(NO)($CH_2SiMe_3$)$_2$.[14] Thus, it forms simple 1:1 adducts with both typical Lewis acids and bases. The notable exception is its behavior upon treatment with water, the subject of this communication. Interestingly, if CpW(NO)(*o*-tolyl)$_2$ is treated with $^{18}OH_2$, the label is incorporated only into **2**. It thus appears that **1** and **2** are formed from CpW(NO)(*o*-tolyl)$_2$ via independent pathways. To date we have yet to find other experimental conditions for the conversion of CpW(NO)(*o*-tolyl)$_2$ to its isomer **1**.[15]

In addition to its unprecedented manner of formation via N–O bond cleavage, **1** is also the first cyclopentadienyl imido oxo complex to have been isolated.[16] It is analogous to the well-known dioxo and alkylidene oxo complexes, CpW(O)$_2$R and CpW(O)(CHR′)R, respectively.[3a] Furthermore, the availability of **1** also affords us, in principle, the opportunity to effect chemistry on a new chiral molecule containing two different types of multiple metal–ligand bonds. Studies directed toward this goal are currently in progress.

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**Supplementary Material Available:** Tables of fractional coordinates and anisotropic thermal parameters for CpW(O)(N-*o*-tolyl)(*o*-tolyl) (**1**) (3 pages). Ordering information is given on any current masthead page.

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## Proton Exchange with Internal Water Molecules in the Protein BPTI in Aqueous Solution

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A high-resolution protein crystal structure usually includes the positions of numerous hydration water molecules. The Brookhaven protein data bank[2] thus includes the locations of over 30 000 water oxygen atoms. The large majority of these water sites form a hydration shell that covers large parts of the molecular surface. In addition, a smaller number of water molecules can be located in the interior of a protein and represent an integral part of the molecular architecture. Using high-resolution NMR[1] experiments we have started investigations of protein hydration in aqueous solution.[3] In 2D [$^1$H,$^1$H]-NOESY and 3D $^{15}$N-correlated [$^1$H,$^1$H]-NOESY experiments and the corresponding measurements in the rotating frame, NOE cross peaks between protein protons and protons of interior water molecules could be identified in several small proteins.[3–5] In contrast, the NOEs expected to arise from close proximity of surface hydration waters to the protein were, as a rule, not observed. Similar observations were recently reported for interleukin 1$\beta$.[6] These data imply that the NOEs between protein protons and surface hydration water are quenched because the effective correlation time for positional rearrangement of the water protons relative to the protein surface, which is determined either by chemical exchange or by independent rotational motions of the water molecules, is much shorter than the rotational correlation time of the protein. For interior water molecules a *lower limit* of 0.3 ns for the lifetime of the protons with respect to exchange with the bulk water was derived from the fact that the observed NOEs have a negative sign.[3] In the present note we report new experiments with paramagnetic shift reagents, which provide an *upper limit* for this lifetime, and thus contribute a further fundamental detail toward a precise characterization of protein hydration in aqueous solution.

(1) Abbreviations used: NMR, nuclear magnetic resonance; 2D, two-dimensional; 3D, three-dimensional; NOESY, two-dimensional nuclear Overhauser enhancement spectroscopy; NOE, nuclear Overhauser enhancement; BPTI, basic pancreatic trypsin inhibitor.
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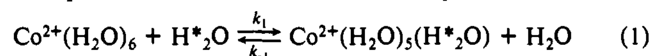
In all the aforementioned experiments[3–6] it was observed that the chemical shifts of interior water protons manifested in the NOE cross peaks with polypeptide protons are identical with that of the bulk water. This could be rationalized by two limiting situations: (i) The water chemical shifts in the protein interior and the bulk water are indeed identical. (ii) The interior water protons experience *conformation-dependent shifts* similar to those observed for the protons of polypeptide chains in globular proteins;[7] the presence of separate $^1$H NMR signals for the interior water molecules would then have to be concealed by proton exchange or exchange of intact water molecules. Because it is not known whether there is indeed a chemical shift difference between interior hydration water and bulk water, a proper distinction between the limiting situations i and ii was so far not possible. In the experiment described in this note a defined chemical shift difference between bulk water and "inaccessible" interior water molecules was therefore established by addition of an extrinsic paramagnetic shift reagent.

In the protein BPTI four internal waters were previously identified in three different crystal structures[8] and by NMR in solution.[3] After addition of $CoCl_2$ to an aqueous solution of BPTI, the equilibria 1 and 2 are of interest in the present context. H*



$$Co^{2+}(H_2O)_6 + H^*_2O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Co^{2+}(H_2O)_5(H^*_2O) + H_2O \qquad (1)$$

$$BPTI(H_2O)_4 + H'_2O \underset{k_{-m}}{\overset{k_m}{\rightleftharpoons}} BPTI(H_2O)_3(H'_2O) + H_2O \qquad (2)$$

and H′ identify bulk water protons that are exchanged into binding sites of $Co^{2+}$, or interior sites in the protein, respectively. $H^*_2O$ and $H'_2O$ are bound water molecules that contain one or two protons from the bulk water. The water molecules bound to $Co^{2+}$ experience large chemical shifts and some broadening of the $^1$H resonance lines due to the interactions with the unpaired electrons. It is known that the exchange $k_1$ is sufficiently fast for these paramagnetic effects to be averaged over the bulk water[9] and that a 30 mM concentration of $Co^{2+}$ should cause a $^1$H shift for the bulk water of approximately $\omega = 900\ s^{-1}$ at 600 MHz and 20 °C.[10]

Our measurements confirmed that the bulk water shift caused by addition of 30 mM $CoCl_2$ is approximately 0.25 ppm (arrows along $\omega_1$ in Figure 1, parts A and B). Under these conditions a line broadening of 130 Hz for the bulk water is observed along $\omega_1$ in Figure 1B, which is sufficiently small to allow observation of most of the previously identified NOEs between protons of the polypeptide chain of BPTI and the four interior water molecules (Figure 1B).[3] The NOESY cross peaks between different polypeptide protons of BPTI are virtually identical in parts A and B of Figure 1, which shows that the conformation of BPTI remained unchanged after addition of $CoCl_2$. The experiment of Figure

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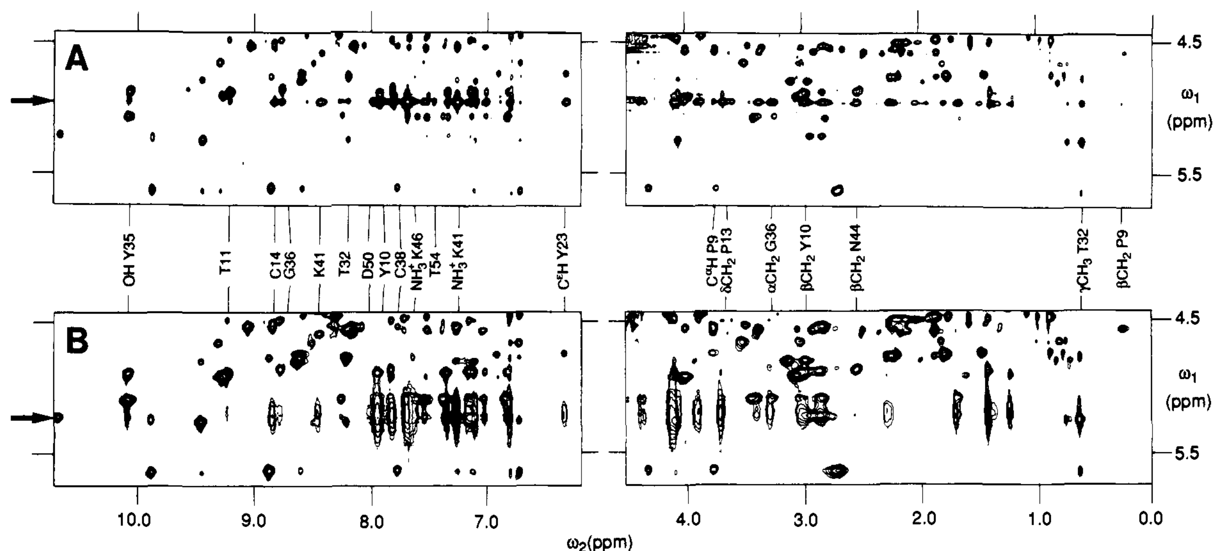


**Figure 1.** Spectral regions ($\omega_1$ = 4.4–5.8 ppm, $\omega_2$ = 0.0–4.5 and 6.2–10.2 ppm) of two homonuclear 2D [$^1$H,$^1$H]-NOESY spectra recorded at 4 °C with a 20 mM solution of BPTI in 90% $H_2O$/10% $D_2O$, pH 3.5, with and without addition of the shift reagent $CoCl_2$, using the experiment described in ref 3 ($^1$H frequency = 600 MHz, $t_{1max}$ = 60 ms, $t_{2max}$ = 150 ms, sweep width in $\omega_1$ and $\omega_2$ 6944 Hz, total measuring time 12 h). The spectra were base-line-corrected in both dimensions, using polynominals. The arrows on the left indicate the $\omega_1$ frequency of the water signal. The contour levels were plotted on an exponentially increasing scale, where each level is $2^{1/2}$ times higher than the preceding one. (A) Without $CoCl_2$, mixing time = 112 ms. (B) With 30 mM $CoCl_2$, mixing time = 90 ms. Lower contour levels are plotted than in A. In the center the cross peaks with the water resonance are identified with the one-letter amino acid symbol, the sequence position, and except for the backbone amide protons, the proton positions in the amino acid residues.[3] Some of the signals seen in A are below the lowest plot level in B because of the line broadening.

1 presents unambiguous evidence that the apparent $^1$H chemical shift equivalence between bulk water and interior water molecules is related to the chemical exchange reaction described by eq 2. For the reaction rate a lower limit of $k_m > 50$ s$^{-1}$ was established from the following considerations. Although the observations in Figure 1 would be compatible with the assumption that the resonance lines of the bulk water and the interior water are coalesced or that the resonances of the interior waters are separated from that of the bulk water but exchange-broadened beyond detection (this would be the case for $k_m > 10^3$ s$^{-1}$), the above, more conservative estimate for $k_m$ is obtained assuming that the resonance lines of the interior waters are resolved, but lie within 0.4 ppm of the bulk water resonance and thus are concealed by the water suppression technique used.[3] In this situation, an exchange rate of $k_m > 50$ s$^{-1}$ would be sufficient to quench the cross peaks with the protein signals, since the magnetization would be rapidly transferred from the interior waters to the bulk water during the NOESY mixing time. The efficient relay of magnetization from the bulk water to the hydration sites would then also account for the cross peaks observed between bulk water and protein protons. Combined with the previously estimated lower limit,[3] we then have that, at 4 °C, 20 ms > $\tau_m$ > 0.3 ns. The available evidence[3-6] supports that these measurements with BPTI present a realistic guideline also for interior hydration of other globular proteins.

Because the experiment of Figure 1 provides direct information only on the upper limit for $\tau_m$ of the *protons* of interior water molecules, we performed similar experiments also with $^{17}$O NMR, using 10% $^{17}$O-enriched water as the solvent and $CoCl_2$ and $DyCl_3$ as shift reagents. In contrast to similar experiments with slowly exchanging water molecules bound to diamagnetic metal ions,[11] no separate $^{17}$O line of BPTI-bound water could be observed. This result would be consistent with the view that the limiting value of $\tau_m$ < 20 ms for the lifetime of the interior hydration water in BPTI is valid not only for the water protons but also for the entire water molecules. It is not clear, however, that this conclusion is warranted, since there is the alternative explanation that the $^{17}$O signal of protein-bound water could be broadened beyond detection due to the efficient nuclear quadrupole relaxation in the slow motional regime, which has not been properly excluded. In this context it should be recalled that, in earlier experiments with BPTI using gel filtration techniques with $^{18}$O-enriched water, the $^{18}$O exchange was complete within the deadtime of the experiment, which was 10 s.[12]

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## Spectroelectrochemical Study of the $C_{60}$ and $C_{70}$ Fullerenes and Their Mono-, Di-, Tri-, and Tetraanions

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The recent synthesis of macroscopic amounts of the third allotropic form of carbon, $C_{60}$ or buckminsterfullerene,[1] has prompted a flurry of research into its structure, properties, and reactivity.[2-8] Some of us reported the first electrochemical study of $C_{60}$ which characterized the $C_{60}^-$ and $C_{60}^{2-}$ buckide anions in $CH_2Cl_2$ by cyclic voltammetry.[9] Subsequently, an electrochemical

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