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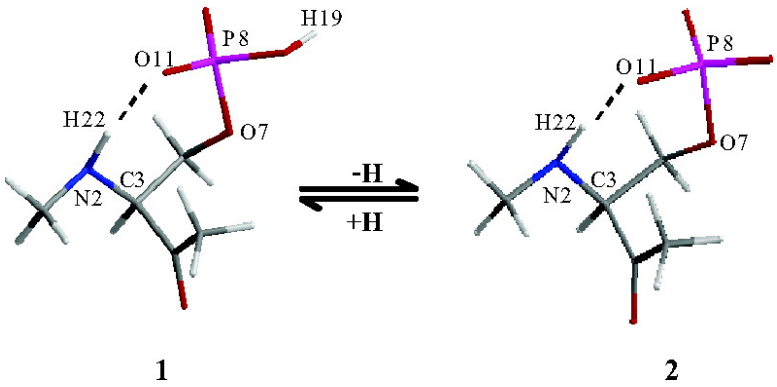

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Low-Barrier Hydrogen Bond between Phosphate and the Amide Group in Phosphopeptide

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Enzymatic catalysis is commonly attributed to the tighter binding between an enzyme and some given reactants in their transition state (TS) than in the initial enzyme−substrate complex. For a stepwise mechanism with only one intermediate (similar to most conventional transition state structures), the intermediate should be more tightly bound than the original unreacted system.[1,2] A short and strong hydrogen bond with lower potential barrier was proposed to contribute 10−20 kcal·mol^{-1} to the TS stabilization energy in enzymatic catalysis. The proposal invokes a large increase in hydrogen bond energy when the pK_a values of the donor and acceptor (where K_a is the acid constant) match in the transition state ($\Delta pK_a = 0$).[3] Usually, the effects of media are of importance because the value of pK_a depends on the solvent sensitively.[2] Under appropriate conditions, the potential energy barrier between the two possible states of a hydrogen bond, in which the hydrogen atom is associated with either one of the donor and acceptor atoms, is lowered so that it creates a single well potential energy system.[4]

As is known, the activity of an enzyme can be modulated by the post-translational covalent modifications. Reversible phosphorylation and dephosphorylation on serine and threonine side chains are a feasible measure commonly used for the purpose. These reactions are catalyzed by the protein Ser/Thr kinases and phosphatases, respectively.[5,6] Modifications such as these have been confirmed to be particularly effective in modulating the activity of the proteins involved in cell cycle regulation, glucose metabolism, and signal transduction.[7] Structural analyses of kinase substrate complexes had provided much insight into the recognition of protein substrates and the mechanism of phosphoryl transfer, yet relatively little is known about how phosphorylation modulates protein structure, which in turn results in the alteration of biological activity.[8] Several models were proposed to explain how phosphorylations of Ser/Thr side chains regulate the functions of a protein. The formation of hydrogen bonds was proposed to explain the effects of phosphorylation.[9−11]

The interest of the present work lies in whether the protonation and deprotonation of the phosphate mentioned above modify the hydrogen bond strength. To answer this question, we have synthesized several peptides and phosphopeptides, including human Tau441 sequence, Tau-(256−273), VKSKIGSTENLKHQPGGG, pTau-(256−273), VKSKIGpSTENLKHQPGGG, Tau-(350−367), VQSKIGSLDNITHVPGGG, pTau-(350−367), VQSKIGpSLDNITHVPGGG, and characterized them with ^{1}H NMR and ^{31}P NMR, respectively. Ser^{262} and Ser^{356} are two of the most likely abnormal phosphorylation sites in the tau protein.[12] In the ^{1}H NMR spectrum, a hydrogen bond is detected between the phosphate group and the amide group of the phosphorylated serine residue. A considerable increment in hydrogen bond strength has been found when the phosphate group changed from its monoionic form (protonated) to its diionic form (deprotonated). Furthermore, a low potential barrier hydrogen bond is found only in the diionic form system. It is suggested that strengthening of hydrogen bonds via the deprotonation of the serine phosphate can substantially enhance catalysis.

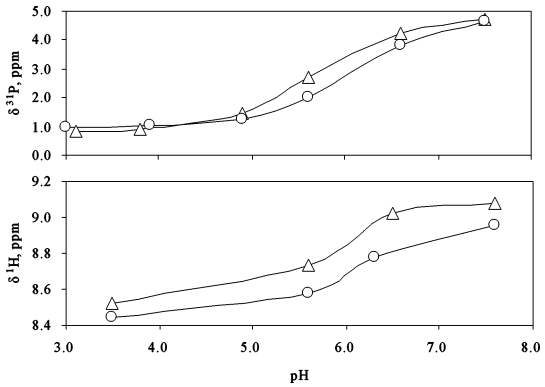

Figure 1. Changes with δ ^{31}P NMR of the phosphate group and δ ^{1}H NMR of amide protons (in Ser^{262} or Ser^{356}) during the pH titration of Tau peptides: pTau-(256−273) (△) and pTau-(350−367) (○).

The ^{1}H NMR spectra were acquired on a Varian Inova-600 spectrometer operating at 599.83 MHz, while the ^{31}P NMR spectra were obtained on a Bruker ACP200 spectrometer with 85% phosphoric acid as the external reference. Sequence-specific resonance assignments were done mainly by using the TOCSY and NOESY spectra. Chemical shift changes were used to identify Ser^{262} and Ser^{356} as the phosphorylated residues and may reflect both the intrinsic electron-withdrawing effects and the hydrogen bonds formed between the pSer^{262} and pSer^{356} amide groups and their respective phosphate groups.[10,12,13]

To better understand the effects of phosphorylation on the local structures of the phosphopeptides, both the ^{1}H and ^{31}P NMR spectra of Tau peptides were obtained over a wide range of pH $\approx$ 3−8. The pK_a values of the phosphate groups for the equilibrium between the monoionic and diionic forms were obtained from the changes in the phosphate chemical shift (Figure 1). Titration of the phosphorylated Tau peptides caused large downfield shifts in the amide proton signals of Ser^{262} and Ser^{356} as the pH increased through the phosphoserine pK_a (Figure 1). In contrast, the amide proton resonances of Ser^{262} and Ser^{356} changed slightly in the nonphosphorylated peptides. Interestingly, through methyl esterification of the phosphate, the corresponding chemical shifts of the amide proton and phosphorus in the phosphopeptides did not change with pH titration.

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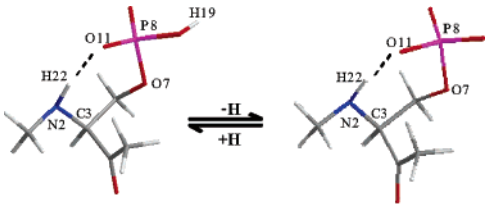

Figure 2. One of the possible regulatory mechanisms of phosphorylation in protein. The equilibrium geometries of the model molecule in monoionic (**1**) and diionic (**2**) form were predicted by MP2/6-31+G(d,p) calculations. The numerals after each element symbol denote the serial numbers of the atoms.

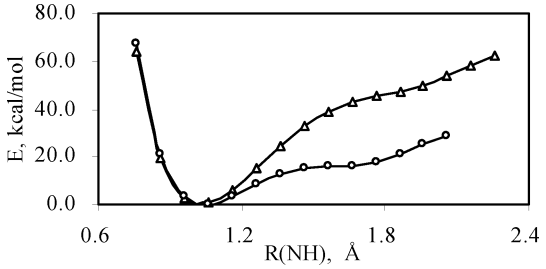

Figure 3. Relative adiabatic potential, *E*, for the proton movement for monoionic **1** (△) and diionic **2** (○) at the MP2/6-31+G(d,p) level.

Ab initio calculations were carried out at the MP2/6-31+G(d,p) level by using the Gaussian 03 program[14] to obtain detailed information of the hydrogen bond energy.[15] To reduce computational time, two model molecules of monoionic (**1**) and diionic form (**2**), as shown in Figure 2, were employed to simulate the compounds pTau-(256−273) and pTau-(350−367), respectively. The initial geometries of the model molecules were constructed according to the NMR data.[16−18] Then full geometry optimizations were performed to obtain the equilibrium geometries of these molecules.

It can be seen that the model molecules **1** and **2** each has an intramolecular hydrogen bond (denoted by dash line), N2−H22···O11. A relatively shorter distance (2.74 Å) between N2 and O11 in molecule **2** than that in molecule **1** (2.92 Å) indicates the formation of a stronger hydrogen bond upon conversion of the monoionic form to the diionic form of the phosphate.

To evaluate the change of the adiabatic potential along with the proton movement in the intramolecular hydrogen bond, local potential surface scan was done with the two model molecules. The scan was performed with the distance of N2−H22 extended step-by-step. In each step, the angle ∠C−N−H and dihedral angle ∠C−C−N−H angle were partially optimized. Such an approach seems to be reasonable in obtaining the potential for a fast transfer of proton.[19] Figure 3 shows the energy profile for proton movement within the hydrogen bridge for the model molecules **1** and **2**. The effect of shortening of the hydrogen bond upon the deprotonation appears to be clear from comparison of the potentials.

In conclusion, through NMR chemical shifts and theoretical calculations, we have observed change in the length and strength of hydrogen bonds between a serine and a phosphate group in several peptides as the phosphate ionizes. We propose that the low-barrier hydrogen bonds may play a role in the control of enzymatic activities. Increasing the pH and subsequently deprotonating the phosphate led to substantial shortening of the hydrogen bond length and a change of the spectroscopic behavior of such systems. It is especially interesting that the phosphate may exist in the intermediate region of enzyme−substrate,[20] where the low potential barrier for proton transfer within hydrogen bonds is anticipated. This reversible protonation of the phosphate group, which changes both the electrostatic properties of the phosphate group and the strength of the hydrogen bond, provides a possible mechanism in regulating protein function.

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Supporting Information Available: NMR spectra, chemical shift assignments, geometries, Cartesian matrixes of the optimized structures, and complete refs 12 and 14. This material is available free of charge via the Internet at http://pubs.acs.org.

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