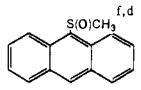
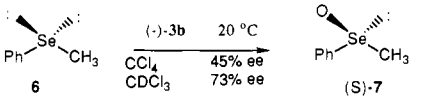
Table I. Asymmetric Oxidation of Prochiral Sulfides to Sulfoxides Using Camphorsulfonyloxaziridine **3** at 20 °C

3a-b + R-S-R′ → R-S(O)-R′ + (−)-**4** or (−)-**5**

entry	sulfoxide	solvent	% ee (configuration) [time (h)] % yield[a] **3a** (X = H)	**3b** (X = Cl)	$[\alpha]^{20}_D$ (in acetone)	modified Sharpless[b]
1	*p*-Tol-S(O)-CH_3[c,d]	CH_2Cl_2	28 (*S*) [1] 80	62 (*S*) [1] 60		96 (*R*)[e]
2		CCl_4	26 (*S*) [40] 22	95 (*S*) [4] 95	−139.0 (*c* 1.6)	
3	*p*-Tol-S(O)-*n*-Bu[f,d]	CH_2Cl_2	11 (*S*) [1] 70	61 (*S*) [1] 90		20 (*R*)[b]
4		CCl_4	8 (*S*) [18] 90	84 (*S*) [3] 90	−162.3 (*c* 3.2)	
5	*p*-Tol-S(O)-*i*-Pr[f,d]	CH_2Cl_2	11 (*S*) [1] 70	54 (*S*) [1] 95		63 (*R*)[b]
6		CCl_4	8 (*S*) [18] 90	66 (*S*) [6] 95	−119.0 (*c* 3.0)	
7		CCl_4	23 (*S*) [40] 23	92 (*S*) [18] 90	−131.6 (*c* 1.1)	95 (*R*)[g]
8		CH_2Cl_2	64 (*S*) [1] 70	95 (*S*) [1] 90	−138.8 (*c* 1.2)	86 (*R*)[g]
9		CCl_4	73 (*S*) [1] 80	95 (*S*) [48] 60		
10	Ph-S(O)-CH=CH_2[d]	CCl_4	21 (*S*) [40] 25	85 (*S*) [48] 60	−262.4 (*c* 1.7)	70 (*R*)[g]
11	Ph-S(O)$CH_2CO_2CH_3$[c,d]	CCl_4	23 (*S*) [40] 18	94 (*S*) [48] 65	−170.0 (*c* 1.5)[h]	64 (*R*)[g]
12	Ph-S(O)$CH_2C(O)CH_3$[c,d]	CCl_4	no reaction	84 (*S*) [48] 52	−183.2 (*c* 1.1)	60 (*R*)[g]
13	Ph-S(O)-CH_2CN[d]	CCl_4	no reaction	>95 (*S*) [48] 45	−170.1 (*c* 1.0)	34 (*R*)[g]
14	$(CH_3)_3C$-S(O)CH_3[c,d]	CCl_4	66 (*S*) [12] 85	94 (*S*) [18] 84	+7.1 (*c* 1.0)[i]	53 (*R*)[b]

[a] Isolated yields. [b] Oxidations at −20 °C for 4-22 h. See ref 6. [c] Ee's determined using Eu(hfc)$_3$. [d] Determined by comparison of the rotation to literature values. [e] Reference 6e. [f] The sulfoxide enantiomers were separated on a Regis Pirkle covalent phenylglycine HPLC column eluting with 95:5 hexane/isopropyl alcohol. The *S*-sulfoxides were the first to be eluted. See ref 4. [g] Reference 6b. [h] In ethanol. [i] In $CHCl_3$.

sulfonyloxaziridines are ideal reagents for the synthesis and study of optically active selenoxides because oxidations can be carried out under anhydrous conditions in the absence of acid.[18] Indeed oxidation of **6** by (−)-**3b** in $CDCl_3$ at 20 °C affords (−)-(*S*)-**7** in 73% ee (95% yield).[20] Oxidation at −60 °C improves the ee to 83%. Interestingly oxidation of **6** by **3b** is faster than methyl *p*-tolyl sulfide (<5 min vs 4 h), and higher ee's were observed in $CDCl_3$ compared to CCl_4.



The stereoselectivities for asymmetric oxidations using enantiomerically pure *N*-sulfonyloxaziridines can be predicted by using steric arguments.[7] However, the uniformly high ee's for a variety of sulfide substrates suggest that factors other than steric are important. The fact that solvent influences the stereoselectivities for asymmetric oxidations with **3b** having polar Cl groups but not for **3a** strongly suggests that electronic or polar elements influence the stereoselectivity. Consistent with this observation are the faster rates of oxidation for **3b** compared to **3a**, despite the fact that the active site in the former is more hindered.

Enantiomerically pure α,α-dichlorocamphorsulfonyloxaziridine (**3b**) is the most effective and generally asymmetric oxidizing reagent developed to date for the asymmetric oxidation of sulfides (selenides) to sulfoxides (selenoxides). Since the configuration of the oxaziridine three-membered ring controls the stereochemistry, both sulfoxide enantiomers are readily available simply by choice of the appropriate oxaziridine. Asymmetric oxidations using (−)-**3b** is now in many cases a viable alternative to the Andersen procedure for the synthesis of enantiomerically pure sulfoxides in both enantiomeric forms.

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Supplementary Material Available: Spectroscopic data and physical constants (yield, mp, IR, and ^{1}H NMR) for **3a**, **3b**, **4**, and **5** (1 page). Ordering information is given on any current masthead page.

(16) Davis, F. A.; Billmers, J. M.; Stringer, O. D. *Tetrahedron Lett.* **1983**, 3191.

(17) Davis, F. A.; Stringer, O. D.; McCauley, J. P., Jr. *Tetrahedron* **1985**, *41*, 4747.

(18) The asymmetric oxidation **6** by **1a** gave **7** in 9% ee.[17] Low to moderate optical properties (18-49% ee) for the asymmetric oxidation of functionalized selenides to selenoxides by a modified Sharpless reagent has been reported.[19]

(19) Teicco, M.; Tingoli, M.; Testaferri, L.; Bartoli, D. *Tetrahedron Lett.* **1987**, 3849.

(20) The isolation and manipulation of optically active methyl phenyl selenoxide (**7**) is described in ref 17. The specific rotation for (−)(*S*)-**7**, 27% ee, is $[\alpha]_D$ = −8.0° (*c* 0.7, $CDCl_3$) isolated in 50% yield.

The Mode of Triple Phosphoryl Group Transfer in Pyruvate Phosphate Dikinase Catalysis. Demonstration of the Intermediacy of Pyrophosphorylated and Phosphorylated Enzyme Species

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Pyruvate phosphate dikinase (PPDK)[1] catalyzes the reversible phosphorylation of pyruvate and orthophosphate utilizing the β- and γ-phosphates of a single molecule of ATP.[2] In C_4 plants where PEP serves as the primary acceptor in CO_2 fixation, PPDK

(1) Abbreviations used include the following: PPDK, pyruvate phosphate dikinase; ATP, adenosine 5′-triphosphate; ADP, adenosine 5′-diphosphate; AMP, adenosine 5′-monophosphate; PEP, phosphoenol pyruvate; P_i, orthophosphate; PP_i, pyrophosphate, K^+Hepes, potassium salt of *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; EPP, pyrophosphoryl PPDK; EP, phosphoryl PPDK.

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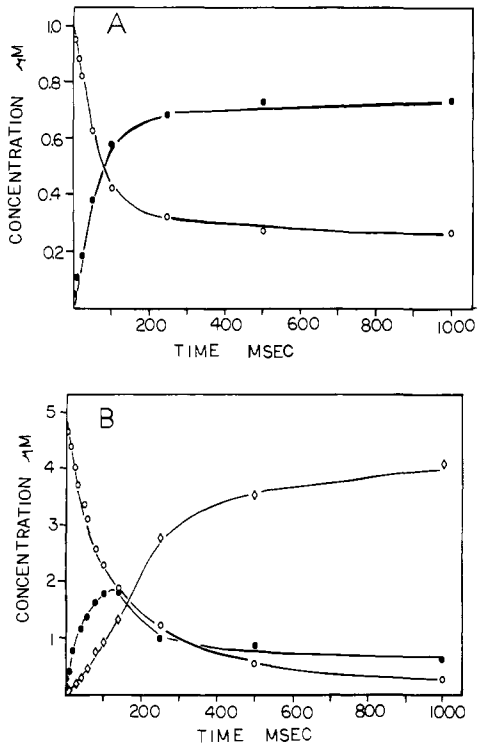
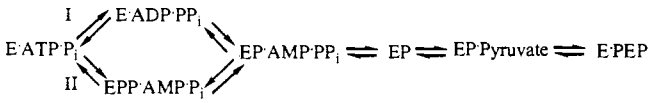
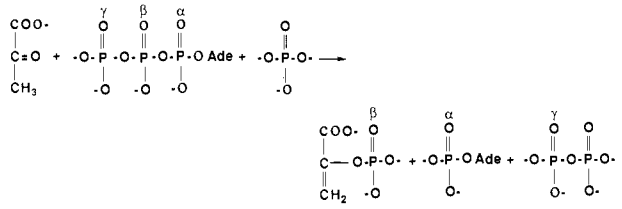


Figure 1. Time course for a single turnover of PEP in the active site of Mg^{2+}/NH_4^+-activated PPDK at 25 °C. (A) The reaction mixture contained 1 μM [^{32}P]PEP, 10 μM PPDK active sites (S.A. ~ 15 μmol/min mg), 5 mM $MgCl_2$, 20 mM NH_4Cl, and 50 mM K^+Hepes (pH 7.0). (B) The reaction mixed contained 5 μM [^{32}P]PEP, 16 μM of PPDK active sites (S. A. ~ 15 μmol/min mg), 5 mM $MgCl_2$, 20 mM NH_4Cl, 20 μM AMP, and 100 μM PP_i: (●) EP, (○) PEP, and (◇) ATP.

Scheme I



potentiates photosynthesis under the control of a light/dark-mediated regulatory mechanism.[3]



During PPDK catalysis the β-P of the ATP is transfered to pyruvate to form PEP, while the γ-P is transfered to P_i. The stereochemistry of the β-P transfer by the bacterial enzyme was shown to be retention, signifying two direct displacements at the β-phosphorus, while the γ-P transfer was shown to occur by a single displacement leading to inversion of configuration.[4] Thus, the PPDK-catalyzed interconversion of ATP and PEP consists of three phosphoryl transfer steps. The kinetic mechanism, bi-(ATP,P_i)bi(AMP,PP_i)uni(pyruvate)uni(PEP), suggests that the first two of the phosphoryl transfer steps are coupled and that they lead to a phosphoryl enzyme which in a third, independent step transfers its phosphoryl group to pyruvate.[5] The phosphoryl

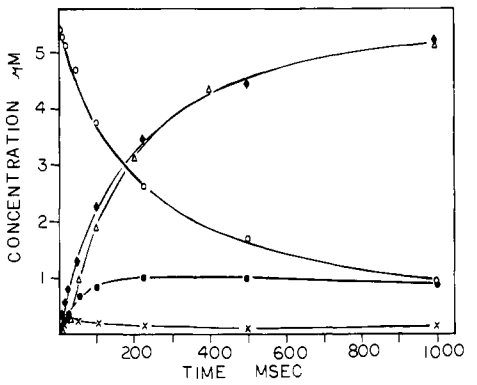


Figure 2. Time course for a single turnover of ATP, P_i, and pyruvate in the active site of Mg^{2+}/NH_4^+-activated PPDK at 25 °C. The reaction mixture contained 6 μM [γ-^{32}P]ATP or [β-^{32}P]ATP, 16 μM PPDK active sites (S.A. ~ 15 μmol/min mg), 2.5 mM $MgCl_2$, 10 mM NH_4Cl, 2 mM P_i, and 1 mM pyruvate, and 50 mM K^+Hepes (pH 7.0): (×) EPP, (●) EP, (○) ATP, (♦) PP_i, and (△) PEP.

enzyme has been isolated from an equilibrium mixture formed from PPDK plus PEP and characterized as the N^3-phosphohistidine adduct.[6]

Upon the basis of these observations we have proposed[5] that the PPDK-catalyzed reaction follows one of the two mechanisms shown in Scheme I. According to mechanism I ATP phosphorylates P_i and generates ADP as an intermediate. The ADP then phosphorylates the enzyme which in turn phosphorylates pyruvate. Mechanism II, on the other hand, involves nucleophilic displacement at the β-P of ATP which has been observed for only a few ATP utilizing enzymes[7] and which leads to an unprecedented pyrophosphoryl enzyme intermediate.[8] The pyrophosphoryl enzyme intermediate then undergoes displacement of its terminal phosphoryl group by P_i to form EP and free PP_i. In this communication we report the results of rapid quench kinetic experiments that allow us to unambiguously identify mechanism II as that followed during PPDK catalysis.

The time course for a single turnover in the PPDK active site was measured by reacting radiolabeled substrate ([^{32}P]PEP, [^{14}C]ATP, [γ-^{32}P]ATP, or [β-^{32}P]ATP)[9] (40 μL) with excess

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(6) Spronk, A. M.; Yoshida, H.; Wood, H. G. *P.N.A.S.* **1976**, *73*, 4415.

(7) Walsh, C. *Enzymatic Reaction Mechanisms*; W. H. Freeman and Company: 1979; p 259.

(8) The formation of a pyrophosphoryl enzyme intermediate was first proposed by Cooper and Kornberg (Cooper, R. A.; Kornberg, H. L. *Biochim. Biophys. Acta* **1967**, *141*, 211 and by Berman et al. (Berman, K. N.; Itada, N.; Cohn, M. *Biochim. Biophys. Acta* **1967**, *141*, 214) for the related phosphoenol pyruvate synthase reaction. Later, Evans and Wood (Evans, H. J.; Wood, H. G. *Fed. Proc. Fed. Amer. Soc. Biol. Chem.* **1968**, *27*, 588) proposed pyrophosphoryl PPDK as an intermediate in the PPDK-catalyzed reaction of ATP, Pi, and pyruvate.

(9) [^{14}C]ATP and [γ-^{32}P]ATP were purchased from Amersham. Before use each nucleotide was purified on a 1-mL DEAE Sephadex column by using a linear gradient of NH_4HCO_3 (0.1 → 0.6 M) as eluant. [β-^{32}P]ATP was prepared by combining 1 mCi of [γ-^{32}P]ATP with 1 μmol AMP, 50 μmol ATP, 1 unit of adenylate kinase, and 1 μmol $MgCl_2$ in 140 μL of 50 mM K^+ Hepes (pH 8). The solution was incubated at 35 °C for 45 min and then applied to a 1.5 × 20 cm DEAE cellulose column. The column was eluted with 1 L of triethylamine bicarbonate (0.05 M → 0.4 M, pH 7.6) at 4 °C. The fractions containing [β-^{32}P]ADP were combined and concentrated with a rotary evaporator. Excess triethylamine bicarbonate was removed by repeated evaporations of small aliquots of water. The resulting [β-^{32}P]ADP was dissolved in 1 mL of 100 mM Tris buffer (pH 7.5) containing 10 mM $MgCl_2$. To this solution were added 10 μL of 100 mM acetylphosphate and 0.5 units of acetate kinase. The reaction solution was incubated at 25 °C for 1 h and then applied to a 2-mL column of DEAE Sephadex equilibrated with 0.1 M NH_4HCO_3. The column was eluted with a step gradient[11] of 20 mL of 0.1 M NH_4HCO_3, 10 mL of 0.23 M NH_4HCO_3, and 6 mL of 1 M NH_4HCO_3. The ATP-containing fractions (from the 1 M NH_4HCO_3 wash) were combined and lyophilized. [^{32}P]PEP was prepared by reacting the [β-^{32}P]ATP (0.08 mM) with 2 mM pyruvate and 2 mM P_i in the presence of 15 mM $MgCl_2$, 15 mM NH_4Cl, 0.5 units/mL of PPDK, and 50 mM K^+Hepes buffer (pH 7.0) for 60 min (27 °C). The reaction solution was chromatographed on a 1.5 × 20 cm DEAE cellulose column with a 1 L linear gradient of triethylamine bicarbonate (0.05 → 0.4 M, pH 7.6).

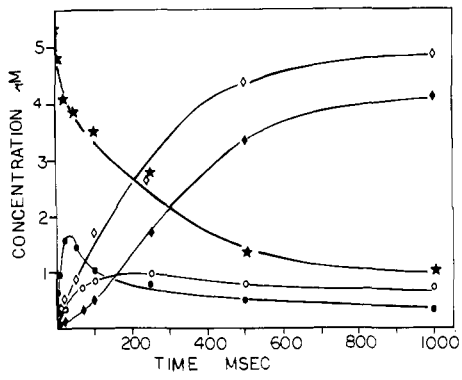

Figure 3. Time course for a single turnover of ATP, P_i, and pyruvate in the active site of Co^{2+}/NH_4^+-activated PPDK at 25 °C. The reaction mixture contained 6 μM [γ-^{32}P]ATP or [β-^{32}P]ATP, 20 μM PPDK active sites (S.A. ~ 15 μmol/min mg), 2.5 mM $CoCl_2$, 10 mM NH_4Cl, 2 mM P_i, 1 mM pyruvate, and 50 mM K^+Hepes (pH 7.0): (●) EPP, (○) EP, (★) ATP, (◇) PP_i, and (◆) PEP.

enzyme (40 μL) in a rapid quench apparatus.[10] The time courses for the reaction of Mg^{2+}/NH_4^+ activated PPDK with limiting [^{32}P]PEP in the absence and presence of the cosubstrates, AMP and PP_i, are depicted in Figure 1. Consistent with the uni-(PEP)uni(pyruvate) portion of the PPDK kinetic mechanism[5] we see that the phosphorylation of the enzyme by PEP occurs independent of PP_i and AMP. In a separate experiment (not shown) where the PEP was reacted with Mg^{2+}/NH_4^+-activated PPDK in a ratio of 1:20, the internal equilibrium constant (K_{eq}^{cat} = [EP·pyruvate]/[E·PEP]) was determined as 1.5.

The time course for the forward direction of the PPDK reaction was measured by using [γ-^{32}P]ATP to monitor EPP and PP_i formation and [β-^{32}P]ATP to monitor EPP + EP, PEP, and ADP formation. [^{14}C]ATP was used to demonstrate that radiolabeled nucleotide did not coprecipitate with the enzyme during the CCl_4 step of the reaction workup. The results obtained with the Mg^{2+}/NH_4^+-activated enzyme are shown in Figure 2. Both EPP and EP were formed as intermediates in the catalyzed reaction of ATP, P_i, and pyruvate, while ADP was not.

Because the level of EPP observed in the single turnover experiment was modest, conditions which would lead to enhanced accumulation of this intermediate were sought. In a separate study of PPDK catalysis the rate of isotope exchange from the P_β–O–P_α to the P_α=O position and from the P_β=O to P_γ–O–P_β position in [β-$^{18}O_2$,β,α-^{18}O]ATP was examined as a possible indicator of the relative rates of P_β–O–P_α and P_γ–O–P_β bond cleavage.[12] The $\beta \rightarrow \beta,\gamma$ and $\beta,\alpha \rightarrow \alpha$ positional isotope exchange (PIX) rates were equivalent for the Mg^{2+}/NH_4^+-activated enzyme, but, with the Co^{2+}/NH_4^+ activated enzyme, the $\beta,\alpha \rightarrow \alpha$ PIX occurred 2-fold faster than the $\beta \rightarrow \beta,\gamma$ PIX, and, with the Mn^{2+}/NH_4^+ enzyme, it was 30-fold faster. These findings suggested that with the Co^{2+}/NH_4^+- or Mn^{2+}/NH_4^+-activated enzyme the rate of EPP formation[13] may exceed the rate of its conversion to EP. Indeed, we found that the level of EPP which accumulates during a single turnover of ATP (6 μM) in the active site (18 μM) of the Mn^{2+}/NH_4^+-activated enzyme (EPP = 3.8 μM) is greater than that observed with the Co^{2+}/NH_4^+-activated enzyme (EPP = 1.7 μM) which in turn is greater than that observed with the Mg^{2+}/NH_4^+-activated enzyme (EPP = 0.3 μM).

The time course for ATP turnover in the active site of Co^{2+}/NH_4^+-activated PPDK is shown in Figure 3. These data clearly show that EPP is the first intermediate formed in this reaction. As the EPP is consumed the second intermediate, EP, and second product, PP_i, appear, and then after a short lag period the final product, PEP, appears. These observations demonstrate that the PPDK reaction proceeds by mechanism II of Scheme I and not by mechanism I.

Acknowledgment. This work was supported by a National Institutes of Health Grant GM-36260. We gratefully thank Kenneth Johnson for helpful discussions of this work and for use of his rapid quench apparatus in preliminary experiments.

(10) Reactions were quenched with 0.6 N HCl (164 μL); and the protein in the quenched sample was precipitated with CCl_4 (100 μL). The radiolabeled reactants and product(s) were separated by HPLC [Beckman Ultrasphere C18 analytical column, 25 mM K^+P_i, 2.5% triethylamine and 5% methanol (pH 6.5) isocratic elution]. The radioisotope content of the protein precipitate (dissolved in boiling 10 N H_2SO_4) and the HPLC fractions[5] was determined by using liquid scintillation techniques.

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(12) Pocalyko, D.; Mehl, A.; Dunaway-Mariano, D., unpublished.

(13) The uncertainty of the conclusion is due to the possibility that tortional equilibration at the α-P limits the P_β–O–$P_\alpha \rightarrow P_\alpha$=O PIX rate.

Doublet–Quartet Intersystem Crossing of Flavin Radical in DNA Photolyase

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DNA photolyases repair UV (200–300 nm)-induced pyrimidine dimers in DNA in a light (300–500 nm)-dependent reaction.[1] The enzyme isolated from *Escherichia coli* has two chromophores, a stable neutral flavo-semiquinone radical (5-hydro-FAD, $FADH^0$)[2] and 5,10-methenyltetrahydrofolate.[3] Nanosecond flash photolysis studies[4,5] on the enzyme revealed a transient absorption band which decays with a rate constant of 0.8×10^6 s^{-1}. This absorption was assigned to an excited state of $FADH^0$, which decays by abstracting a hydrogen atom from a tryptophan residue[6] of the apoenzyme. This means that the radical must have an excited state with an intrinsic lifetime more than 1 μs. Even though this transient absorption was tentatively assigned to the lowest doublet state (D_1) of the flavin radical[5] we had reasons to believe that it may actually be the lowest quartet (Q_1) of $FADH^0$.[5] Since quartet states have not previously been identified in organic free radicals, we undertook a more detailed analysis of the excited states of the enzyme-bound $FADH^0$. In this study we have carried out picosecond laser photolysis on *E. coli* DNA photolyase in order to understand the dynamics of the photophysical processes in more detail. Our results indicate that the excited state species with 1 μs lifetime is the quartet of flavin radical.

E. coli DNA photolyase was prepared as described previously.[7] Enzyme concentration was 1.4×10^{-4} M with respect to the flavin radical. The enzyme was in a buffer containing 5×10^{-2} M Tris HCl, pH 7.5, 5×10^{-2} M NaCl, 10^{-3} M EDTA, 10^{-2} M dithiothreitol, and 50% glycerol.

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