an ORTEP diagram of its molecular structure is shown in Figure 2. In contrast with **7**, treatment of **8** with 3 N HCl readily gave H_2SiPh_2. Although the reaction of **8** with 1-octene was very sluggish at 25 °C, it proceeded smoothly at 60 °C to give *n*-OctSiHPh$_2$ in 80% yield in 6 h. Similarly, the reaction of **4** with H_2SiPh_2 (2 equiv) in the presence of PPh_2Me (1.3 equiv) gave **3** in quantitative yield and a $ZrCp_2$ species (70%), tentatively identified as $Cp_2ZrH(SiHPh_2)(PMePh_2)$ (**9**) on the basis of a ^1H NMR Cp signal at δ 5.27 (d, J = 3 Hz). This complex reacted with 1-octene even at 25 °C to give a 75% yield of *n*-OctSiHPh$_2$ (**1**). The corresponding reactions of **9** with 2-octene also gave **1** in 90% yield. However, attempts to isolate and purify **9** were unsuccessful. These results suggest that, under the catalytic conditions, the monomer of **7**, i.e., $Cp_2ZrH(SiHPh_2)$ (**10**), probably is generated in situ and reacts with alkenes to give hydrosilated products before dimerization to produce **7**. Such a hydrosilation may involve either hydrozirconation[11] or silylzirconation[12] followed by reductive elimination to form a C-Si or C-H[8] bond. All but reductive elimination to form a C-Si bond are precedented. However, elucidation of these mechanistic details requires further investigation.

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Supplementary Material Available: Experimental details for the syntheses of **1**, **7**, and **8**, ^1H and ^{13}C NMR, IR, and mass spectral data for **7**, **8**, and various diphenylsilanes, and ORTEP representations of **7** and **8** (5 pages). Ordering information is given on any current masthead page.

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Spectrophotometric Detection of a Modified Flavin Mononucleotide (FMN) Intermediate Formed during the Catalytic Cycle of Chorismate Synthase

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Chorismate synthase (EC 4.6.1.4), the seventh enzyme in the shikimate pathway,[1] catalyzes the conversion of 5-enolpyruvylshikimate 3-phosphate (EPSP) to chorismate in a reaction that involves the trans 1,4-elimination of the C-6-*pro-R* proton and the C-3 phosphate ion[2-4] (Scheme I). Although this reaction does not involve an overall oxidation or reduction, a reduced flavin cofactor (i.e., $FMNH_2$) is required for activity.[5-7] The enzyme from *Escherichia coli* is therefore routinely assayed in the presence of 10 μM FMN and 1 mM sodium dithionite ($Na_2S_2O_4$).[8] The availability of purified enzyme either from *Neurospora crassa* or overproducing strains of *E. coli* has previously restricted mechanistic studies to steady-state kinetic determinations of K_m for EPSP (2.7 μM[9] and 2.2 μM[10]) and K_i for iso-EPSP (8.7 μM),[9] the detection of an isotope effect with (6*R*)-[6-^{2}H]EPSP,[10] and one pre-steady-state rapid-quench experiment which failed to detect either a lag or a burst phase for P_i release.[11] The role of

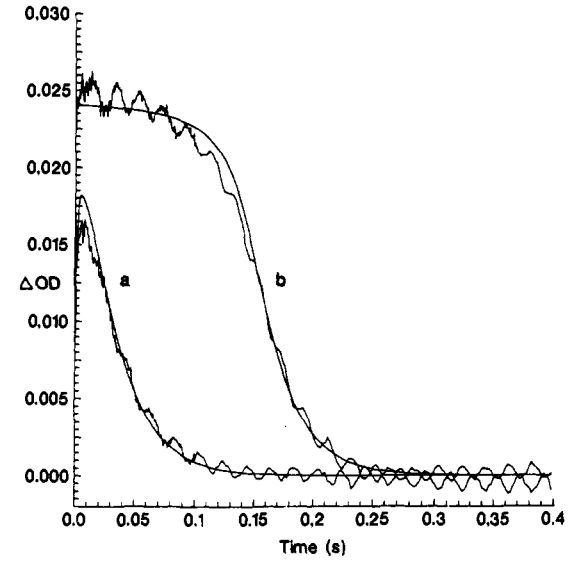
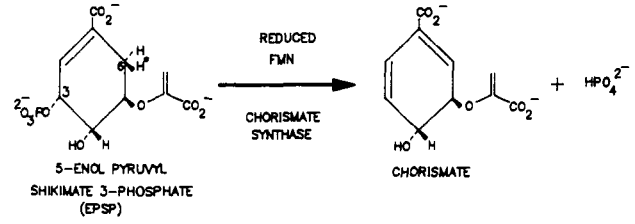


Figure 1. Transient absorbance change at 400 nm for (a) a single-turnover experiment at 25 °C, pH 7.0 [chorismate synthase, 17 μM (sp act. 13 μmol of chorismate produced min^{-1} mg^{-1});[19,20] EPSP, 20 μM; $FMNH_2$, 40 μM; $Na_2S_2O_4$, 1 mM] and (b) a multiple-turnover experiment (concentrations as for Figure 1a except EPSP increased to 100 μM). The lines through the data were simulated using The values of

Scheme I. The C6-H* Proton and the C3-Phosphate Are Eliminated



$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} E{\cdot}S^* \xrightarrow{k_{+2}} E + P$$

The values of the rate constants were constrained so that k_{+1} was 5 × 10^7 M^{-1} s^{-1} (it must be >10^7 M^{-1} s^{-1} as judged from the rise time observed in the traces) and so that k_{-1} = 70 s^{-1} gave a K_s for EPSP of 2.2 μM (the same as the K_m determined for the *N. crassa* enzyme[10]). The best fit value of k_{+2} = 38 s^{-1} is relatively insensitive to the precise values of k_{+1} and k_{-1} in these ranges. The absorbance change was assumed to be proportional to the concentration of the intermediate E·S*. We do not wish to imply that this intermediate is the only transient form of the enzyme nor that k_{+2} is necessarily associated with product release.

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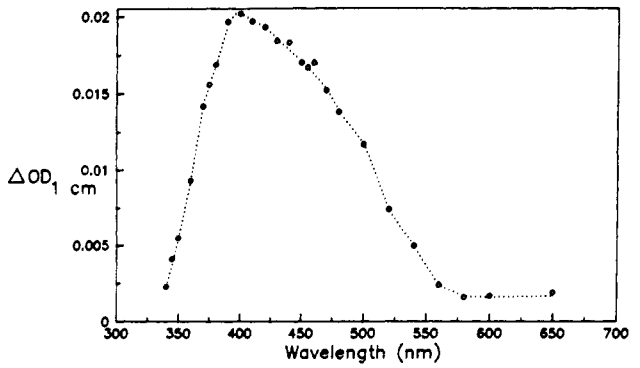

Figure 2. The difference spectrum of the enzyme-bound intermediate formed during the conversion of EPSP (75 μM) to chorismate catalyzed by chorismate synthase (17 μM) at 25 °C, pH 7.0. The data points are the amplitudes of traces similar to that of Figure 1b recorded at various wavelengths.

$FMNH_2$ is not known, and there is no consensus with respect to the overall mechanism.[12]

We report herein EPSP-induced, transient changes in the absorbance spectrum of $FMNH_2$ consistent with the formation of a flavin-C_4 adduct, thereby demonstrating for the first time direct involvement of redox-active $FMNH_2$ in the catalytic cycle.

Figure 1a shows a single-turnover experiment in which the absorbance at 400 nm was monitored using a Hi-Tech SF-51 stopped-flow spectrophotometer installed and operated in an anaerobic glovebox as previously described.[13] A rapid increase in the absorbance, with a rise time close to the dead time of the apparatus, is followed by a decrease in absorbance that occurs with $k_{obsd} \approx 30\ s^{-1}$. The final absorbance was that observed when EPSP was omitted from syringe B when no transient absorbance changes were detected. No effects were observed when $FMNH_2$ was omitted or when chorismate synthase was shot against $FMNH_2$ alone. However, when $Na_2S_2O_4$ was omitted and the $FMNH_2$ generated photochemically in the presence of EDTA,[14] absorbance changes similar to those shown in Figure 1a were observed.

Figure 1b shows the effect of increasing the EPSP concentration such that ca. 6 catalytic cycles of the enzyme (17 μM) are required for the complete conversion of EPSP to chorismate. The concentration of the intermediate is now maintained in a quasi steady state for ca. 100 ms before decaying with the same rate constant ($k_{obsd} \approx 30\ s^{-1}$).

The amplitude of the effects shown in parts a and b of Figure 1 depended in a linear manner on the $FMNH_2$ concentration up to a value of 20 μM with a final chorismate synthase concentration of 17 μM and a saturating EPSP concentration of 100 μM. At higher $FMNH_2$ concentrations, no further increase occurred (data not shown). This indicates stoichiometric, tight binding ($K < 0.5$ μM) of 1 equiv of $FMNH_2$/39K subunit of chorismate synthase. Maintaining the $FMNH_2$ concentration at 40 μM, with 17 μM chorismate synthase, but varying the EPSP concentration from 0 to 50 μM caused the amplitude to increase from 0 to that shown in Figure 1b. At EPSP concentrations in the range 50-100 μM, no further increase occurred. We conclude that the transient increase in absorbance at 400 nm is associated with an intermediate form of FMN that is present on the enzyme only when EPSP is being converted to chorismate. We have obtained a difference spectrum (Figure 2) for this FMN species that is not characteristic of free FMN/$FMNH_2$ nor of protein-bound FMN/$FMNH_2$ calculated from the spectra of *Klebsiella pneumoniae* flavodoxin in its oxidized and hydroquinone states.[15] The essentially 0 amplitude at 580 nm indicates that FMN semiquinone[14] is not formed at detectable levels.

We conclude that the $FMNH_2$ does not simply undergo a one- or two-electron oxidation during the catalytic cycle. The relatively small absorbance change ($\Delta\epsilon_{400} = 1.3\ mM^{-1}\ cm^{-1}$) associated with the decay of the intermediate could reflect either its low steady-state concentration or its intrinsic spectral characteristics. A charge-transfer complex is a possibility, although we also note the similarity of the difference spectrum of the intermediate to that of the flavin-C_4 adduct.[16] Since all four cysteinyl thiol groups predicted from the DNA sequence[17] were quantitatively detected using 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB)[18] in 8 M urea, we do not consider that the role of $FMNH_2$ is to reduce a disulfide as recently reported for mercuric ion reductase.[18]

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Direct Observation of $MeRh(CO)_2I_3^-$: The Key Intermediate in Rhodium-Catalyzed Methanol Carbonylation

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One of the most important industrial processes utilizing homogeneous transition-metal catalysis is the rhodium and iodide promoted carbonylation of methanol to acetic acid. Extensive research by the Monsanto group[2] and others[3,4] indicated that the active catalyst precursor was $[Rh(CO)_2I_2]^-$, A, and that the reaction proceeded as shown in Scheme I.

In this cycle the step which determines the overall rate is the oxidative addition of MeI to $Rh(CO)_2I_2^-$.[5] However, the methyl-rhodium complex, B, was never detected, and the first product observed from a stoichiometric oxidative addition of MeI to A was the acetyl complex C.[6]

We now report that, when the oxidative addition of MeI to $Rh(CO)_2I_2^-$ is carried out in neat MeI solvent, the crucial intermediate $MeRh(CO)_2I_3^-$, B, can be detected at low concentration using FTIR and FTNMR spectroscopy. The observation of B has also allowed estimation of the rate of the key methyl migration

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