Communication

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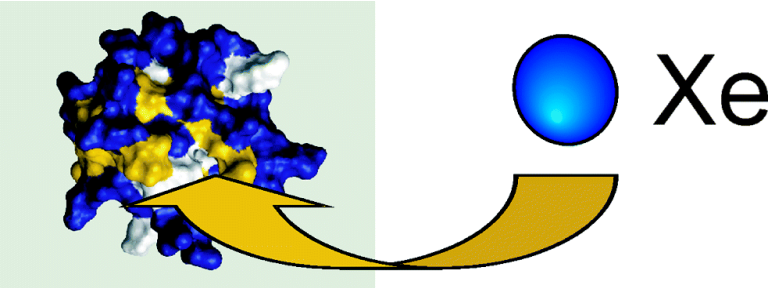

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NMR-Spectroscopic Mapping of an Engineered Cavity in the I14A Mutant of HPr from *Staphylococcus carnosus* Using Xenon

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Xenon binding into preexisting cavities in proteins was first detected by X-ray crystallography.[1,2] Single crystals of native sperm whale myoglobin and horse hemoglobin were subjected to xenon at moderate pressures. Different sites capable of binding noble gases such as xenon or krypton have been discovered[3−5] in a variety of proteins since then. The binding of xenon, argon, krypton, and neon into so-called engineered cavities of phage T4 lysozyme mutants was studied by X-ray crystallography.[6] Apart from specific binding into preexisting cavities, xenon also exhibits nonspecific interactions with the protein surface. NMR spectroscopy, in principle, offers different opportunities for the study of the interaction between xenon atoms and protein molecules in aqueous solution. On one hand, the influence of this interaction upon the chemical shift of xenon can be studied by ^{129}Xe NMR spectroscopy.[7−12] The use of laser-polarized ^{129}Xe greatly enhances the sensitivity of this method.[7] Another possibility is offered by the spin-polarization induced nuclear Overhauser effect[13] (SPINOE) resulting in a transfer of nuclear spin polarization from laser-polarized ^{129}Xe to nuclei located at the protein molecule. This effect could be used to identify the hydrophobic cavity of wheat nonspecific lipid transfer protein.[14] A further possibility is the observation of chemical shift changes of nuclei such as ^{1}H, ^{15}N, or ^{13}C induced by xenon atoms bound to the protein molecules. The existence of such chemical shift changes was described recently by Rubin et al. for the phage T4 lysozyme mutant L99A.[12] In the present contribution, we demonstrate the combined use of xenon-induced ^{1}H and ^{15}N NMR chemical shifts to map residues affected by the presence of a hydrophobic cavity. The cavity was artificially introduced into the histidine-containing phosphocarrier protein (HPr) from *Staphylococcus carnosus* via the replacement of isoleucine 14 by alanine (HPr(I14A)). I14 is a residue of special importance for HPr. High-pressure NMR studies[15] revealed an unusually small pressure response of the ^{1}H and ^{15}N NMR signals of I14. Its mutation to alanine results in a significantly reduced stability and a pronounced decrease of the phosphocarrier efficiency of the molecule without changing the general protein fold.[16] The complete spectral assignment as well as the solution NMR structures of wild-type HPr and HPr(I14A) are available.[16,17] Uniformly ^{15}N-enriched HPr was prepared following the procedure described by Görler et al.[17] The mutant HPr(I14A) was prepared and isolated analogously. A sapphire tube equipped with a titanium valve similar to the apparatus described by Roe[18] was used to dissolve a defined amount of xenon in the samples. After the required amount of xenon (natural abundance) was condensed into the tube, the valve was closed to maintain the desired xenon pressure of 1.4 MPa within the sample. The measurements were carried out on a DMX-500 spectrometer (Bruker, Karlsruhe, Germany) at a temperature of 295 K. The real sample temperature was calibrated by using the temperature-dependence of the ^{1}H NMR signals of methanol.[19] ^{1}H NMR chemical shifts were referenced to internal 2,2-dimethyl-2-silapentane sulfonic acid (DSS), while ^{15}N NMR chemical shifts were referenced indirectly.[20] Spectra processing, visualization, and peak picking was carried out using the computer programs XWINNMR (Bruker, Karlsruhe, Germany) and AUREMOL.[21] Figure 1 shows a selected region from the ^{1}H−^{15}N heteronuclear single-quantum coherence (HSQC) spectrum of HPr(I14A). As can be seen, for example, for amino acid residues A19 and T25, considerable signal shifts in the ^{1}H and ^{15}N dimension are induced by xenon for HPr(I14A).

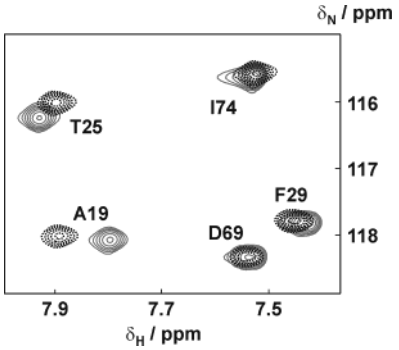

Figure 1. Selected region from the ^{1}H−^{15}N HSQC spectrum (contour plot) of HPr(I14A). The spectra measured for the xenon-free sample (dotted black lines) and for the sample pressurized with xenon (solid gray lines) are superimposed.

To quantitatively evaluate the shift of the $^{1}H^{N}$−^{15}N amide group cross-peaks in the ^{1}H−^{15}N HSQC spectra, a "total xenon-induced shift", Δ, is defined as $\Delta = [(w\Delta\delta_H)^2 + \Delta\delta_N^2]^{1/2}$. Here, $\Delta\delta_H$ and $\Delta\delta_N$ denote the induced chemical shifts measured in ppm in the ^{1}H and ^{15}N dimension, respectively (see Supporting Information). To account for the different sensitivity of $^{1}H^{N}$ and ^{15}N chemical shifts of the amide groups, a weight factor, w, is introduced which amounts to 7.3 and 7.7 for wild-type HPr and HPr(I14A), respectively. Figure 2 shows Δ as a function of the amino acid residue number for wild-type HPr (top) and HPr(I14A) (middle). For both samples, purely pressure-induced effects can be excluded. Such changes of the chemical shift[15] are smaller than 0.004 and 0.0004 ppm for ^{15}N and $^{1}H^{N}$, respectively, at the pressure of 1.4 MPa applied to the samples. The observed signal shifts are, therefore, due to the interaction between protein molecules and

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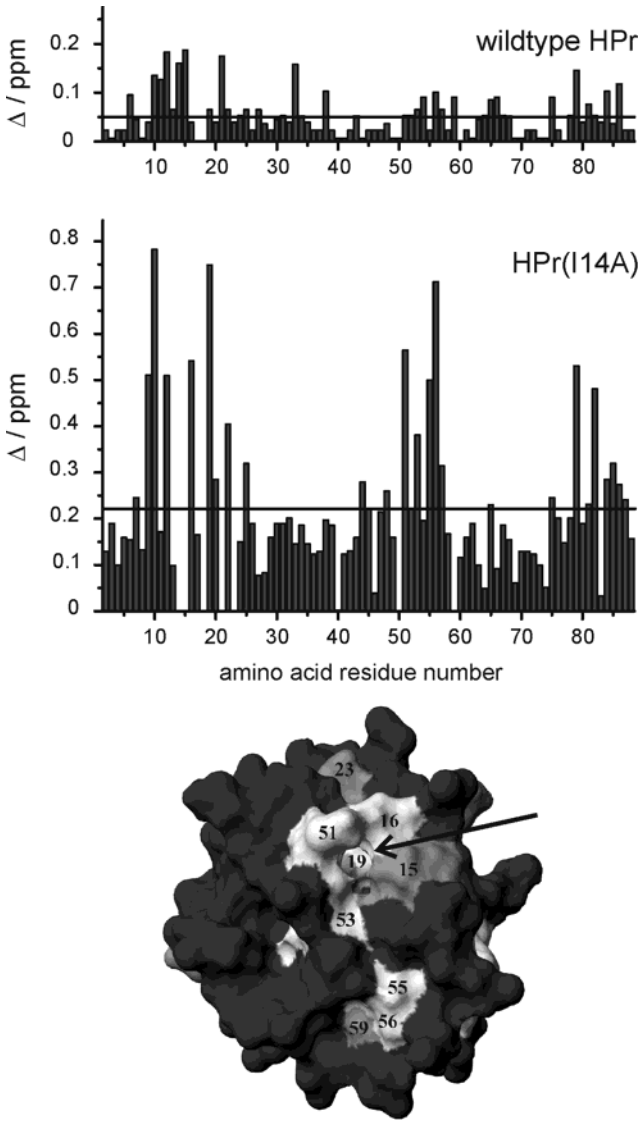

Figure 2. Total xenon-induced shift, Δ, observed for wild-type HPr (top) and HPr(I14A) (middle) as a function of the amino acid residue number. The average values of Δ are indicated by horizontal black lines. A surface model of HPr(I14A) (bottom, the computer program MOLMOL[22] was used to draw this figure) demonstrates the location of the hydrophobic cavity (see arrow). Residues with a total xenon-induced shift exceeding the sum of the mean value and the standard deviation of Δ (0.38 ppm) are shown in white. For residues shown in light gray, Δ could not be measured.

dissolved xenon atoms. For wild-type HPr, only nonspecific interactions are expected. Indeed, Δ is relatively small for the wild-type protein; the average value of Δ amounts to only 0.05 ppm. In contrast, considerably higher values of Δ are observed for the mutant, and the differences between the individual residues are much larger. The average total xenon-induced shift amounts to 0.22 ppm for the mutant, suggesting that the specific interaction of xenon atoms with the mutated protein results in structural changes of the molecule. The highest Δ-values of ca. 0.4−0.8 ppm occur for amino acid residues located within three limited regions of HPr(I14A) (amino acid residues 9−22, 51−56, and 79−82). An inspection of the molecular structure of HPr shows that most of these amino acid residues are close to the engineered cavity. The point mutation I14A results in the formation of a hydrophobic cavity due to the shorter side chain of alanine. The analysis of the structure of HPr(I14A) predicts an approximate diameter of 3−4 Å for this cavity which is close to but somewhat smaller than the diameter of a xenon atom (4.4 Å). It is, however, known that the insertion of such engineered cavities into proteins results in a "relaxation" of the environment, that is, in the contraction of the cavity.[6] Ligand binding then tends to reexpand the cavity, that is, to reverse this contraction. The observation of larger Δ-values for HPr(I14A) than for the wild-type protein in the entire molecule—and not only for residues close to the hydrophobic cavity—shows that the expansion of the hydrophobic cavity caused by xenon binding results in a slight but detectable structural rearrangement of the entire molecule. In summary, the comparison of the total xenon-induced shift, Δ, between mutant and wild-type HPr confirms the presence of specific xenon binding in HPr(I14A). The largest shifts correspond to well-defined regions close to the mutation site. In addition, xenon-induced shifts throughout the molecule indicate structural rearrangements in response to xenon binding into the hydrophobic cavity. Work is in progress to study whether the stability and biological activity of the HPr(I14A) is restored in the xenon-bound state.

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Supporting Information Available: Description of the total xenon-induced shift, Δ (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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