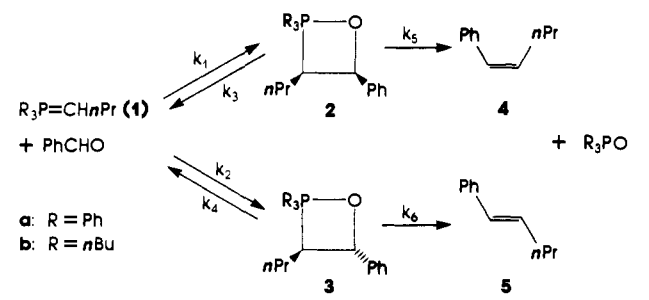


Figure 1. Kinetic plot for the reaction of **1a** and benzaldehyde (1:1 molar ratio), 0.36 M in THF-d_8 at −30 °C, derived from ^{31}P-^{1}H NMR data. (Broad-band proton decoupling in the ^{31}P data collection was not used to avoid differential heating.) The dotted lines indicate extrapolation. Only every third data point is displayed. Proton NMR and ^{31}P NMR data were quantitated absolutely by using external references in glass capillaries: trimethyl orthobenzoate and trimethyl phosphite, respectively. Legend: (▲) triphenylphosphine oxide, (○) **2a**, (●) **3a** [from ^{31}P NMR]; (□) **4**, (■) **5** [from ^{1}H NMR].

Scheme I



Oxaphosphetanes **2a** and **3a**, from **1a** and benzaldehyde under lithium salt (LiBr) conditions in THF, decompose to alkenes at a rate similar to the rate of their interconversion,[1d] presumably by reversal to reactants followed by recombination.[4] To evaluate these overlapping processes, we monitored the disappearance of oxaphosphetanes and the appearance of alkenes with time at −30 °C by 145.8-MHz ^{31}P and 360-MHz ^{1}H NMR in a broad-band probe (Bruker AM-360). Quantitation was achieved by integration of the phosphorus resonances (singlets) for **2a** at −61.4 ppm, **3a** at −63.8 ppm, and triphenylphosphine oxide–LiBr at 28 ppm,[2] and of the vinylic proton signals (multiplets) for **4** at δ 5.65 and for **4** and **5** at δ 6.2–6.5.[5] Data for the first two half-lives are presented in Figure 1 (only every third data point is displayed). Differential equations based on the reaction profile depicted in Scheme I, containing the six rate constants, were set up.[6] Since no ylide was detected by NMR, its concentration was indeterminate. With this in mind, we applied a steady-state approximation to the concentration of ylide (d[Y]/dt = 0), which simplified the rate expressions. The equations were solved by iterative

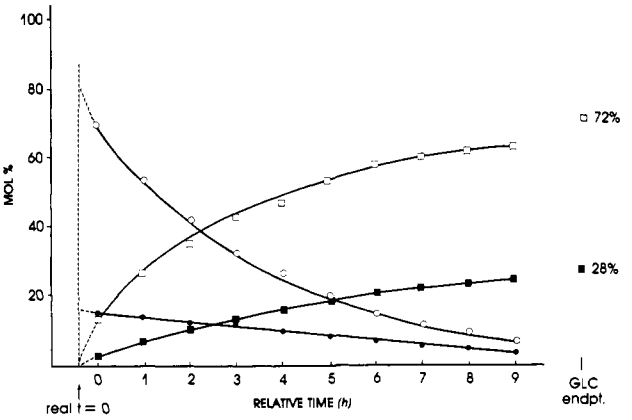


Figure 2. Kinetic plot for the reaction of **1a** (labeled at the ylidic carbon with ^{13}C) and benzaldehyde (1:1 molar ratio), 0.25 M in THF-d_8 at −30 °C, derived from ^{13}C NMR data. Dotted lines indicate extrapolation. Only every third data point is displayed. Legend: (○) **2a**, (●) **3a**, (□) **4**, (■) **5**.

methods.[7] Optimization of curve fitting (by offsetting the time scale) gave an effective zero point ("real" t = 0) of the reaction, at which the ratio of **2a** and **3a** is 78:22, contrasting with a 55:45 Z/E alkene ratio from GLC analysis after the reaction was complete. This generated the following rate constants (10^{-5} s^{-1}): k_3 = 13.9, k_4 = 0.93, k_5 = 4.8, and k_6 = 7.9.[8] Thus, E alkene **5** is generated at nearly the same rate as Z alkene **4** (ca. 1.6 times faster), and cis oxaphosphetane converts to trans oxaphosphetane at a greater rate than the reverse reaction (ca. 15 times faster). The larger k_3 is clearly the origin of "stereochemical drift" toward E alkene.

To verify these results, we conducted the same type of study with **1a** enriched to 92 atom % in ^{13}C at the ylide carbon, monitoring the reaction by 90.5-MHz $^{13}C\{^{1}H\}$ NMR. Quantitation was achieved by integration of the labeled-carbon doublets for **2a** (at 71.5 ppm, J = 85.0 Hz) and **3a** (at 75.0 ppm, J = 83.7 Hz) and the labeled-carbon singlets for **4** (at 132.5 ppm) and **5** (at 130.0 ppm).[5] Data for the first three half-lives are presented in Figure 2 (only every third point is shown). Analogous optimization of curve fitting[6] gave an initial 82:18 ratio of **2a**/**3a**, which compares with a final alkene Z/E ratio of 72:28 (GLC). The iterative solution gave the following rate constants (10^{-5} s^{-1}): k_3 = 9.2, k_4 = 1.2, k_5 = 5.7, and k_6 = 6.8.[8] Again, E alkene is produced at nearly the same rate as Z alkene, and conversion of cis to trans oxaphosphetane is faster than the reverse (by ca. 8 times). The diminished "stereochemical drift" in the labeled (82:18 → 72:28) vs. unlabeled (78:22 → 55:45) experiments appears to be associated with the difference in k_3/k_4 ratios, perhaps reflecting an isotope effect.[8] In any event, the salient point is the diversion of **2a** to **3a** during the reaction course because of the *relatively* large value for k_3.

The "(lithium) salt-free" reaction of **1a**, from phosphonium bromide and NaHMDS, with benzaldehyde gave predominantly **2a** (ca. 95%) and did not exhibit "stereochemical drift".[2,9] However, this was not the case for the reaction of trialkylphosphorus ylide **1b** with benzaldehyde. Salt-free ylide **1b**, generated from the corresponding phosphonium bromide with *n*-butyllithium and distilled in vacuo as a pale yellow liquid (δ ^{31}P 8.7

(3) (a) Meyers, A. I.; Lawson, J. P.; Carver, D. R. *J. Org. Chem.* **1981**, *46*, 3119. (b) Bissing, D. E. *Ibid.* **1965**, *30*, 1296.

(4) This is the same reaction reversal responsible for crossover products. For crossover chemistry, see: (a) Reference 1d. (b) Schlosser, M.; Christmann, K. F. *Justus Leibigs Ann. Chem.* **1967**, *708*, 1. (c) Vedejs, E.; Fang, H. W. *J. Org. Chem.* **1984**, *49*, 212.

(5) Relaxation times were checked for representative ^{31}P and ^{13}C NMR spectra to determine proper spectroscopic conditions for quantitating various experiments. For the rate studies, we used a temperature that would allow spectral acquisition for each data point, on a 0.2–0.4 M solution, in a time period less than 5% (3–4%) of the reaction half-life. The low-temperature phosphorus chemical shifts are referenced against the signal for 85% phosphoric acid at −30 °C.

(6) The equations, along with their derivation and some related discussion, are presented in the microfilm supplement (see paragraph at the end of this paper regarding supplementary material).

(7) Computations were performed with a statistical program for estimating the parameters of nonlinear equations: Metzler, C. M.; Elfring, G. L.; McEwen, A. J. *Biometrics* **1974**, *30*, 562. We employed the 1976 revision, available from The Upjohn, Co., Kalamazoo, MI.

(8) Because of error introduced from (1) NMR integration (accurate to ±3%), (2) variation in the temperature of the NMR probe (±1 °C), and (3) a strong concentration effect (to be discussed later in a full paper), the values for the rate constants are not rigorously exact. This makes a comparison of rate data from separate experiments inadvisable; the rate data are best viewed in a relative sense within a particular experiment. Nevertheless, analogous results were obtained in duplicate studies.

(9) Although we detected no "stereochemical drift", this reaction has been reported to yield crossover products (see ref 1d and: Piskala, A.; Rehan, A. H.; Schlosser, M. *Collect. Czech. Chem. Commun.* **1983**, *48*, 3539).

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*

Pui Shing Ho, Carol Sutoris, Nong Liang, E. Margoliash, and Brian M. Hoffman*

Department of Chemistry and Department of Biochemistry, Molecular Biology and Cell Biology Northwestern University, Evanston, Illinois 60201

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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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