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Hydrogen Bonds in Human Ubiquitin Reflected in Temperature Coefficients of Amide Protons

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Analysis of amide proton temperature coefficients ($\Delta\sigma_{HN}/\Delta T$) in human ubiquitin shows their usefulness in indicating hydrogen bonds. The availability of a very accurate solution structure of ubiquitin enables the precise determination of hydrogen bonds and increases the reliability of the analysis of chemical shift temperature gradients. Values of $\Delta\sigma_{HN}/\Delta T$ more positive than −4.6 ppb/K are very good indicators of hydrogen bonds. Additionally, a weak temperature dependence of non-hydrogen-bonded amides was observed for amide protons that are significantly shifted upfield. We observed that temperature gradients of amide protons involved in short hydrogen bonds are related to donor–acceptor distances.

Key Words: **amide proton temperature coefficients; chemical shifts; hydrogen bonds; ubiquitin.**

INTRODUCTION

Amide proton temperature coefficients ($\Delta\sigma_{HN}/\Delta T$) carry valuable information about hydrogen bonding properties of amide protons. In general, formation of intramolecular hydrogen bonds causes chemical shifts to be less affected by temperature compared to solvent-exposed or non-hydrogen-bonded amides (*1*). As a consequence, weak negative values of temperature gradients ($\Delta\sigma_{HN}/\Delta T \geq -4.6$ ppb/K) are expected for hydrogen-bonded amides and strong negative gradients ($\Delta\sigma_{HN}/\Delta T < -4.6$ ppb/K) should be observed for non-hydrogen-bonded amides (*2, 3*). Detailed analysis of $\Delta\sigma_{HN}/\Delta T$ for two proteins, bovine pancreatic trypsin inhibitor (BPTI) and hen egg-white lysozyme, showed almost linear changes of chemical shifts up to 15° below denaturation temperature (*4*). To date, temperature-induced changes of amide proton chemical shifts in proteins have been measured only occasionally. Recently, we carried out more comprehensive analysis of the factors contributing to values of amide proton temperature gradients (*5*). Nevertheless, the detailed analysis is limited by the lack of data on proteins for which highly accurate solution structures are available. On the other hand, interpretation based on x-ray structures may be affected by small changes due to crystal packing.

One very attractive target for this purpose is the 76-residue protein ubiquitin. It possesses a stable tertiary fold and a high-quality structure has been determined using NMR spectroscopy with the use of an unusually large number of experimental restraints (distance and dihedral angle restraints, dipolar couplings, and directly detected hydrogen bonds) (*6*). In this work, we utilized this high-resolution structure and analyzed the factors contributing to amide proton temperature coefficients in ubiquitin.

RESULTS AND DISCUSSION

Temperature gradients were measured for 63 out of 72 backbone amides in ubiquitin. For a small number of resonances, signal broadening was observed with increasing temperature, and it was not possible to make an accurate determination of chemical shifts for these signals. Values of $\Delta\sigma_{HN}/\Delta T$ span the range between −12 and 0 ppb/K and are clearly related to the presence of hydrogen bonds (Fig. 1). An empirical delineation can be made whereby a value of −4.6 ppb/K differentiates between most hydrogen-bonded and non-hydrogen-bonded amides. More than 90% (43 out of 47) of amide protons with temperature gradients more positive than −4.6 ppb/K form hydrogen bonds. There are four exceptions: Ser20, Ile36, Asp39, and Glu16. The H^N chemical shifts of two outliers (Ser20 and Ile36) are 7.07 and 6.19 ppm, respectively. These values are shifted upfield by more than 1 ppm relative to the random coil values, and the resonances exhibit weak negative temperature gradients. Similar behavior was previously observed in different proteins for amide protons that are strongly deshielded by aromatic rings (*3, 5*). In those cases, the large ring current effects on H^N chemical shifts correlated with weak negative temperature gradients of amide protons and the absence of involvement in hydrogen bonds. However, in ubiquitin there are no aromatic rings in the vicinity of the Ser20 and Ile36 amide protons nor are the amide protons involved in hydrogen bonds; nevertheless, the observed temperature gradients were found within the range between −2 and 0 ppb/K. Inspection of the ubiquitin solution structure revealed that the two amide protons are located in close proximity to the main chain C′–N

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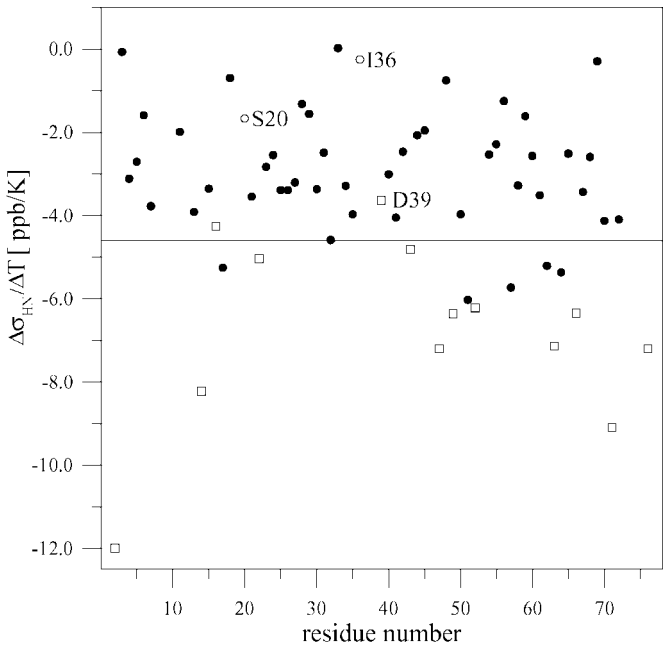

FIG. 1. Values of amide proton temperature coefficients in human ubiquitin. Filled circles (●) stand for hydrogen-bonded amides, opened squares (□) denote non-hydrogen-bonded amides, and open circles (○) indicate strongly deshielded non-hydrogen-bonded amides. The solid line corresponds to $\Delta\sigma_{HN}/\Delta T = -4.6$ ppb/K. Three amide protons with temperature gradients more positive than −4.6 ppb/K and not involved in hydrogen bonds are labeled (see text).

and C′–O bonds between Glu18–Pro19 and Glu34–Gly35 for Ser20 and Ile36, respectively. Thus, one explanation of their upfield chemical shifts could be the effect of magnetic anisotropies of neighboring peptide bonds (*7*). However, we were unable to quantitatively predict this shielding effect by empirical chemical shift calculations using SHIFTS (*7*). The predicted shifts are, indeed, upfield, but the magnitudes of the shifts (−0.4 and −0.5 ppm) do not match the observed values. While the trend is correct, further investigations are required to provide a quantitative interpretation of the observed effects. The third amide with a weak negative temperature gradient ($\Delta\sigma_{HN}/\Delta T = -3.6$ ppb/K) that is not involved in a hydrogen bond is the H^N of Asp39. Analysis of the ubiquitin NMR structure showed that in 2 out of 10 conformers, the solvent-exposed side chain of Asp39 satisfies the criteria for a hydrogen bond with its own backbone amide. Thus, the value of $\Delta\sigma_{HN}/\Delta T$ may reflect this potential transient hydrogen bond. In conclusion, there is a very good agreement between weak negative temperature gradients of amide protons (values more positive than −4.6 ppb/K) and the presence of hydrogen bonds. Three out of four outliers (labeled in Fig. 1) can be explained in a reasonable way.

On the other hand, strong negative temperature gradients ($\Delta\sigma_{HN}/\Delta T < -4.6$ ppb/K) do not necessarily correspond to non-hydrogen-bonded amides (Fig. 1). The majority of amides in this category are not hydrogen-bonded; however, there are a significant number of amides that are hydrogen-bonded. Hence, this category is less deterministic then that of amides with weak negative temperature gradients. One of the possible reasons for this behavior is the presence of very short hydrogen bonds (see below). Numerous examples of such short hydrogen bonds are observed in protein α-helices, and these effects have been described previously (*5*).

In our previous work, we observed a relationship between hydrogen bond length and the value of amide proton temperature coefficients (*5*). It would be of interest and value to establish a general correlation between hydrogen bond length and amide proton temperature coefficient. However, there is still a paucity of proteins for which both an ultra-high-resolution crystal structure and the appropriate NMR data exist. A quantitative relationship between backbone–backbone hydrogen bond lengths and $^{3h}J_{NC'}$ couplings across hydrogen bonds has been recently described (*8*). While there is not an ultra-high-resolution crystal structure of ubiquitin available to allow the measurements of hydrogen bond lengths directly, a set of 27 $^{3h}J_{NC'}$ coupling constants measured across backbone–backbone hydrogen bonds have been reported for ubiquitin (*9*), and we may infer a hydrogen bond length based on the reported relationship. We have correlated the $^{3h}J_{NC'}$ coupling constants measured for ubiquitin (*9*) with the observed amide proton temperature coefficients (Fig. 2). The correlation suggests that the amide protons within this set can be separated into two groups based on the value of the $^{3h}J_{NC'}$ coupling constant. The first group is defined by $^{3h}J_{NC'} \leq -0.4$ Hz and corresponds to short hydrogen bonds,

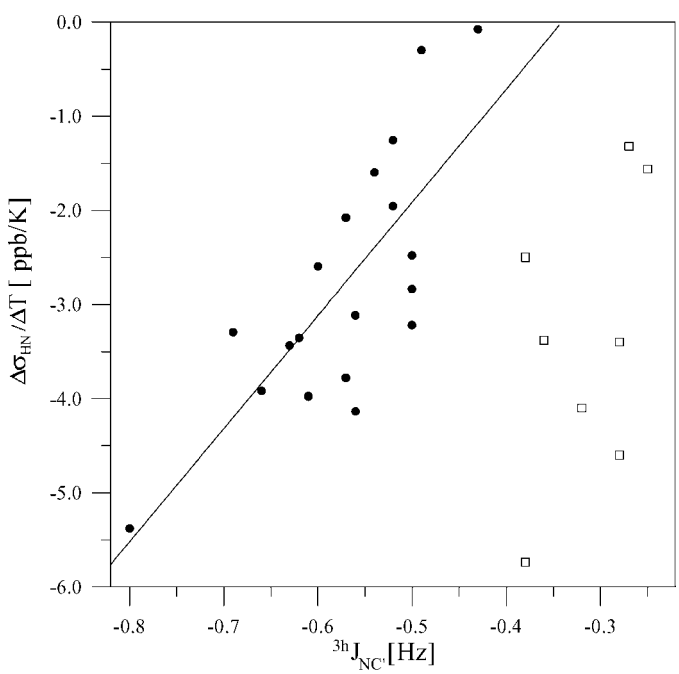

FIG. 2. Correlation of amide proton temperature coefficients ($\Delta\sigma_{HN}/\Delta T$) and coupling constants across hydrogen bonds ($^{3h}J_{NC'}$). $^{3h}J_{NC'}$ values were taken from the work of Cornilescu *et al.* (*9*). Solid line shows a linear fit to the data for $^{3h}J_{NC'}$ stronger than −0.4 Hz ($R = 0.77$).

and the second group, with ${}^{3h}J_{NC'} > -0.4$ Hz, corresponds to longer hydrogen bonds. Amide protons in the first group exhibit a correlation between the large negative coupling constants and the amide proton temperature coefficients (Fig. 2). According to the empirical equation relating ${}^{3h}J_{NC'}$ and hydrogen bond lengths, this group corresponds to N···O distance shorter than ~3 Å (*8*). The correlation of $\Delta\sigma_{HN}/\Delta T$ with hydrogen bond length was previously observed for helical peptides (*10*). The origin of this correlation is based on the inverse third power dependence of amide proton chemical shift on hydrogen bond length (*11*). A temperature-induced increase in the hydrogen bond length causes the amide proton to be less deshielded by the acceptor atom. This effect is stronger for short hydrogen bonds and, consequently, results in stronger negative temperature gradients of the amide protons. However, we observe this correlation only for N···O distances shorter than about 3 Å. For longer hydrogen bonds, the amide proton temperature coefficients exhibit no correlation with the measured ${}^{3h}J_{NC'}$ values (Fig. 2).

To summarize, amide proton temperature coefficients can be classified into four groups: I. amide protons involved in short hydrogen bonds ($d_{N\cdots O} \leq 3$ Å); II. amide protons forming longer hydrogen bonds ($d_{N\cdots O} > 3$ Å); III. amides not forming hydrogen bonds; IV. amide protons shifted strongly upfield and not involved in hydrogen bonds. In the first group, the amide proton temperature gradients are related to hydrogen bond lengths and amides involved in very short hydrogen bonds may exhibit strong negative temperature gradients ($\Delta\sigma_{HN}/\Delta T < -4.6$ ppb/K). Group II generally exhibits $\Delta\sigma_{HN}/\Delta T$ weaker than −4.6 ppb/K. However, the correlation is not unique, and there are some hydrogen bonded amides with temperature coefficients stronger than −4.6 ppb/K. A strong temperature dependence of chemical shifts is observed for non-hydrogen-bonded amides (group III). Analysis of the ubiquitin structure shows that almost all amide protons that are not involved in intramolecular hydrogen bonds have temperature gradients stronger than −4.6 ppb/K. In some cases non-hydrogen-bonded amides may exhibit significantly upfield shifted chemical shifts (group IV). The strong deshielding effect is observed to correlate with a weak temperature gradient ($-2 \leq \Delta\sigma_{HN}/\Delta T \leq 0$).

EXPERIMENTAL

Experiments were carried out on a Varian UnityPlus 500 spectrometer. The NMR sample contained of 1.5 mM solution of ${}^{15}N;{}^{13}C$-labeled ubiquitin in 25 mM sodium acetate, pH 6.0. Sets of ${}^{1}H–{}^{15}N$ HSQC and ${}^{1}H–{}^{13}C$ HSQC spectra (*12*) were measured at 25, 30, 35, 40 and 45°C. Because of a very weak dependence of aliphatic proton chemical shifts on temperature, ${}^{1}H–{}^{13}C$ HSQC were treated as reference spectra. Superimposed methyl regions of the spectra were used for calibration of proton chemical shifts in ${}^{1}H–{}^{15}N$ HSQC spectra. Temperature coefficients were calculated as the best fit to measured ${}^{1}H^{N}$ chemical shifts versus temperature. The solution structure of human ubiquitin (PDB code: 1d3z) was used for identification of hydrogen bonds. Hydrogen bonds were identified using the program MOLMOL (*13*). The criteria were (I) the acceptor atom was found within a distance of 2.5 Å from the amide proton, (II) the angle between the vector connecting the backbone nitrogen atom and the amide proton and the vector connecting the backbone nitrogen atom and the acceptor atom was smaller than 45°, and (III) criteria (I) and (II) were present in at least 8 out of 10 structures.

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