Kinetic Scheme I implies a quantitative relationship among k_{i} , the absorbance changes associated with B, and the concentration of A* formed by the actinic flash;²⁶ analysis based on this relationship demonstrates that within experimental error, triplet-state quenching is associated only with the electron-transfer process, eq 2, and that other mechanisms leading to deactivation of the excited state in A* (e.g., energy transfer) are relatively insignificant (<20%).22

With these results in hand, the effects of heme ligation, porphyrin substituents, and the protein matrix on the $Fe^{2+}P \rightarrow (MP)^+$ long-range electron-transfer process now can be investigated.

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(26) When monitoring triplet decay at 475 nm, $\Delta A(0) = A_0^*(\epsilon_A^* - \epsilon_A)$. Thus, $k_t = (\epsilon_A^* - \epsilon_A)\delta/\Delta A(0)(\epsilon_B - \epsilon_A)$ where terms are defined in footnote

Synthesis of Seryl Threonyl Phosphate. A Model Compound Designed To Study the Spectroscopic and Chemical Features of a Phosphodiester Linkage Similar to the One Proposed To Exist in Azotobacter Flavodoxin

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A well-established post-translational event in proteins is the formation of intermolecular and (or) intramolecular disulfide linkages which, inter alia, confer¹ on these molecules their unique structure and physiological activity. On the other hand, phosphorylation is another post-translational modification of increasing importance² in proteins. For example, the regulatory action of phosphorylation was nicely illustrated³ in the conversion of glycogen phosphorylase b to phosphorylase a by phosphorylation of serine-14.

Some years ago Edmondson and James⁴ proposed on the basis of chemical degradation and ³¹P NMR studies the presence of a phosphodiester linkage between the hydroxyl groups of serine and threonine in Azotobacter flavodoxin. Evidence supporting the occurrence of this unusual linkage was recently corroborated by Live and Edmondson⁵ by using ${}^{1}H^{-31}P$ two-dimensional NMR. Although this appears to be the only example sofar⁶ to support the existence of a phosphodiester linkage between the hydroxyamino acids serine and threonine in a protein, it is not excluded that phosphodiester bonds may also serve a similar purpose in proteins as disulfide linkages.

As part of a program directed toward the preparation of phosphopeptides⁷ and nucleopeptides,⁸ we report the synthesis of

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(6) The enzyme glucose oxidase [James, T. L. et al. Biochemistry 1981,

20, 617] also contains a disubstituted covalently bound phosphorus residue; however, the nature of its linkage has not been determined.

model compound 6 having a phosphodiester bond between an isolated serine and threonine and evaluate the chemical properties and spectroscopic (NMR) features of 6 with those published on the protein-bound seryl threonyl phosphodiester bond. The synthetic route⁹ to 6 is depicted in Scheme I and consists of the following steps.

Phosphitylation of the L-serine derivative¹⁰ 1 with benzyloxy $bis(N,N-diisopropylamino)phosphine^{11}$ (2), in the presence of 1H-tetrazole, resulted, after column chromatography, in the isolation of phosphite derivative 3 (80% yield, δ_p 149.05 and 149.35 ppm). 1H-Tetrazole-mediated coupling of 3 with the L-threonine derivative¹⁰ 4 gave an intermediate phosphite triester, which was oxidized in situ with tert-butyl hydroperoxide.¹² Purification of the crude phosphotriester product afforded homogeneous 5 (δ_p -1.56 and -1.68 ppm) in a yield of 80%. Hydrogenolysis of 5 (0.19 mmol) in the solvent mixture $tBuOH/H_2O/HOAc$ (4/1/1, v/ v/v), followed by workup and conversion into the sodium salt (via SP-Sephadex C25, Na⁺ form) yielded 6 (Na⁺ salt, 56 mg, δ_n -1.28 ppm, D₂O, pD 7.1, external standard: 85% H₃PO₄). The identity of 6 was unambiguously determined by ¹H NMR¹³ (see Figure 1 and Table I), ¹³C NMR¹⁴ and ³¹P NMR spectroscopy.

The presence of a seryl threonyl phosphodiester in Azotobacter flavodoxin was supported⁵ from the proton-detected ¹H-³¹P multiquantum 2D NMR spectral data. Thus the proton projection of the 2D spectrum reveals an AB type pattern with major peaks at 3.4 and 3.7 ppm and a doublet at 4.0 ppm, which were assigned to the two geminal β -protons of serine and the α -proton of threonine, respectively. The supposed absence of a cross peak for the α -proton of serine with phosphorus was attributed to the smaller H α -P coupling constant in serine than in threenine. The latter assumption¹⁶ is to some extent supported by the observed difference in magnitude between these coupling constants in model compound 6 (see Table I). On the other hand, the assumed nonappearance in the spectrum of the phosphothreonyl β -proton, which has a higher H β -P coupling constant than the phosphoseryl β -proton in compound 6, cannot be explained solely by a reduction in peak height arising from the multiple couplings of this proton.

Apart from the NMR characteristics of compound 6, we also observed that the phosphodiester bond in 6 was rather resistent toward alkaline treatment. This finding is in contrast with the result of Edmondson and James⁴ who found that base treatment of the protein resulted in the relative fast elimination of phosphate and concomitant formation of the dehydro forms of the appropriate hydroxyamino acids. Our finding may be explained by the presence of two free carboxylic functions in 6 which will decrease

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(13) The simulated ¹H NMR spectrum (PANIC program from Bruker) of 6 was in excellent agreement with the experimental one.

(15) Marginal changes in chemical shifts (0.01-0.21 ppm) and coupling constants (0.2-0.6 Hz) were observed for model compound 6 at pD 2. (16) It is, however, not excluded that the motion of the phosphodiester

bond in the protein may be restricted and would not necessarily give identical coupling constants as the model compound.

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^{(14) &}lt;sup>13</sup>C NMR data (δ values, D₂O, pD 7.1) 19.42 (C γ , Thr), 55.74 (d, C α , Ser, $J_{C\alpha,P}$ = 8.8 Hz), 60.12 (d, C α , Thr, $J_{C\alpha,P}$ = 8.8 Hz), 64.84 (d, C β , Ser, $J_{C\beta,P}$ = 4.4 Hz), 72.49 (d, C β , Thr, $J_{C\beta,P}$ = 4.4 Hz), 172.13 and 172.84 (2 × C=O, Ser and Thr).



Figure 1. A. ¹H NMR spectrum of fully deprotected seryl threonyl phosphate 6. B. ¹H 31 P} NMR spectrum of 6.



$$Z = -C - 0 - CH_2 - OBz = -CH_2 - OBz = -C$$

Table I. ¹H NMR Data of Compound 6 (Na⁺ Salt, 0.16 mM in D_2O , pD 7.1)¹⁵

unit	δ values (ppm)	coupling constants (Hz)
		Seryl
Ηα	3.95	$J_{\text{H}\alpha,\text{P}} = 1.2, J_{\text{H}\alpha,\text{H}\beta(a)} = 6.3, J_{\text{H}\alpha,\text{H}\beta(b)} = 3.6$
Hβ(a)	4.14	$J_{H\beta(a),P} = 5.0, J_{H\beta(a),H\beta(b)} = 11.3$
Hβ(b)	4.22	$J_{\mathrm{H}\beta(\mathrm{b}),\mathrm{P}} = 5.1$
Threonyl		
Hα	3.70	$J_{\text{H}\alpha,\text{P}} = 1.9, J_{\text{H}\alpha,\text{H}\beta} = 3.3$
Hβ	4.74	$J_{\rm HB,P} = 7.4, J_{\rm HB,CH_2} = 6.6$
СН,	1.42	

the rate of the elimination reaction.

In conclusion, the NMR data and virtually inertness of com-

pound 6 toward base do not a priori exclude the presence of an unusual phosphodiester bond in *Azotobacter* flavodoxin; the assignment of the individual peaks, however, may be subject to further investigation. To this end, we are at present actively engaged in preparing model compounds which contain, apart from an intermolecular or intramolecular phosphodiester linkage, also peptide bonds.

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Alkoxyl Radicals in 1,5-Hydrogen Shifts for Site-Specific, Stereocontrolled Alkylation of Carbohydrates¹

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Two areas of continued interest in our laboratory in developing the organic chemistry of carbohydrates are concerned with enhancing stereoselectivity at (normally) "off-template" sites⁴ and with the application of radical processes for mild transforma-

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