Filtered NOESY and water-amide NOE to detect conformational differences in a protein complexed with highly analogous inhibitors

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In the era of state-of-the-art inhibitor design and high-resolution structural studies, detection of significant but small protein structural differences among inhibitor-bound forms is critical when developing potent inhibitors. Comparison of protein structures bound to highly analogous inhibitors has been mostly done using X-ray crystallographic analyses or MD simulations. Solution NMR spectroscopy is less explored for this purpose, presumably because protein NMR structures and dynamics among the "rigid" inhibitor bound forms are similar to one another. Here, we applied 3D ^{15}N-half filtered NOESY experiments, in which NOE cross peaks from non-^{15}N-bonded protons to ^{15}N-bonded protons are detected, and 2D ^{15}N-edited water-amide NOE experiments, in order to identify how a subtle changes in in a few chemical moieties of the inhibitors affect their interactions with HIV-1 protease (PR). Because these experiments detect signal intensities caused by dipolar interaction and water-amide exchange, comparison of the data among homologous inhibitor-bound forms gives insight into differences in the water environment at and near the protein-inhibitor interaction sites (Figure).

3D ^{15}N-half filtered NOESY experiments for two inhibitor-bound forms were carried out with perdeuterated ^{15}N-labeled PR with a 120 ms NOE mixing time on a 900 MHZ spectrometer (1, 2). We did not apply a ^{13}C-half filter using a ^{13}C-labeled protein, to avoid any residual aliphatic ^{13}C-^{1}H protein signals that may overlap with the inhibitor signals. 2D water-NOE experiments for analogous inhibitor-bound forms of ^{15}N-labeled PR were recoded with a T_2-filter (1, 3). Since the latter experiments were performed for several analogous inhibitors, non-deuterated PR was used.

In the 2D water-NOE experiments, two sets of 2D water-NOE experiments were recorded for each inhibitor-bound PR, with 7.2 ms and 20 ms T_2-filters, that are water-selective 90°- τ -180°- τ duration. The ratio of the two NOE intensities was calculated using the following equation.

filtered/unfiltered ratio = [NOE_filtered] / [NOE_unfiltered]

Here, [NOE_unfiltered] is the NOE intensity at 7.2 ms T_2-filter, which is occupied by water shaped pulses, and [NOE_filtered] is the NOE intensity at 20 ms T_2-filter. Note, NOE intensities themselves were obtained in individual 2D water-NOE experiments. The ratio obtained using the above equation discards magnitude information of the HN-water NOE, but provides information on whether the NOE is from Hα that is inverted together with water protons, or from water protons. Thus, when the filtered/unfiltered ratio is close to 1, the observed NOE is expected mostly from HN-water NOE, and when the filtered/unfiltered ratio is close to 0, the observed NOE is expected mostly from Hα-HN NOE. The ratio qualitatively agreed with the results obtained by 3D half-filtered NOESY and was useful to analyze ambiguous water-protein NOEs. The work is supported by NIH GM109767.

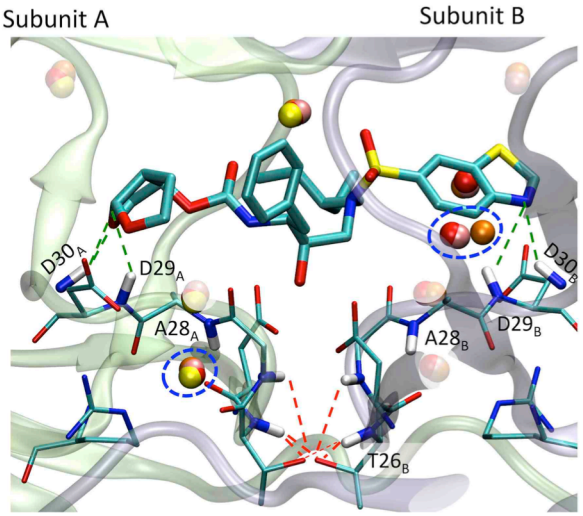

Figure. Graphical presentation of the active-site region of PR, showing inhibitor-PR NOE connectivity (green dashed line) and intra-PR NOE connectivity (red dashed line). Crystallographic water positions of four analogous inhibitor-bound PR structures (red, orange, pink, yellow, taken from PDB: 3O9A, 3O9B, 3O9F, and 3O9I, respectively (4)) are shown as frosted (not observed in solution) or non-frosted (predicted in our experiments). The blue dashed circles indicate possible water positions that differ when chemical moiety differs between two inhibitors.

References

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