Kinetic Scheme I implies a quantitative relationship among k_t, 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.

Acknowledgment. We thank Professor J. Torkelson for the use of his phosphorimeter. This research has been supported by a NIH NRSA postdoctoral fellowship (HL07531) to M.J.N. and by NIH Grants HL 13531 and HL 40453 and NSF Grant DMG 8606575 to BMH.

(26) When monitoring triplet decay at 475 nm, $\Delta A(0) = A_o^*(\epsilon_A^* - \epsilon_A)$. Thus, $k_t = (\epsilon_A^* - \epsilon_A)\delta/\Delta A(0)(\epsilon_B - \epsilon_A)$ where terms are defined in footnote 21.

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

C. M. Dreef-Tromp, C. Erkelens, G. A. van der Marel, and J. H. van Boom*

Gorlaeus Laboratory, P.O. Box 9502
2300 RA Leiden, The Netherlands

Received March 23, 1989

A well-established post-translational event in proteins is the formation of intermolecular and (or) intramolecular disulfide linkages which, inter alia, confer[1] on these molecules their unique structure and physiological activity. On the other hand, phosphorylation is another post-translational modification of increasing importance[2] in proteins. For example, the regulatory action of phosphorylation was nicely illustrated[3] in the conversion of glycogen phosphorylase *b* to phosphorylase *a* by phosphorylation of serine-14.

Some years ago Edmondson and James[4] proposed on the basis of chemical degradation and ^{31}P 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 Edmondson[5] by using ^1H-^{31}P two-dimensional NMR. Although this appears to be the only example sofar[6] 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 phosphopeptides[7] and nucleopeptides,[8] we report the synthesis of 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 route[9] to **6** is depicted in Scheme I and consists of the following steps.

Phosphitylation of the L-serine derivative[10] **1** with benzyloxybis(*N*,*N*-diisopropylamino)phosphine[11] (**2**), in the presence of 1*H*-tetrazole, resulted, after column chromatography, in the isolation of phosphite derivative **3** (80% yield, δ_p 149.05 and 149.35 ppm). 1*H*-Tetrazole-mediated coupling of **3** with the L-threonine derivative[10] **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 *t*BuOH/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_p −1.28 ppm, D_2O, pD 7.1, external standard: 85% H_3PO_4). The identity of **6** was unambiguously determined by ^1H NMR[13] (see Figure 1 and Table I), ^{13}C NMR[14] and ^{31}P NMR spectroscopy.

The presence of a seryl threonyl phosphodiester in *Azotobacter* flavodoxin was supported[5] from the proton-detected ^1H-^{31}P 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 Hα–P coupling constant in serine than in threonine. The latter assumption[16] 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 Hβ–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 James[4] 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

(1) Light, A. *Proteins, Structure and Function*; Prentice Hall, Inc.: Englewood Cliifs, 1975.
(2) Krebs, E. G. *The Enzymes*, 3rd ed.; Boyer, P. D., Krebs, E. G., Eds.; Academic: New York, 1986; Vol. 17, pp 3–20. Cohen, P. *Eur. J. Biochem.* **1985**, *151*, 439.
(3) Sprang, S. R.; Acharya, K. R.; Goldsmith, E. J.; Stuart, D. I.; Varvill, K.; Fletterich, R. J.; Hudson, N. B.; Johnson, L. N. *Nature* **1988**, *215*, 336.
(4) Edmondson, D. E.; James, T. L. *Flavins and Flavoproteins*; Massey, V.; Williams, C. H., Eds.; Elsevier: North Holland, Amsterdam, 1982; p 111.
(5) Live, D. H.; Edmondson, D. E. *J. Am. Chem. Soc.* **1988**, *110*, 4468.
(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.
(7) de Bont, H. B. A.; Veeneman, G. H.; van Boom, J. H.; Liskamp, R. M. J. *Recl. Trav. Chim. Pays-Bas* **1987**, *106*, 641. Kuyl-Yeheskiely, E.; Tromp, C. M.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1987**, *28*, 4461. de Bont, H. B. A.; Liskamp, R. M. J.; O'Brian, C. A.; Erkelens, C.; Veeneman, G. H.; van Boom, J. H. *Int. J. Pept. Protein Res.* **1989**, *33*, 115.
(8) Kuyl-Yeheskiely, E.; van der Klein, P. A. M.; Visser, G. M.; van der Marel, G. A.; van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1986**, *105*, 69. Schattenkerk, C.; Wreesman, C. T. J.; de Graaf, M. J.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1984**, *25*, 5197. Kuyl-Yeheskiely, E.; Tromp, C. M.; Schaeffer, A. H.; van der Marel, G. A.; van Boom, J. H. *Nucl. Acid Res.* **1987**, *15*, 1807. Kuyl-Yeheskiely, E.; Tromp, C. M.; Lefeber, A. W. M.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron* **1988**, *44*, 6515. Kuyl-Yeheskiely, E.; Dreef-Tromp, C. M.; Geluk, A.; van der Marel, G. A.; van Boom, J. H. *Nucl. Acids Res.* **1989**, *17*, 2897.
(9) The synthetic route to **6** proved also to be suitable to couple serine or threonine with tyrosine: Dreef-Tromp, C. M. et al., to be published.
(10) Commercially available from Novabiochem (Switzerland).
(11) Dreef, C. E.; Elie, C. J. J.; Hoogerhout, P.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1988**, *29*, 6513.
(12) Engels, J.; Jäger, A. *Angew. Chem. Suppl.* **1982**, 2010.
(13) The simulated ^1H NMR spectrum (PANIC program from Bruker) of **6** was in excellent agreement with the experimental one.
(14) ^{13}C NMR data (δ values, D_2O, pD 7.1) 19.42 (Cγ, Thr), 55.74 (d, Cα, Ser, $J_{C\alpha,P}$ = 8.8 Hz), 60.12 (d, Cα, Thr, $J_{C\alpha,P}$ = 8.8 Hz), 64.84 (d, Cβ, Ser, $J_{C\beta,P}$ = 4.4 Hz), 72.49 (d, Cβ, Thr, $J_{C\beta,P}$ = 4.4 Hz), 172.13 and 172.84 (2 × C=O, Ser and Thr).
(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.

0002-7863/89/1511-6470$01.50/0

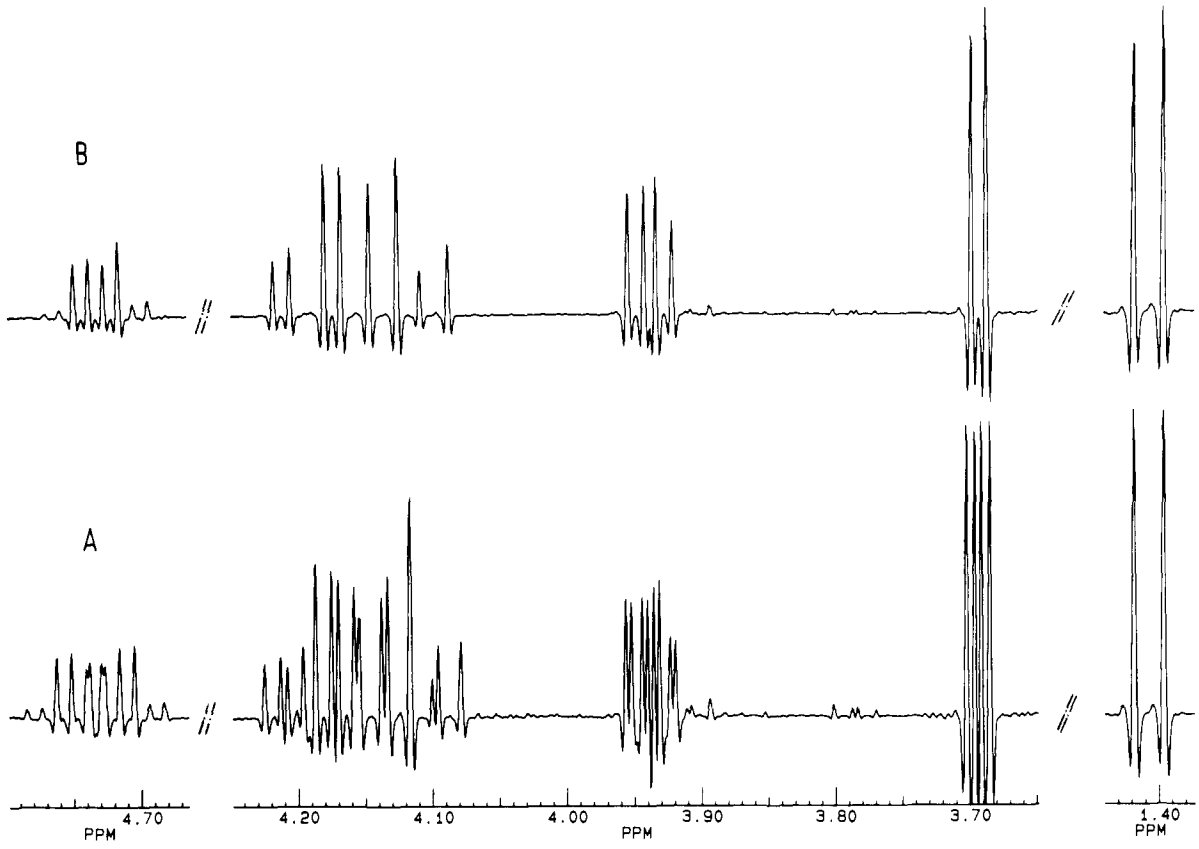

Figure 1. A. ^1H NMR spectrum of fully deprotected seryl threonyl phosphate **6**. B. $^1H\{^{31}P\}$ NMR spectrum of **6**.

Scheme I

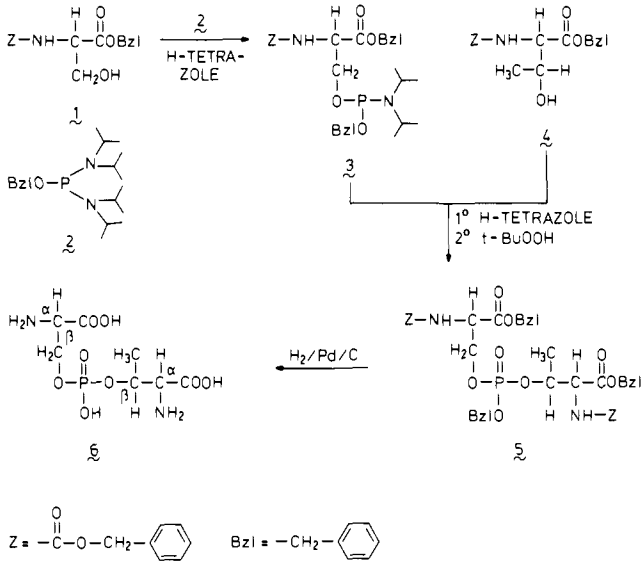

Table I. ^1H NMR Data of Compound **6** (Na^+ Salt, 0.16 mM in D_2O, pD 7.1)[15]

unit	δ values (ppm)	coupling constants (Hz)
		Seryl
Hα	3.95	$J_{H\alpha,P}$ = 1.2, $J_{H\alpha,H\beta(a)}$ = 6.3, $J_{H\alpha,H\beta(b)}$ = 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	$J_{H\beta(b),P}$ = 5.1
		Threonyl
Hα	3.70	$J_{H\alpha,P}$ = 1.9, $J_{H\alpha,H\beta}$ = 3.3
Hβ	4.74	$J_{H\beta,P}$ = 7.4, $J_{H\beta,CH_3}$ = 6.6
CH_3	1.42	

the rate of the elimination reaction.

In conclusion, the NMR data and virtually inertness of compound **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.

Acknowledgment. We thank Prof. Dr. D. E. Edmondson for his critical remarks and helpful discussions.

Alkoxyl Radicals in 1,5-Hydrogen Shifts for Site-Specific, Stereocontrolled Alkylation of Carbohydrates[1]

J. Cristobal Lopez,[2] Ricardo Alonso,[3] and Bert Fraser-Reid*

Department of Chemistry
Paul M. Gross Chemical Laboratory
Duke University, Durham, North Carolina 27706

Received March 13, 1989

Two areas of continued interest in our laboratory in developing the organic chemistry of carbohydrates are concerned with enhancing stereoselectivity at (normally) "off-template" sites[4] and with the application of radical processes for mild transforma-

(1) This work was supported by a grant from the National Science Foundation (Grant CHE 8703916).

(2) Holder of Research Fellowship from the Consejo Superior de Investigaciones Cientificas of Spain.

(3) Research Associate from the Universidad de Santiago de Compostela (Spain); acknowledges a fellowship from the Ministerio de Educacion y Ciencia (Spain).

(4) (a) Fraser-Reid, B.; Magdzinski, L.; Molino, B. *J. Am. Chem. Soc.* **1984**, *106*, 731. (b) Magdzinski, L.; Cweiber, B.; Fraser-Reid, B. *Tetrahedron Lett.* **1983**, *24*, 5823. (c) Molino, B. F.; Magdzinski, L.; Fraser-Reid, B. *Tetrahedron Lett.* **1983**, *24*, 5819.

0002-7863/89/1511-6471$01.50/0