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Transverse relaxation-optimized HCN experiment for tautomeric states of histidine sidechains

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Abstract **:** Function of protein is frequently related with tautomeric states of histidine sidechains. Thus, several NMR experiments were developed to determine the tautomeric states of histidines. However, poor sensitivity of these experiments caused by long duration of magnetization transfer periods is unavoidable. Here, we alleviate the sensitivity of HCN experiment for determining the tautomeric states of histidine residues using TROSY principle to suppress transverse relaxation of ^{13}C spins during long polarization transfer delays involving ^{13}C-^{15}N scalar couplings. In addition, this experiment was used to assign the sidechain resonances of histidines. These assignments can be used to follow the pH-titration of histidine sidechains.
Keywords : HCN, Histidine, NMR, Tautomeric state, TROSY

INTRODUCTION

In exploring structure/function relationships in proteins, it is of keen interest to determine the tautomeric states of histidine residues since histidine residues are frequently responsible for the functions of proteins, mainly due to the chemical versatility of the imidazole ring. For investigation of the chemistry of histidine, long-range HMQC, long-range HSQC[1] and HCN[2] experiments based on either proton-nitrogen remote coupling or carbon-nitrogen coupling in the imidazole ring have been extensively used. Due to the small values of scalar coupling, coherence transfers have long durations of 40-100 ms.[3]

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Transverse relaxation-optimized spectroscopy (TROSY) can be used for reducing transverse relaxation in ^{13}C of aromatic systems by using the interference between dipole-dipole (DD) coupling and chemical shift anisotropy (CSA).[4-7] Here, we report an experiment for determining the tautomeric states of histidine residues using TROSY principle to suppress transverse relaxation of ^{13}C spins during long polarization transfer delays involving ^{13}C-^{15}N scalar couplings. In addition to determining the tautomeric states of histidines, we were able to assign the sidechain resonances of histidiens. These assignments can be used to follow the pH-titration of histidine sidechains.

EXPERIMENTALS

Sample preparation

Recombinant vectors harboring the *Bombyx mori* pheromone-binding protein (BmPBP) gene cotrolled by the T7 promoter were transferred into BL21(DE3) expression hosts (Novagen). Expression and purification of BmPBP were done according to the protocol published elsewhere (Lee, ETH Zurich, 2003). The final NMR sample contained ~ 7 mg of uniformly $^{13}C/^{15}N$-labeled BmPBP in 0.6 ml of 50 mM potassium phosphate, 95 % H_2O/5% D_2O with 2 mM NaN_3

RESULTS and DISCUSSION

Figure 1 shows the experimental scheme for the TROSY-HCN, which is based on the TROSY-HCN experiment developed for assigning nucleotides.[6,7] The major addition in our TROSY-HCN pulse scheme is the refocusing of the $^1J^{CC}$ coupling during the C-N and N-C transfer periods, which is not necessary in nucleotides.

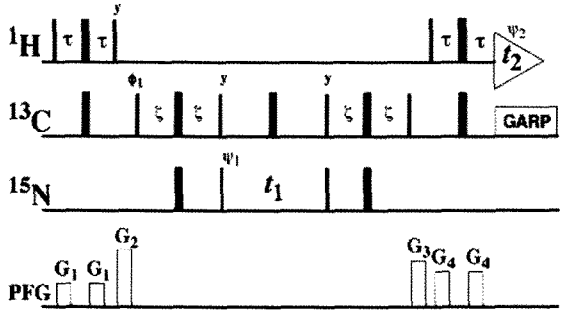

Fig. 1. Experimental scheme for the TROSY-HCN experiment for determining the tautomeric state of histidine residues. The radio-frequency pulses on 1H, 13C, and 15N are applied at 4.7, 125, 205 ppm, respectively. Narrow and wide black bars stand for nonselective 90° and 180° radio-frequency pulses, respectively. The ^{1}H and ^{15}N carrier frequencies are set at the water resonance and at 118 ppm, respectively. The delays τ = 1.3 ms and z = 7.14 ms were adjusted for optimal sensitivity. The line marked PFG (pulsed field gradient) indicates the magnetic field gradients applied along the z-axis: G_1, amplitude = 10.8 G/cm, duration = 0.5 ms; G_2, amplitude = 17.1 G/cm, duration = 0.5 ms; G_3, amplitude = 37 G/cm, duration = 1 ms; G_4, amplitude = 45.6 G/cm, duration = 0.5 ms. Phases are ϕ_1 = {x,x,-x,-x}, ψ_1 = {x,-x,x,-x}, ψ_2 = {-x,x,x,-x} and {x} otherwise. The phase-sensitivity in the t_1 dimension is obtained by the States-TPPI method applied to the phase ψ_1.

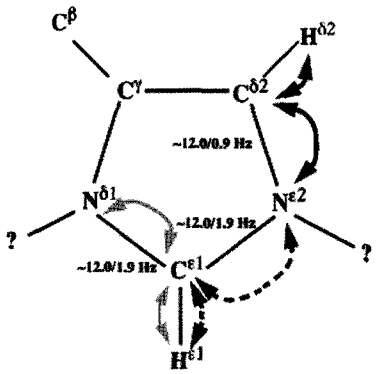

Fig. 2. Scheme showing the three magnetization transfer pathways in a histidine sidechain exploited in the TROSY-HCN experiment. Solid arrows indicate the transfers between $H^{\delta 2}$, $C^{\delta 2}$ and $N^{\varepsilon 2}$, dotted arrows the transfers between $H^{\varepsilon 1}$, $C^{\varepsilon 1}$ and $N^{\varepsilon 2}$, and grey arrows the transfers between $H^{\varepsilon 1}$, $C^{\varepsilon 1}$ and $N^{\delta 1}$. The $^1J^{NC}$ couplings are given for the protonated/un-protonated states of ^{15}N atoms involved in the coupling. The $^1J^{CC}$ coupling between C^{γ} and $C^{\delta 2}$ is ~70 Hz.

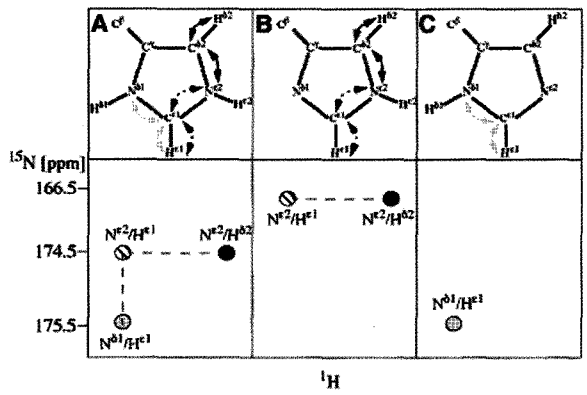

Fig. 3. Schematic peak patterns expected from the TROSY-HCN experiment for different tautomeric states of histidine residues. (A) All nitrogens protonated (charged), (B) $N^{\varepsilon 2}$ protonated, and (C) $N^{\delta 1}$ protonated states of histidine residues. The chemical shifts indicated for nitrogen are taken from the random coil value. Expected peak patterns (observable due to large coupling constants as shown in Figure 2) are indicated below the relevant histidine state.

Similar to the conventional HCN experiment[2] for determining the tautomeric states of histidine residues, the TROSY-HCN pulse sequence is based on three possible coherence patways $H^{\delta 2} \rightarrow C^{\delta 2} \rightarrow N^{\varepsilon 3} \rightarrow C^{\delta 2} \rightarrow H^{\delta 2}$ (solid arrows), $H^{\varepsilon 1} \rightarrow C^{\varepsilon 1} \rightarrow N^{\varepsilon 2} \rightarrow C^{\varepsilon 1} \rightarrow H^{\varepsilon 1}$ (dotted arrows), and $H^{\varepsilon 1} \rightarrow C^{\varepsilon 1} \rightarrow N^{\delta 1} \rightarrow C^{\varepsilon 1} \rightarrow H^{\varepsilon 1}$ (grey arrows) (Figure 2) and depends on $^1J^{NC}$ couplings which vary depending on tautomeric states of histdine residues. Thus, for the $N^{\delta 1}$-$H^{\delta 1}$ tautomer, we expect one peak corresponding to the correlation $N^{\delta 1}$-$H^{\varepsilon 1}$. For the $N^{\varepsilon 2}$-$H^{\varepsilon 2}$ tautomer, two peaks are expected, due to the correlations $N^{\varepsilon 2}$-$H^{\delta 2}$ and $N^{\varepsilon 2}$-$H^{\varepsilon 1}$. In addition, three peaks from all pathways are expected for the charged form (Figure 3). Therefore, the identification of the tautomeric states of the histidine residues can be achieved by simply counting the number of peaks which have correlations between ^{15}N and proton chemical shifts.

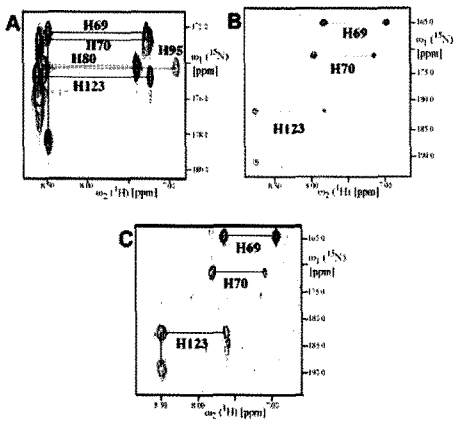

Fig. 4. Contour plots from 2D TROSY-HCN spectra of (A) $BmPBP^A$, (B) $BmPBP^B$, and (C) the BmPBP-bombykol complex. Sample conditions were as follows: approximately 0.5 mM protein in 50 mM sodium phosphate buffer at 20 °C. Spectra in A, B, and C were obtained at pH 4.5, 6.5, and 6.5, respectively, and the BmPBP-bombykol complex was approximately stochiometric. Based on the 2D TROSY-HCN spectrum shown in (A), all five histidine residues in $BmPBP^A$ are in the fully protonated state. In $BmPBP^B$ and the BmPBP-bombykol complex, two histidine residues (H69 and H70) occur as $N^{\varepsilon 2}$ protoanted tautomers and H123 is fully protonated, whereas the two other histidines, H80 and H95, were not detected possibly due to exchange-mediated line broadening. The three spectra were measured with different number of scans and t_{1max} values.

Figure 4 shows contour plots of 2D TROSY-HCN spectra of *Bombyx mori* pheromone-binding proteins at low ($BmPBP^A$) and high pH ($BmPBP^B$) and complexed with its natural ligand (BmPBP-bombykol). All five histidine residues in $BmPBP^A$ are in the fully protonated state whereas in both $BmPBP^B$ and the BmPBP-bombykol complex two histidine residues (H69 and H70) occur as $N^{\varepsilon 2}$ protonated tautomers and H123 is fully protonated (Figure 4). The two other histidines, H80 and H95, were not detected in BmPBPB and the BmPBP-bombykol complex possibly due to exchange-mediated line broadening.

We have shown that the tautomeric state of histidine residue can be achieved simply by counting the number of peaks using the TROSY-HCN experiment. In addition, it is demonstrated that the assignment of histidine sidechain resonances of BmPBP is achieved by the TROSY-HCN experiment. Thus, we anticipate that the TROSY-HCN experiment will play a key role in the functional studies of proteins involving the histidine sidechain.

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