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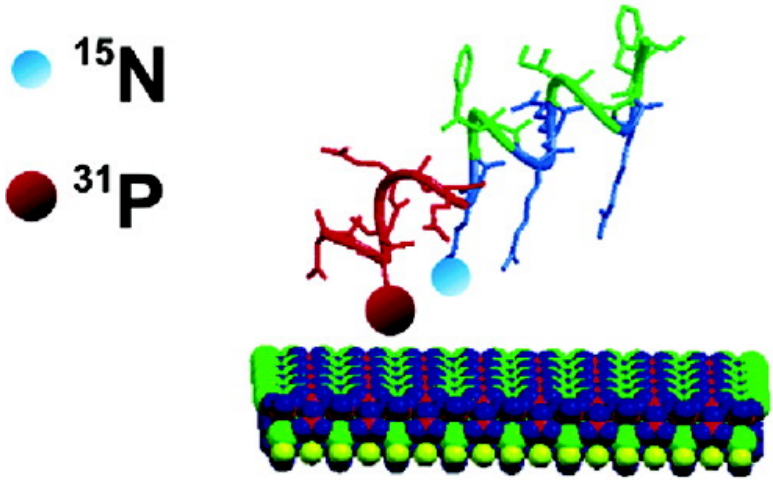

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A REDOR NMR Study of a Phosphorylated Statherin Fragment Bound to Hydroxyapatite Crystals

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Acidic proteins found in mineralized tissues act as nature's crystal engineers, where they play a key role in promoting or inhibiting the growth of minerals such as hydroxyapatite (HAP), $Ca_{10}(PO_4)_6(OH)_2$, the main mineral component of bone and teeth. There is remarkably little known about the protein structure−function relationships and the recognition processes governing hard tissue engineering. It is well-known that several salivary proteins (statherin) and peptides (SN-15, N-terminal 15 amino fragment of statherin) bind strongly to HAP to regulate crystal growth.[1] In this work, we describe how solid-state NMR can be used to identify which amino acid side chains of SN-15 (DpSpSEE^{15N}KFLRRIGRFG) interact with the HAP surface, even in the presence of phosphorylated side chains. Prior structural studies have indicated that the second through twelfth amino acids are α-helical in full length statherin on HAP, while the SN-15 fragment is in an extended structure toward the N-terminus, only gaining α-helical structure at the seventh amino acid. Additionally, prior dynamics studies have indicated that the region from the seventh amino acid to the C-terminus interacts less strongly with the HAP surface than the first six amino acids.[2]

Our NMR strategy is based on ^{15}N{^{31}P} rotational-echo double-resonance (REDOR),[3] whose pulse sequence is shown in Figure 1. ^{15}N and/or ^{13}C labels are placed on selected side chains of amino acids believed to be exposed to the HAP surface. ^{15}N{^{31}P} REDOR is used to detect dipolar couplings between ^{15}N spins in amino acid side chains and ^{31}P spins in the HAP surface. A possible complication is the presence of ^{31}P spins in the phosphoserine (pS) side chains, which if close enough to the targeted amino acid side chain may couple to the ^{15}N spin and complicate analysis. As we traverse the peptide primary sequence, the distances from the side chains to the HAP surface can be measured, and a quantitative picture of statherin−HAP interactions can be obtained. Locations for isotopic enrichment on amino acid side chains are guided by binding models and prior NMR data. It has been proposed that pS chelates to the calcium in HAP, based on ^{31}P NMR experiments.[1a] It is also known that lysine and arginine side chains are capable of hydrogen bonding to phosphate groups in DNA and RNA molecules and inorganic phosphates,[4] suggesting an additional binding mechanism that may occur.

Here, we present the first solid-state NMR study that accounts for multiple ^{31}P spins of the interaction between a selected amino acid side chain in the HAP binding domain of a phosphorylated biomineralization protein and the phosphate groups of the native mineral surface. For initial study, we select the unique lysine in SN-15 and statherin (i.e., K6). K6 is somewhat removed from the phosphate groups of pS2 and pS3 in the primary structure, while still closely associated with the acidic HAP binding region. The proximity of phosphorylated protein side chains to the lysine side chain results in a more complex analysis than that described in a recent article.[5]

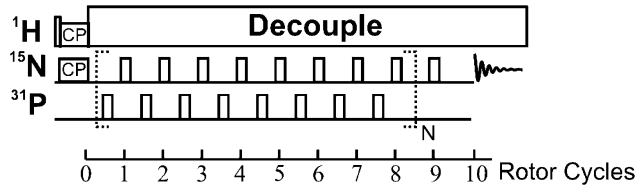

Figure 1. The version of REDOR with alternating π-pulses and XY-8 phase cycling on both channels was used.

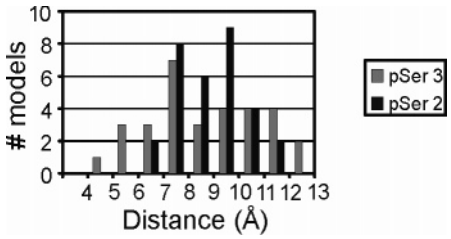

Figure 2. Statistical models derived from the Rosetta program suggest that it is unlikely for the pS side chains and the K6 side chain to be near each other in unbound SN-15.

To investigate the likely proximity of a pS side chain to the K6 side chain, statistical analysis was performed on a cluster of 31 structures predicted using de novo energy minimization techniques from the Rosetta program.[6] The structures reproduced the essential conformational features as deduced from earlier NMR experiments. All structures showed a distance greater than 4 Å between the K6 ^{15}N and the pS3 oxygen in an unphosphorylated SN-15 sample, as demonstrated in Figure 2, and it is therefore unlikely that a pS side chain is near a K side chain. In addition, we chose to perform REDOR on the unbound SN-15 sample, the results of which are shown in Figure 3a. The best fit simulation involves a bimodal distribution where 80% of the ^{15}N spins from the K6 side chain are not coupled to a ^{31}P spin from a pS side chain, while 20% of the ^{15}N spins from the K6 side chain are about 3.2 Å from a ^{31}P spin on a pS side chain, resulting in a dipolar coupling of 150 Hz. Due to the statistical analysis, we believe this is more likely due to interpeptide interactions, but we will still allow for the possibility of intrapeptide interactions in the analysis of the bound peptide.

The SN-15 peptide was bound to HAP, and REDOR was performed on both hydrated (and frozen at −40 °C) and lyophilized versions of the sample. The REDOR results for these experiments are shown in Figure 3b. The two samples have results that are not statistically different for the REDOR experiment performed.

In the case of the unbound sample, a simple bimodal distribution was utilized where a significant fraction of the labeled spins were uncoupled, and the remaining fraction was fit to a well-known analytical function for a spin pair.[7] However, in the case of the bound SN-15 sample we want to consider cases beyond a spin pair.

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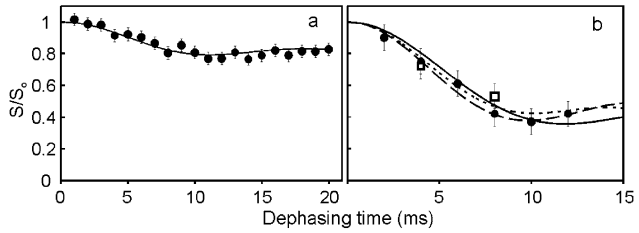

Figure 3. $^{15}N\{^{31}P\}$ REDOR was performed on an (a) unbound SN-15 sample to assess the likelihood of interference from the phosphoserine side chain. A best fit scenario with 80% of the K6 ^{15}N spins away from the ^{31}P spins and 20% of the K6 ^{15}N spins 3.2 Å from a pS ^{31}P spin fit the data. $^{15}N\{^{31}P\}$ REDOR was also performed an a (b) SN-15 sample bound to HAP. (●) Experimental data from the lyophilized SN-15 bound to HAP; (□) experimental data from the hydrated sample. Three possible models that fit our REDOR data are shown. The solid line represents a system where the K6 ^{15}N is 3.7 Å from one ^{31}P spin and 4.4 Å from a second with the two ^{31}P spins being 2.2 Å apart. The long dashed line represents the system where a bimodal distribution exists: 40% of the ^{15}N K6 spin remain uncoupled and 60% are 3.1 Å from a ^{31}P spin. The short dashed line represents a system that is nearly linear in its geometry with $^{15}N-^{31}P$ distances of 3.7 and 4.0 Å.

REDOR on systems with multiple dephasing nuclei has been shown to fit a geometrically dependent integral in the absence of large interactions other than heteronuclear coupling.[8] Due to the complex phosphate network of the HAP system and the pS side chains, we must consider the potential effect of the homonuclear $^{31}P-^{31}P$ couplings. Several articles have addressed the issues of strong homonuclear couplings on the observed nucleus in a REDOR experiment[9] in the form of a SEDRA[10] effect. Few articles, however, have discussed in detail a homonuclear coupling effect on the dephasing channel. A recent article by Goetz and Schaefer addresses homonuclear coupling of the dephasing nuclei, but it is experimentally limited to the case of dephasing of a single ^{13}C spin by a trifluoromethyl group, the rotational motion of which equalizes the three heteronuclear couplings and chemical shifts, thereby removing the effect of homonuclear couplings from the REDOR data.[8a] We have used numerical simulations with SIMPSON[11] to analyze how REDOR is affected by homonuclear coupling of the dephasing nuclei in cases where structural symmetry and/or symmetrical motions are absent. Briefly, a significant amount of homonuclear coupling will lessen the apparent dephasing effect of the REDOR experiment.

We consider three models that all result in agreement with the experimental data. The first model we consider is the bimodal distribution, similar to that found for the unbound peptide. In this case, the best fit results in 40% of the K6 ^{15}N spins remaining uncoupled and 60% of the K6 spins coupling to a ^{31}P spin at 175 Hz, a distance of 3.1 Å. The large distributional change from 20% coupled spins to 60% coupled spins is unlikely, unless the distribution involves coupling to surface phosphate groups.

The second model considered is a near-linear geometry where the K6 ^{15}N is coupled at 100 Hz to one ^{31}P and at 80 Hz to a second ^{31}P, resulting in distances of 3.7 and 4.0 Å, respectively, with a P−N−P angle of 170°. In this model, it is possible that the K6 ^{15}N hydrogen bonds with two phosphate networks. It is not possible for the K6 side chain to be between the two pS side chains, meaning that at least one phosphate group in this model must be from the HAP surface.

The final model we consider involves a multiple spin system with a strong $^{31}P-^{31}P$ homonuclear coupling. In this case, the data fit with N−P distances of 3.7 and 4.4 Å and a P−P distance of 2.2 Å. Given the constraints of HAP crystal geometry, this scenario is unlikely and probably the result of using too small a $^{31}P-^{31}P$ coupling network. Other scenarios with weaker homonuclear couplings and more ^{31}P dephasing spins were also considered, although a unique solution is not easily obtainable. Because the ^{31}P spins of the phosphoserine side chains in the extended structure of SN-15 are at least 8 Å away from each other, we know this scenario also involves at least one HAP ^{31}P spin and likely more HAP ^{31}P spins because a larger $^{31}P-^{31}P$ coupling network is necessary to simulate accurately the HAP crystal geometry.

All three scenarios require at least one ^{31}P spin from the HAP surface, and we therefore conclude that the K6 side chain interacts with the HAP surface. Further studies of SN-15 interactions with HAP are ongoing.

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Supporting Information Available: Procedures for preparing the samples and performing the NMR experiments and a sample NMR spectrum. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Naganagowda, G. A.; Gururaja, T. L.; Levine, M. J. *J. Biomol. Struct. Dyn.* **1998**, *16*, 91−107. (b) Schlesinger, D. H.; Hay, D. I. *J. Biol. Chem.* **1977**, *252*, 1689−1695. (c) Raj, P. A.; Johnsson, M.; Levine, M. J.; Nancollas, G. H. *J. Biol. Chem.* **1992**, *267*, 5968−5976. (d) Schwartz, S. S.; Hay. D. I.; Schlukebier, S. K. *Calcif. Tissue Int.* **1992**, *50*, 511−517. (e) Ramasubbu, N.; Thomas, L. M.; Bhandary, K. K.; Levine, M. J. *Crit. Rev. Oral Biol. Med.* **1993**, *4*, 363−370. (f) Gururaja, T. L.; Levine, M. J. *Pept. Res.* **1996**, *9*, 283−289.

(2) (a) Long, J. R.; Dindot, J. L.; Zebroski, H.; Kiihne, S.; Clark, R. H.; Campbell, A. A.; Stayton, P. S.; Drobny, G. P. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12083−12087. (b) Long, J. R.; Shaw, W. J.; Stayton, P. S.; Drobny, G. P. *Biochemistry* **2001**, *40*, 15451−15455. (c) Shaw, W. J.; Long, J. R.; Dindot, J. L.; Campbell, A. A.; Stayton, P. S.; Drobny, G. P. *J. Am. Chem. Soc.* **2000** *122*, 1709−1716. (d) Shaw, W. J.; Long, J. R.; Dindot, J. L.; Campbell, A. A.; Stayton, P. S.; Drobny, G. P. *J. Am. Chem Soc.* **2000**, *122*, 7118−7119.

(3) (a) Gullion, T.; Schaefer, J. *J. Magn. Reson.* **1989** *81*, 196−200. (b) Gullion T.; Schaefer, J. *Adv. Magn. Reson.* **1989**, *13*, 57.

(4) (a) Nadassy, K.; Wodak, S. J.; Janin, J. *Biochemistry* **1999**, *38*, 1999−2017. (b) Burget, U.; Zundel, G. *J. Mol. Struct.* **1986** *145*, 93−109.

(5) Shaw, W. J.; Campbell, A. A.; Paine, M. L.; Snead, M. L. *J. Biol. Chem.* **2004**, *279*, 40263−40266.

(6) Rohl, C. A.; Strauss, C. E. M.; Misura, K. M. S.; Baker, D. *Methods Enzymol.* **2004**, *383*, 66−93.

(7) (a) Mueller, K. T.; Jarvie T. P.; Aurentz, D. J.; Roberts, B. W. *Chem. Phys. Lett.* **1995**, *242*, 535−542. (b) Mueller, K. T.; Jarvie T. P.; Aurentz, D. J.; Roberts, B. W. *Chem. Phys. Lett.* **1995**, *254*, 281−282.

(8) (a) Goetz, J. M.; Schaefer, J. *J. Magn. Reson.* **1997**, *127*, 147−154. (b) Arshava, B.; Breslav, M.; Antohi, O.; Stark, R. E.; Garbow, J. R.; Becker, J. M.; Naider, F. *Solid State Nucl. Magn. Reson.* **1999**, *14*, 117−136. (c) Fyfe, C. A.; Lewis, A. R. *J. Phys. Chem. B* **2000**, *104*, 48−55. (d) Vogt, F. G.; Gibson, J. M.; Mattingly, S. M.; Mueller, K. T. *J. Phys. Chem. B* **2003** *107*, 1272−1283. (e) Mueller, L. J.; Elliott, D. W. *J. Chem. Phys.* **2003,** *118*, 8873−8881.

(9) (a) Schaefer, J. *J. Magn. Reson.* **1999**, *137*, 272−275. (b) Jaroniec, C. P.; Tongue, B. A.; Herzfeld, J.; Griffin, R. G. *J Am. Chem Soc.* **2001**, *123*, 3507−3519. (c) Jaroniec, C. P.; Tongue, B. A.; Rienstra, C. M.; Herzfeld, J.; Griffin, R. G. *J. Am. Chem. Soc.* **1999**, *121*, 10237−10238. (d) Mehta, A. K.; Segelski, L.; O'Connor, R. D.; Schaefer, J. *J. Magn. Reson.* **2003**, *163*, 182−187.

(10) Gullion, T.; Vega, S. *Chem. Phys. Lett.* **1992**, *194*, 423−428.

(11) Bak, M.; Rasmussen, J. P.; Nielsen, N. C. *J. Magn. Reson.* **2000**, *147*, 296−330.

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