Solid-State NMR Determination of Intra- and Intermolecular ^{31}P–^{13}C Distances for Shikimate 3-Phosphate and [1-^{13}C]Glyphosate Bound to Enolpyruvylshikimate-3-phosphate Synthase†

Allyson M. Christensen‡ and Jacob Schaefer*

Department of Chemistry, Washington University, St. Louis, Missouri 63130

Received October 13, 1992; Revised Manuscript Received January 4, 1993

ABSTRACT: Rotational-echo, double-resonance (REDOR) ^{31}P NMR was used to obtain internuclear distances for shikimate 3-phosphate (S3P) and *N*-(phosphonomethyl)-[1-^{13}C]glycine (glyphosate) bound to 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, a 46-kDa enzyme essential for the synthesis of aromatic amino acids in plants and microorganisms. An intermolecular ^{31}P–^{13}C distance of 7.2 Å was measured between the phosphate of S3P and the labeled carbon of glyphosate. This means that S3P and glyphosate are in proximity in the binding site of the enzyme. An intramolecular ^{31}P–^{13}C distance of 5.6 Å was measured between the phosphonate ^{31}P and the labeled carbon of glyphosate. This distance can be achieved only if glyphosate is completely extended when bound to EPSP synthase.

The molecular mechanism by which 5-enolpyruvylshikimate-3-phosphate (EPSP)[1] synthase catalyzes the reversible condensation of shikimate 3-phosphate (S3P) and phosphoenolpyruvate (PEP) to form EPSP in the synthesis of aromatic amino acids in plants and microorganisms is not known, although the reaction itself is well-characterized [for a review, see Anderson and Johnson (1990)]. This reaction is inhibited by the nonselective herbicide *N*-(phosphonomethyl)glycine (glyphosate), $HO_3PCH_2NHCH_2COOH$, which requires S3P in order to bind to EPSP synthase (Steinrücken & Amrhein, 1980). It has been proposed that the S3P–glyphosate complex is an analog of the transition state of the enzyme-catalyzed S3P–PEP condensation (Figure 1) which is known to pass through a tetrahedral intermediate (Bondinell et al., 1971; Anderson et al., 1988a,b). Knowledge of how glyphosate inhibits the condensation of S3P and PEP might lead to a better understanding of the molecular mechanism of catalysis by EPSP synthase.

Attempts to obtain specific binding-site information for this enzyme in the past have been unsuccessful. Complexes of EPSP synthase containing both S3P and glyphosate are extremely difficult to crystallize. An X-ray crystal structure has been obtained for the enzyme without substrates bound (Stallings et al., 1991), and a low-resolution structure was determined for EPSP synthase with only S3P bound (W. C. Stallings, private communication). However, if the enzyme structure changes significantly upon the binding of glyphosate, which seems likely (Stallings et al., 1991), information from the available X-ray structures may not be relevant to the ternary complex.

The 46-kDa EPSP synthase is not conducive to solution-state ^{1}H NMR studies because of its size and complexity. Solution-state ^{31}P NMR experiments on S3P and glyphosate bound to EPSP synthase are possible (S3P and glyphosate are the only sources of ^{31}P), but their interpretation in terms of substrate geometry is problematic because all the ^{31}P relaxation parameters are dominated by complicated interactions with the abundant protons. However, solid-state ^{31}P NMR is suitable for study of the conformation of EPSP synthase-bound substrates. Both the intra- and intermolecular ^{31}P–^{13}C dipolar couplings of S3P and ^{13}C-labeled glyphosate bound to natural-abundance EPSP synthase can be measured using REDOR NMR (Gullion & Schaefer, 1989a,b). The dipolar coupling constants obtained from REDOR are directly translated into internuclear distances. These distances are determined with accuracy and without interferences from protons. Because of the inherent accuracy of the REDOR experiment, just a few distances provide important constraints

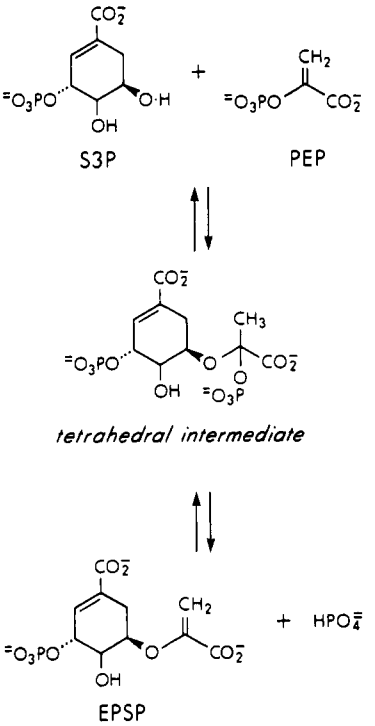

FIGURE 1: Reaction scheme for the condensation of S3P and PEP catalyzed by EPSP synthase.

† This work was supported by NIH Grant GM40634.

‡ Present address: Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, UT 84132.

[1] Abbreviations: EPSP, 5-enolpyruvylshikimate 3-phosphate; S3P, shikimate 3-phosphate; glyphosate, *N*-(phosphonomethyl)glycine; PEP, phosphoenolpyruvate; NMR, nuclear magnetic resonance; REDOR, rotational-echo double-resonance; CPMAS, cross-polarization magic-angle spinning.

0006-2960/93/0432-2868$04.00/0

in defining the geometry of the S3P–glyphosate complex in the binding site.

REDOR was developed to measure weak heteronuclear dipolar couplings between isolated spin-1/2 pairs in the presence of large chemical shift anisotropies (Gullion & Schaefer, 1989a,b). REDOR has been used to measure a 4-Å C–N internuclear distance in a helical peptide with an accuracy of ±0.1 Å (Marshall et al., 1990) and an 8-Å C–F internuclear distance in the same peptide with an accuracy of ±0.3 Å (Holl et al., 1992). REDOR has also been used to detect the presence of directly bonded ^{13}C–^{15}N pairs in specific cross-links in insect cuticle (Christensen et al., 1991).

^{31}P–^{13}C REDOR involves the observation of ^{31}P rotational echoes in the presence and absence of rotor-synchronized ^{13}C π pulses. The REDOR difference (the difference between spectra obtained with and without ^{13}C pulses) has a strong dependence on the strength of the ^{31}P–^{13}C dipolar coupling. This experiment does not depend on ^{31}P or ^{13}C chemical shift tensors, does not require resolution of the ^{31}P–^{13}C dipolar coupling in the chemical shift dimension, and is not limited by the size or solubility of the molecules being studied. REDOR can be performed on a variety of solid samples including lyophilized or microcrystalline powders.

MATERIALS AND METHODS

Cell Culture. EPSP synthase was isolated from an *Escherichia coli* construct, W3110/pMON5537, which overproduces the enzyme. This engineered bacterial strain was provided by Alan Easton of Monsanto Co. (St. Louis, MO). The parent W3110 strain is described by Bachmann (1972). The plasmid pMON5537 (Olins et al., 1988) is under the control of the recA promoter which is induced with nalidixic acid. The media was modified from Bogosian et al. (1989): 38.0 mM K_2HPO_4, 27.8 mM NaH_2PO_4, 10.0 mM $(NH_4)_2SO_4$, 3.0 mM $MgSO_4$, 77.0 mM glucose, 0.23 mM thiamine hydrochloride, 0.22 mM $FeCl_3$, 20.0 mL/L 50X trace elements, 200 mg/L ampicillin, and 100 mg/L of each of the 20 common amino acids. The 50X trace elements stock solution contained 40 μM $ZnSO_4$, 80 μM Na_2MoO_4, 90 μM $CuSO_4$, 90 μM H_3BO_3, 80 μM $MnSO_4$, and 80 μM $CoCl_2$. The media was brought to pH 7.0 with potassium hydroxide and filter sterilized. Aliquots (475 mL) of media in 1.0-L Erlenmeyer flasks were inoculated with 12.5 mL of saturated culture grown at 37 °C. The cells were grown at 37 °C with moderate shaking in a water bath. The cells were induced to overproduce EPSP synthase by adding 75 μg/mL nalidixic acid (as a 15 mg/mL solution in 0.1 N NaOH) during the logarithmic growth phase (A_{600} = 0.15). The cells were harvested after 20–24 h by centrifuging for 30 min at 12200*g*. The media was reinoculated and cells were grown as described above. Yields were typically 10–15 g of wet cell paste/L of media.

EPSP Synthase Purification. The enzyme was purified using a substantially modified version of the protocol described by Lewendon and Coggins (1983). The entire procedure was carried out at 5 °C and all centrifugation was for 20 min at 41000*g*. *E. coli* cells (20–30 g) were melted in homogenization buffer (0.5 g of cells/mL) containing 100 mM Tris-HCl, pH 7.5, 50 mM KCl, 1 mM EDTA, 10% (v/v) glycerol, 5 mM 2-mercaptoethanol, 0.1 mg/mL type II-T trypsin inhibitor, and 0.25% Tween 20. The cells were lysed for 15 min with 0.2 mg/mL egg-white lysozyme and then subjected to osmotic shock by adding sucrose to a 15% (w/v) final concentration. Cell debris was pelleted by centrifugation. Nucleic acids were precipitated from the supernatant by adding 20 mL of 2% (w/v) protamine sulfate in homogenization buffer and stirring for 20 min. The nucleic acids were pelleted by centrifugation. The supernatant was then brought to 50% saturation with ammonium sulfate (enzyme grade), stirred for 20 min, and centrifuged. This supernatant was brought to 70% saturation with ammonium sulfate, stirred for 20 min, and centrifuged. The pellet was resuspended in buffer A (100 mM Tris-HCl, pH 7.5, 1 mM EDTA, 10% (v/v) glycerol, 5 mM 2-mercaptoethanol, and 1.0 M ammonium sulfate) and then filtered through a 0.20-μm filter. The protein solution was then loaded onto a 2.5 cm × 16 cm phenyl-Sepharose (Pharmacia) column equilibrated with buffer A. The column was eluted with a 500-mL linear gradient from buffer A to buffer B (100 mM Tris-HCl, pH 7.5, 1 mM EDTA, 10% (v/v) glycerol, and 5 mM 2-mercaptoethanol) at a rate of 2 mL/min. EPSP synthase eluted in the latter half of the gradient. The protein was concentrated by ultrafiltration under N_2(g). The concentrated enzyme solution was stored at –80 °C. Typical yields were 3–5 mg of enzyme/g of cell paste, determined using an extinction coefficient at 280 nm of 0.77 cm^2/mg (Anderson et al., 1988b).

Lyophilization. Purified EPSP synthase was exchanged into a buffer containing 2 mM MOPS (4-morpholinepropanesulfonic acid), pH 7.5, 0.5 mM KCl, 10 μM EDTA, and 1 mM dithiothreitol and then concentrated to 9 mg/mL (0.2 mM) by ultrafiltration. Samples were also prepared with an enzyme concentration of 0.05 mM. The concentrated enzyme was reacted with a small excess of S3P and glyphosate. Solution-state ^{31}P NMR spectra showed no detectable unbound glyphosate when a nominal 37% molar excess of S3P was used. (The estimate of excess was based on an assumption of no phosphate hydrolysis of S3P.) The reaction mixture was maintained at room temperature for 1.5 h and then divided into 200-μL aliquots in 1.5-mL Eppendorf microcentrifuge tubes and maintained at room temperature for another hour. The aliquots were then frozen by immersion in liquid nitrogen and lyophilized. Some samples were frozen from larger volumes. Samples were completely freeze-dried within 24 h. Between 50% and 75% of EPSP synthase activity was recoverable following lyophilization.

Natural-abundance glyphosate was obtained from James Sikorski of Monsanto Co. and [1-^{13}C]glyphosate (99 atomic % ^{13}C taken as 100% labeled) was obtained from MSD Isotopes. Natural-abundance shikimate 3-phosphate was obtained from Karen Anderson (Monsanto Co.).

Nuclear Magnetic Resonance. Cross-polarization, magic-angle spinning (CPMAS) ^{31}P NMR spectra were obtained at room temperature at 121.3 MHz on a Chemagnetics spectrometer using a home-built four-channel (^1H, ^{31}P, ^{13}C, ^{15}N) probe. The single, 9-mm-i.d. radiofrequency coil was connected by a low-loss transmission line to a quadruple-resonance tuning circuit outside the magnet (McKay, 1984). Cross-polarization transfers were performed at 50 kHz and proton decoupling at 70 kHz with a sequence-repetition time of 2 s. A sample-spinning speed of 5000 Hz was used for all experiments. The magic-angle stators were obtained from Chemagnetics (Fort Collins, CO). Rotors with nominal 9-mm diameters were made from zirconia barrels and had Kel-F end caps.

The REDOR pulse sequence (Figure 2) utilized pulses on both the phosphorus and carbon channels, with the ^{31}P π pulses at the completion of each rotor period (to refocus isotropic chemical shifts) and the ^{13}C π pulses at each half rotor period. These pulses were phase-cycled using the xy-8 scheme to eliminate resonance offset effects (Gullion et al., 1990; Gullion & Schaefer, 1991). The π pulses change the sign of the dipolar

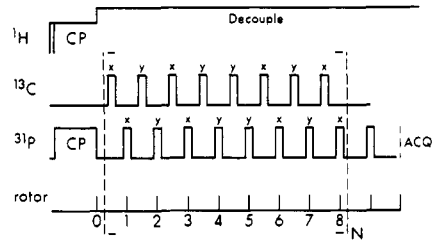

FIGURE 2: REDOR pulse sequence with dephasing π pulses on both the phosphorus and carbon channels. The pulses are applied using an xy-8 phase-cycling scheme to eliminate off-resonance effects. Signal acquisition begins two rotor cycles after the completion of a full 8N rotor cycles of dephasing.

interaction, causing a net dephasing of the transverse magnetization for phosphorus nuclei dipolar coupled to carbons. This results in a REDOR difference signal (ΔS, the difference between ^{31}P rotational-echo spectra obtained with and without dephasing ^{13}C pulses). The full-echo signal (obtained with no ^{13}C π pulses) is S_0. The $\Delta S/S_0$ ratio depends on the product of the number of rotor cycles of dephasing, the rotor period, and the dipolar coupling constant. A measurement of $\Delta S/S_0$ therefore determines the dipolar coupling because the other parameters are known. The internuclear distance is determined directly from the dipolar coupling constant, D, which is equal to $\gamma_P\gamma_C\hbar/r^3$, where γ_P and γ_C are the magnetogyric ratios of ^{31}P and ^{13}C, respectively, $\hbar$ is Planck's constant divided by 2π, and r is the internuclear distance.

For the ^{31}P REDOR experiments performed on complexes of EPSP synthase, the full-echo and dephased spectra were obtained under the same experimental conditions with dephasing ^{13}C π pulses applied on alternate scans. Phosphorus-observe REDOR experiments were performed with 32, 40, and 64 rotor cycles of dephasing π pulses. Signal intensities were measured separately for the REDOR spectra with and without dephasing pulses and their arithmetic difference was used to determine ΔS. REDOR difference spectra were used for display purposes but were not used to determine ΔS. First and second spinning sidebands were included in the determination of $\Delta S/S_0$. A 5% reduction in D due to averaging by ultra-high-speed small-amplitude molecular motion was assumed (Marshall et al., 1990).

RESULTS

The ^{31}P CPMAS NMR spectrum for S3P and [1-^{13}C]-glyphosate bound to natural-abundance EPSP synthase shows four sharp, well-resolved lines (Figure 3, bottom) corresponding to bound S3P (δ_P 3 ppm), unbound S3P (δ_P 5 ppm), bound glyphosate (δ_P 12 ppm), and unbound glyphosate (δ_P 9 ppm). The chemical shift of the free glyphosate peak matches that of glyphosate in the presence of enzyme but absence of S3P (Figure 3, top). The 2 ppm upfield shift for bound relative to unbound S3P and the 3 ppm downfield shift for bound relative to unbound glyphosate are consistent with the 1.6 ppm upfield and 3.5 ppm downfield chemical shift differences observed with solutions NMR (Castellino et al., 1989). The sum of integrated intensities of the centerband and sidebands arising from bound glyphosate equals 0.83 that arising from bound S3P, consistent with a 1:1 stoichiometry at 83% of the binding sites. The remaining 17% of the sites have bound S3P only.

The ^{31}P NMR resonances of EPSP ternary complexes were not always sharp. Broad ^{31}P NMR lines are sometimes observed (Figure 4, top), suggesting a disordered binding site. Low concentrations of EPSP synthase ternary complex (0.05

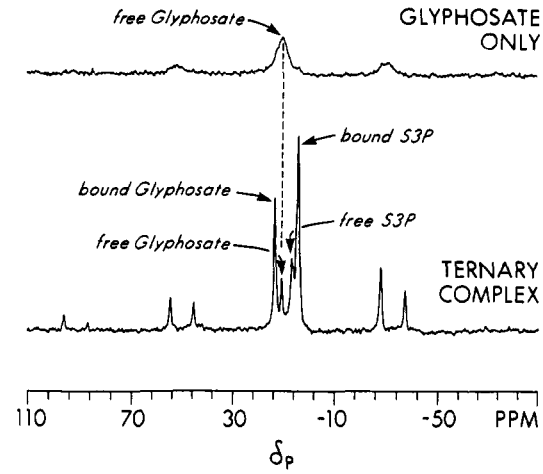

FIGURE 3: The 121.3-MHz CPMAS ^{31}P NMR spectra of glyphosate and natural-abundance EPSP synthase (top) and 5 μmol of S3P plus 3.5 μmol of [1-^{13}C]glyphosate bound to 3.2 μmol (143 mg) of natural-abundance EPSP synthase (bottom). Both samples were lyophilized in pH 7.5 buffer. The ^{31}P chemical shift of bound S3P is 2 ppm upfield from that of free S3P. The ^{31}P chemical shift of bound glyphosate is 3 ppm downfield from that of free glyphosate. The shift reference is external phosphocreatin.

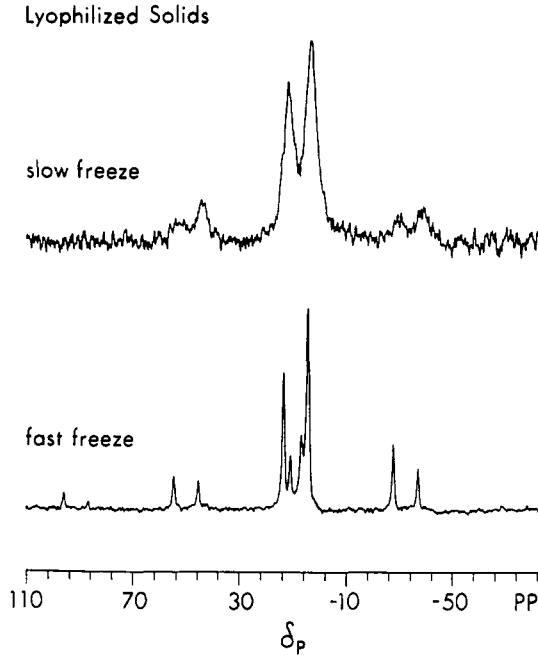

FIGURE 4: The 121.3-MHz CPMAS ^{31}P NMR spectra of S3P and glyphosate bound to EPSP synthase. The top spectrum is of a sample which was frozen from a 10-mL volume prior to lyophilization; the bottom spectrum is of a sample which was frozen in 200-μL aliquots prior to lyophilization.

mM) frozen quickly in small volumes generally had spectra characterized by sharp ^{31}P NMR lines similar to those shown in Figure 4 (bottom).

Attempts to obtain enzyme–substrate complexes in non-lyophilized forms amendable to solids NMR experiments were unsuccessful. Water-soluble EPSP synthase crystals bathed in a low ionic-strength solvent suitable for NMR became white and did not bind S3P and glyphosate (Christensen, 1992). Crystals that had been cross-linked by glutaraldehyde for stability and bathed in water also did not bind S3P and glyphosate (Christensen, 1992).

The REDOR ^{31}P NMR spectra of S3P and [1-^{13}C]-glyphosate bound to natural-abundance EPSP synthase are shown in Figure 5 (left). After 64 rotor cycles of dephasing

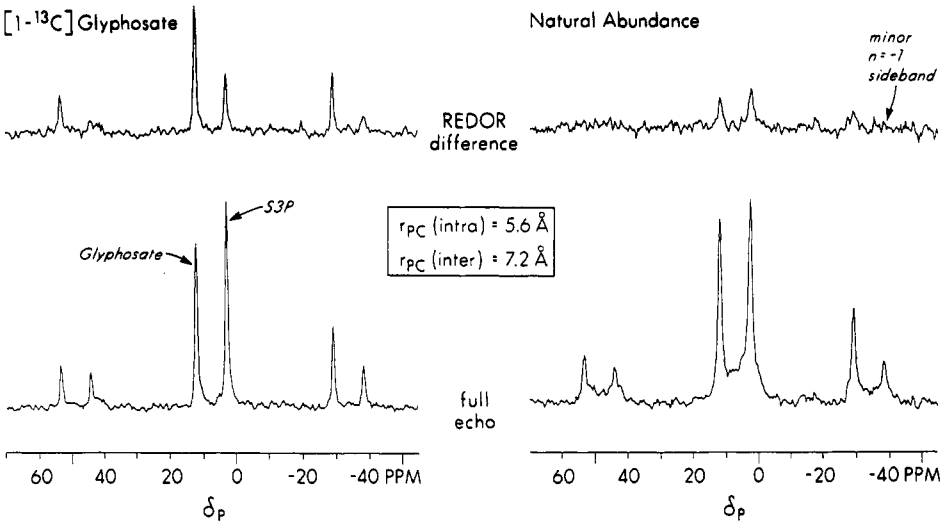

FIGURE 5: The 121.3-MHz REDOR ^{31}P NMR spectra of S3P and [1-^{13}C]glyphosate (left) and S3P and natural-abundance glyphosate (right) bound to natural-abundance EPSP synthase after 64 rotor cycles of dephasing. Magic-angle spinning was at 5 kHz.

Table I: REDOR Parameters and Distances for S3P and [1-^{13}C]Glyphosate Bound to EPSPS

P observed	N_c	$\Delta S/S_0$[a]	$\Delta S/S_0$[b] (nat abnd)	corrected $\Delta S/S_0$[c]	λ_D (N_cT_rD)	D_{PC} (Hz)	r_{PC} (Å)
S3P	32	0.152	0.110	0.042	0.20	31	7.2
S3P	40	0.170	0.089	0.081	0.28	35	7.5
S3P	64	0.351	0.206	0.145	0.38	30	7.1
glyphosate	32	0.280	0.094	0.186	0.44	69	5.6
glyphosate	40	0.349	0.068	0.281	0.54	68	5.6
glyphosate	64	0.735	0.170	0.565	0.84	66	5.7

[a] Observed S3P $\Delta S/S_0$ multiplied by 0.83^{-1}. [b] For EPSP synthase ternary complex with natural-abundance S3P and glyphosate. [c] Difference of columns 3 and 4.

pulses, the ratio of the REDOR difference (ΔS) to the full echo signal (S_0) associated with dipolar coupling between the phosphate ^{31}P of S3P and the labeled carbon of glyphosate is 0.291. The $\Delta S/S_0$ ratio due to the intramolecular ^{31}P–^{13}C coupling between the phosphonate ^{31}P and the labeled carbon of glyphosate is 0.735.

Before distances can be calculated from these ratios, two corrections must be made. The first correction applies only to S3P and takes into account sites that have bound S3P (and contributions to the S3P S_0) but no bound [1-^{13}C]glyphosate (and so no contribution from the ^{13}C label to ΔS). This correction was made by multiplying the observed $\Delta S/S_0$ by the inverse of the experimentally determined fraction of doubly occupied binding sites (Table I, footnote *a*). The second correction is for the contributions to the REDOR differences for both S3P and glyphosate from natural-abundance carbons. To determine these corrections, ^{31}P REDOR experiments were performed on S3P and natural-abundance glyphosate bound to natural-abundance EPSP synthase. Results for 64 rotor cycles of dephasing are shown in Figure 5 (right). REDOR ^{31}P NMR experiments were also performed with 32 and 40 rotor cycles of dephasing for both the labeled and natural-abundance samples. The results of all the REDOR experiments are summarized in Table I. All three experiments yield the same value for the internuclear distances within experimental error assuming that the dephasing from the natural-abundance background and from the label are additive. The observed dependence of $\Delta S/S_0$ on λ_D (the product of the number of rotor cycles of dephasing, N_c, the rotor period, T_r, and the dipolar coupling constant, D) agrees with the theoretical prediction (Figure 6) and is consistent with the presence of one structure for the ternary complex with well-defined internuclear distances.

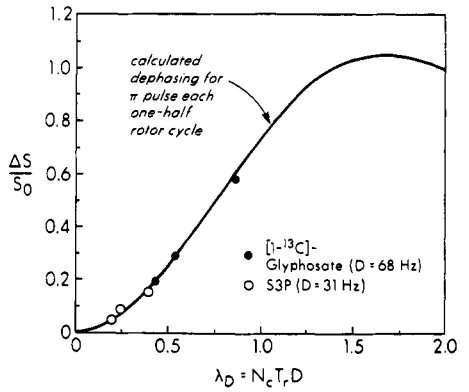

FIGURE 6: Observed dependence of $\Delta S/S_0$ on λ_D for the enzyme-bound S3P–labeled-glyphosate complex. λ_D is the product of N_c (the number of rotor cycles of dephasing), T_r (the rotor period), and D (the dipolar coupling constant). The results for the 68-Hz intramolecular ^{31}P–^{13}C coupling in labeled glyphosate (filled circles) and for the 31-Hz intermolecular ^{31}P–^{13}C coupling between the S3P ^{31}P and the labeled C-1 carbon of glyphosate (open circles) are in agreement with the theoretical REDOR dephasing calculated for π pulses each half rotor cycle (solid line).

DISCUSSION

EPSP synthase is a large protein which may have an open, flexible clam-shaped conformation when substrates are not bound (Stallings et al., 1991). This shape leaves ample room for the association of water at the binding site, which is probably in the cleft between the two domains of the protein. The presence of positive charges on the protein in the cleft (Stallings et al., 1991) and negative charges on the substrate and inhibitor enhance the tendency for the binding site to have associated water. This water may crystallize upon slow freezing for lyophilization, thus distorting the binding site. The distortions produce a distribution of structures contributing to ^{31}P NMR line broadening. Fast freezing of dilute solutions captures the ordered bound complex, avoids aggregation by concentration, inhibits crystallization of water at the binding site, and prevents multiple freeze–thaw cycles during lyophilization. This combination of effects eliminates distortions of binding-site geometry and so broad lines. We are confident that those enzyme complexes having sharp ^{31}P lineshapes, and chemical shifts that match those of bound

substrate and inhibitor in solution, have native geometry at the binding site.

The absence of spinning sidebands for free S3P and glyphosate (Figure 3, bottom) indicates that the unbound species are more mobile than bound S3P and glyphosate, both of which have large spinning sidebands consistent with complete immobilization. The rotational echoes for free (mobile) S3P and glyphosate also decay faster than those for the bound species, with virtually no intensity from unbound species left after 64 rotor cycles (Figure 5, bottom left). The fact that the peaks for free S3P and glyphosate are sharp means that all the mobile molecules are in the same uniformly averaged environment. It is possible that they may be trapped in the cleft near the binding site of the enzyme, and, with time, may shift into the binding site. This transition would account for the slight decrease in unbound intensity and increase in bound intensity observed in ^{31}P NMR spectra of complexes obtained several months after their preparation (Christensen, 1992).

The results of REDOR ^{31}P NMR experiments for [1-^{13}C]-glyphosate and S3P bound to natural-abundance EPSP synthase (summarized in Table I) translate into an internuclear distance of 7.2 Å (±0.4 Å) between the phosphate ^{31}P of S3P and the labeled carbon of glyphosate. This means that S3P and glyphosate are near one another in the binding site of EPSP synthase. The intramolecular distance between the phosphonate ^{31}P and labeled carbon in glyphosate is 5.6 Å (±0.2 Å). This four-bond distance can be achieved only if glyphosate is fully extended when bound to the enzyme. This C–P distance in crystals of glyphosate is 4.707 Å (Knuuttila & Knuuttila, 1979). The experimental uncertainty in the intramolecular distance determined for glyphosate is smaller than that in the intermolecular distance determined for S3P because the natural-abundance correction for glyphosate is less important and because there is no correction for binding-site occupancy.

The hypothesis that the S3P–glyphosate complex acts as a transition-state analog for the native reaction is consistent with the fully extended conformation of bound glyphosate required by the experimentally determined 5.6-Å intramolecular distance. According to this hypothesis, bound S3P–glyphosate mimics the transition-state conformation of S3P–PEP (Figure 1) with charges located in similar positions and the glyphosate nitrogen in the tetrahedral center. A possible structure for the bound S3P–glyphosate complex is shown in Figure 7. The structure was obtained using the SEARCH module and standard parameter tables of the SYBYL software package (Tripos Associates, St. Louis, MO). A systematic search of all accessible conformations was constrained by the two distances measured with REDOR NMR and an assumed hydrogen bond between a glyphosate NH proton and the C5 oxygen of S3P. (The hydrogen-bond constraint is based on the transition-state analog model proposed for the S3P–glyphosate complex.) The structure shown in Figure 7 is just one of many glyphosate orientations and S3P ring conformations possible with these distance constraints. Additional constraints are needed to specify more exactly the geometry of the complex in the binding site of EPSP synthase. These constraints can be obtained from measurements on EPSP synthase ternary complexes made using [2-^{13}C]glyphosate, [3-^{13}C]glyphosate, or [^{15}N]glyphosate. In addition, the ^{31}P–^{31}P distance in ternary complexes can be measured by NMR experiments specifically designed to detect homonuclear dipolar coupling within isolated pairs of spins (Tycko & Dabbagh, 1990). Experiments to obtain these additional distances are in progress.

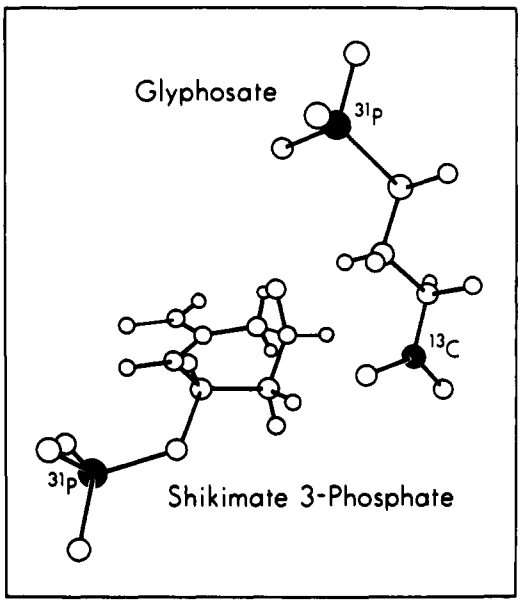

FIGURE 7: Possible structure of the S3P–glyphosate complex bound to EPSP synthase. The intramolecular ^{31}P–^{13}C distance for this structure is 5.6 Å, and the intermolecular ^{31}P–^{13}C distance is 7.2 Å. A hydrogen bond between the glyphosate NH proton and the C5 oxygen of S3P is assumed.

ACKNOWLEDGMENTS

We are happy to acknowledge Denise Beusen (Washington University) for assistance with molecular modeling of the bound S3P–glyphosate complex and Asher Schmidt (Washington University) for assistance with the ^{31}P NMR data reduction. We thank James A. Sikorski (Monsanto Co.) for sharing many of his insights into the structure of EPSP synthase and for his encouragement and support during the course of this work.

REFERENCES

Anderson, K. S., & Johnson, K. A. (1990b) *Chem. Rev. 90*, 1131–1149.

Anderson, K. S., Sikorski, J. A., Benesi, A. J., & Johnson, K. A. (1988a) *J. Am. Chem. Soc. 110*, 6577–6579.

Anderson, K. S., Sikorski, J. A., & Johnson, K. A. (1988b) *Biochemistry 27*, 7395–7406.

Bachmann, B. J. (1972) *Bacteriol. Rev. 36*, 525–557.

Bogosian, G., Violand, B. N., Dorward-King, E. J., Workman, W. E., Jung, P. E., & Kane, J. F. (1989) *J. Biol. Chem. 264*, 531–539.

Bondinell, W. E., Vnek, J., Knowles, P. F., Sprecher, M., & Sprinson, D. B. (1971) *J. Biol. Chem. 246*, 6191–6196.

Castellino, S., Leo, G. C., Sammons, R. D., & Sikorski, J. A. (1989) *Biochemistry 28*, 3856–3868.

Christensen, A. M. (1992) Ph.D. Thesis, Washington University.

Christensen, A. M., Schaefer, J., Kramer, K. J., Morgan, T. D., & Hopkins, T. L. (1991) *J. Am. Chem. Soc. 113*, 6799–6802.

Gullion, T., & Schaefer, J. (1989a) *J. Magn. Reson. 81*, 196–200.

Gullion, T., & Schaefer, J. (1989b) *Adv. Magn. Reson. 13*, 55–83.

Gullion, T., & Schaefer, J. (1991) *J. Magn. Reson. 92*, 439–442.

Gullion, T., Baker, D., & Conradi, M. S. (1990) *J. Magn. Reson. 89*, 479–482.

Holl, S. M., Marshall, G. R., Beusen, D. D., Kociolek, K., Redlinski, A. S., Leplawy, M. T., McKay, R. A., Vega, S., & Schaefer, J. (1992) *J. Am. Chem. Soc. 114*, 4830–4833.

Knuuttila, P., & Knuuttila, H. (1979) *Acta Chem. Scand. B 33*, 623–626.

Lewendon, A., & Coggins, J. R. (1983) *Biochem. J. 213*, 187–191.

Marshall, G. R., Beusen, D. D., Kociolek, K., Redlinski, A. S., Leplawy, M. T., Pan, Y., & Schaefer, J. (1990) *J. Am. Chem. Soc. 112*, 963–966.

McKay, R. A. (1984) U.S. Patent 4446431.

Olins, P. O., Devine, C. S., Rangwala, S. H., & Kavka, K. S. (1988) *Gene 73*, 227–235.

Steinrücken, H. C., & Amrhein, N. (1980) *Biochem. Biophys. Res. Commun. 94*, 1207–1212.

Stallings, W. C., Abdel-Meguid, S. S., Lim, L. W., Shieh, H. S., Dayringer, H. E., Leimgruber, N. K., Stegman, R. A., Anderson, K. S., Sikorski, J. A., Padgette, S. R., & Kishore, G. M. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 5046–5050.

Tycko, R., & Dabbagh, G. (1990) *Chem. Phys. Lett. 173*, 461–465.