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ARTICLE

Probing water-protein contacts in a MMP-12/CGS27023A complex by nuclear magnetic resonance spectroscopy

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Abstract Using the case of the catalytic domain of MMP-12 in complex with the known inhibitor CGS27023A, a recently assembled 3D ^{15}N-edited/^{14}N,^{12}C-filtered ROESY experiment is used to monitor and distinguish protein amide protons in fast exchange with bulk water from amide protons close to water molecules with longer residence times, the latter possibly reflecting water molecules of structural or functional importance. The ^{15}N-edited/^{14}N,^{12}C-filtered ROESY spectra were compared to the original ^{15}N-edited/^{14}N,^{12}C-filtered NOESY and the conventional amide-water exchange experiment, CLEANEX. Three protein backbone amide protons experiencing direct dipolar cross relaxation with water in the ^{15}N-edited/^{14}N,^{12}C-filtered ROESY spectrum were assigned. In an ensemble of six crystal structures, two conserved water molecules within 3 Å of the three amide protons were identified. These two water molecules are buried into cavities in the protein surface and thus sufficiently slowed down by the protein topology to account for the observed dipolar interaction. Structural analysis of an ensemble of six crystal structures ruled out any exchange-relayed contributions for the amide-water interactions of interest.

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Abbreviations

MMP	Matrix metalloprotease
NMR	Nuclear magnetic resonance
NOESY	Nuclear overhauser effect spectroscopy
ROESY	Rotating frame overhauser effect spectroscopy

Introduction

The role of the solvent with regard to both protein structure and function is known to be important, although elusive. High resolution crystal structures always include large numbers of trapped water molecules in well-defined positions, but their actual significance has been repeatedly questioned over the years. Some of the concerns that have been voiced are that the localization of exposed hydration sites is to some extent subjective (Badger 1997; Ohlendorf 1994), that crystal structure water molecules carry no dynamic information (Halle 2002), and that a large percentage (30–40 % for a small protein) of the solvent accessible surface is in fact buried in crystal contacts (Islam and Weaver 1990). NMR spectroscopy is in this respect a complementary method that is frequently used to investigate protein hydration in aqueous solution. The available NMR methods are sensitive to a wide range of dynamic time scales but carry less *direct* structural information in terms of water molecule coordination.

The pioneering works of Otting et al. (1991a) and (1991b), Clore et al. (1990) and Grzesiek and Bax (1993) have shown us already in the early 90′ies that 3D NOESY/ROESY-TOCSY and ROESY-HMQC type of NMR experiments can be used to monitor cross relaxation between

protein protons and water molecules. Two kinds of waters were detected. On the one hand, there are well-ordered interior waters embedded in the protein structure and characterised by long-residence times, often referred to as 'structural waters'. On the other hand, there is the hydration water on the protein surface with residence times in the subnanosecond range. Of this, at best about 60 % could be monitored by NMR techniques (Otting et al. 1991a). A recent development, encapsulating the protein in a reverse micelle, has however enabled the study of a much higher fraction of the water molecules by significantly slowing down the water mobility (Nucci et al. 2011).

The hydration toolbox was later expanded by Hwang et al. (1997), with the publication of the CLEANEX experiment, designed to monitor pure exchange between water and labile protein protons by using a mixing scheme that suppresses contributions from dipolar cross relaxation, such as intramolecular NOEs and exchange-relayed NOEs. It has also been observed that cross relaxation from groups with fast (internal) dynamics can pass through the CLEANEX mixing scheme as they don't fully satisfy the NOE/ROE ratio required for perfect cancellation, thus the experiment can under some conditions also detect intermolecular NOEs to water or intramolecular NOEs to methyl groups. For example, the CLEANEX experiment has been applied successfully to verify the role of water in stabilizing a structurally crucial hydrogen bond in RNA pseudouridine (Newby and Greenbaum 2002). Thus, the information content of CLEANEX is complementary to NOESY and ROESY type of experiments. Further developments of hydration experiments include, for instance, the use of selective excitation of water using Q-switched probes (Otting and Liepinsh 1995) or pulse field gradients (Böckmann and Guittet 1996; Dalvit and Hommel 1995). Alternatively, selective excitation of water combined with the purging of any underlying H^{α} protons (Grzesiek and Bax 1993) has been proposed. Reduction of the dimensionality, and hence experiment time, can also be achieved by allowing the protein to relax before acquisition (Mori et al. 1996).

The aim of this work was initially to investigate by means of NMR whether there are water molecules present in the binding pocket of the MMP12 catalytic domain that are slowed down enough to allow detection and, if this was the case, to determine whether they mediate hydrogen bonds between the inhibitor and the enzyme. An interesting observation in this respect is that in the crystal structure (denoted 1Y93 in the Brookhaven database) of the MMP-12 catalytic domain co-crystallized with a very small inhibitor (acetohydroxamic acid, HAE), the ligand-binding zinc atom is coordinated by a water molecule inside the active site (Bertini et al. 2005). Recently, a low occupancy of about 0.30 has been reported by Borsi et al. (2010) for this particular water molecule located at 2.65 Å distance from the zinc ion. Here, we report our findings concerning MMP-12 in complex with the hydroxamic acid based inhibitor CGS27023A (MacPherson et al. 1997), using the complementary information from ^{15}N-edited/^{14}N,^{12}C-filtered ROESY and NOESY, and CLEANEX type NMR experiments. Since this study focuses on identifying water molecules that have long residence times in solution, the effect of long range dipolar couplings with water, that can potentially be a dominating factor for the relatively highly mobile surface hydration water, is not expected to affect the conclusions (Halle 2003; Modig et al. 2004).

Methods

Ligand synthesis

The hydroxamic acid based MMP-inhibitor, CGS27023A, N-hydroxy-2(R)-((4-methoxysulfonyl)(3-picolyl)amino)-3-methylbutaneamide hydrochloride, was synthesized according to the method of MacPherson et al.(1997).

Protein cloning, expression and purification

The catalytic domain of human MMP-12, corresponding to G106-G263, was cloned into the expression vector pET11a. The mutation F171D was made to improve protein solubility for NMR experiments. A second construct with the additional inactivating mutation E219A was engineered for long term stability. A third construct, comprising the inactivating mutation but lacking the F171D mutation, was produced for completeness. This last form was subsequently used in crystallographic experiments. Uniformly ^{15}N/^{13}C-labeled MMP-12 was produced in *Escherichia coli* BL21(DE3) grown in M9 medium (Sambrook et al. 1989) with 0.5 g/L of ^{15}N-NH_4Cl and 2 g/L ^{13}C-D-glucose as nitrogen and carbon source, respectively. For expression and purification essentially the procedure of Morales et al. (2004) was followed.

NMR sample preparation

The purified ^{15}N/^{13}C-labelled MMP-12 NMR sample was prepared by buffer exchange into an NMR-buffer containing 10 mM deuterated TRIS–HCl, 100 mM NaCl, 5 mM $CaCl_2$, 0.1 mM $ZnCl_2$, 2 mM NaN_3, 10 mM deuterated DTT in 90 % H_2O/10 % D_2O. Buffer exchange was carried out on a pre-packed PD-10 column (Sephadex G-25; GE Healthcare, Sweden). The sample was concentrated to 0.7 mM in a Vivaspin 15R Centrifugal Filter Unit (VivaScience, Germany). In experiments with inhibitor this was added in a 2:1 ratio (inhibitor:enzyme). Care was taken not to exceed 5 % DMSO in the NMR-samples.

NMR data collection

All experiments were performed at 22 °C, using a Bruker Avance II spectrometer operating at 600 MHz ^{1}H-frequency equipped with a cryogenically cooled inverse triple resonance probe. The ^{1}H-, ^{13}C- and ^{15}N- resonances of the protein backbone and aliphatic amino acid side chains were assigned using standard protocols (Isaksson et al. 2009) and verified with the previously published assignment (Bertini et al. 2005). Solvent interactions were monitored by means of 3D ^{15}N-edited/^{14}N,^{12}C-filtered ROESY spectra (60 and 125 ms spinlock time) that was compared to 2D ^{15}N-correlated CLEANEX (Hwang et al. 1997) and 3D ^{15}N-edited/^{14}N,^{12}C-filtered NOESY (60 and 125 ms mixing time) (Zwahlen et al. 1997). The pulse program and the experimental parameters of the ROESY were identical to the corresponding 3D ^{15}N-edited/^{14}N,^{12}C-filtered NOESY experiment except that the NOESY mixing time had been replaced by a ROESY spinlock pulse of 2,400 Hz during 10–150 ms. The CLEANEX spinlock was applied at 2,500 Hz field for 25–150 ms and the number of transients was typically 128 resulting in experiment times of about 9 h. All multidimensional spectra were processed by multiplying the FIDs with a 90-degree-shifted squared sine bell function in each dimension.

Structure analysis

All inter-atom distances were harvested with an in house awk/shell script, using deposited coordinates from the RCSB protein databank, accession codes: 1JIZ (Nar et al. 2001), 2W0D (Isaksson et al. 2009), 2W09 (Holmes et al. 2009), 3LJG (Devel et al. 2006), 3NX7 (Bertini et al. 2007) and 1Y93 (Bertini et al. 2005). Distance measurements were made on the explicit hydrogens for all protein residues while the oxygen atoms were used for all water molecules because of their orientations being undefined in the crystal structures. Any missing protein hydrogen atoms were added using PyMol. The degree of burial was estimated in PyMol by exporting the normalized difference of the available surfaces of all water molecules before and after all non-waters in the coordinate file had been deleted. The difference represents the surface that is contact with the protein, metal ions and inhibitors, but not with other water molecules.

Results and discussion

NMR observations

In search for indications for water molecules associating with the protein at distinct positions, a 3D ^{15}N-edited/^{14}N,^{12}C-filtered ROESY spectrum (Fig. 1a) was first acquired. In contrast to the corresponding NOESY experiment, the ^{1}H-^{1}H-plane of this ROESY spectrum is completely dominated by signals at the water frequency in the indirect ^{1}H-dimension, whereas the protein–ligand crosspeaks are practically absent because of fast protein transverse relaxation during the spinlock. Fig. 1b shows the amide proton region extracted at the horizontal H_2O-frequency from the ^{15}N-edited/^{14}N, ^{12}C-filtered NOESY (top) and ROESY (bottom) experiments, respectively. Although the two rows are similar, the H_2O-row from the ROESY also contains four negative signals (marked with an asterisk) compared to the H_2O-row from the NOESY.

In the 2D ^{1}H-^{15}N-projection plane of ^{15}N-edited/^{14}N, ^{12}C-filtered ROESY, the amide proton signals found on the water line in the indirect ^{1}H dimension are dispersed according to their ^{15}N-frequency. This spectrum (Fig. 2a) was used to correlate the four observed negative ROESY peaks to the amides of residues G166, H172 and G176, all being situated in loop 5 (see Fig. 4), as well as one amide that could neither be assigned in our data set, nor in the previously published NMR assignment (Bertini et al. 2005). No structural water molecules with long residence times could however be detected near amides in the active site (residues 181-184, 215-219 and 239-242) or in the immediate proximity to any of the metal ions, neither in the CGS27023A complex nor in the apo form of MMP-12 (data not shown). This is not because of lack of water access as will be discussed further below (Fig. 3). The entire protein surface, including the active site, has contact with water molecules. The absence of detectable water to protein cross-relaxation rather reflects that out of all the water molecules that are trapped in the crystal structures, only two are slowed down enough to allow detection in aqueous solution at room temperature.

Negative cross peaks in the ^{15}N-edited/^{14}N,^{12}C-filtered ROESY can arise by two mechanisms, either (1) by direct cross relaxation between the amide proton and a water molecules with sufficiently long residence time or, (2) by cross relaxation to a near labile proton (OH or NH) that in turn relays the correlation to water by chemical exchange, resulting in a false negative ROESY peak. The CLEANEX mixing scheme, on the other hand, is designed to efficiently suppress both inter- and intra-molecular cross relaxation and exchange-relayed contributions (Hwang et al. 1997). This is achieved by placing the magnetization along the Z-axis 2/3 of the time and in the transverse plane 1/3 of the time during the CLEANEX spinlock, resulting in a cancellation of the NOE- and the ROE contributions to the net cross relaxation in the spin diffusion regime. For this reason, Hwang et al. state that any negative peaks that go through the CLEANEX spinlock do not originate from immobilized waters incorporated inside the protein, but rather arise from interaction with water molecules on the

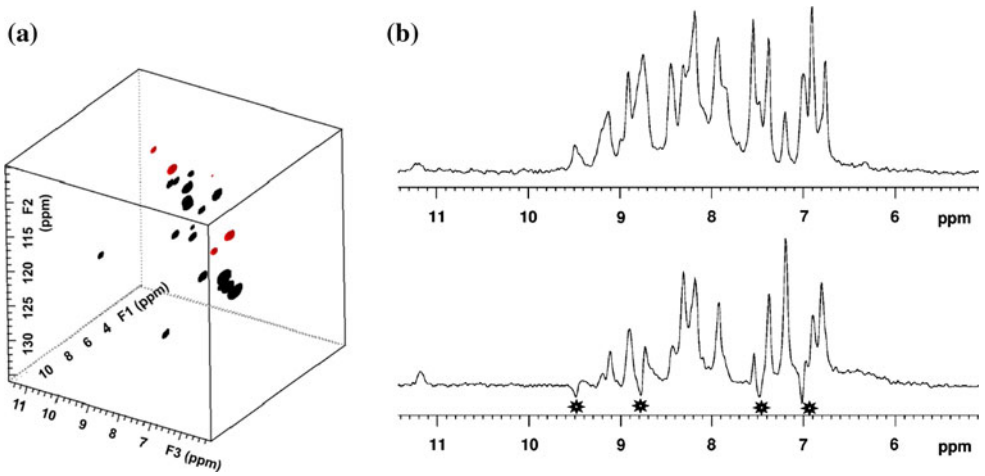

Fig. 1 **a** The 3D ^{15}N-edited/^{14}N,^{12}C-filtered ROESY spectrum displays positive exchange peaks (*black*) and negative protein amide—water ROESY peaks (*red*) at the indirect water frequency. The dimensions are ^{15}N-edited (F3), ^{15}N-separated (F2), ^{14}N,^{12}C-filtered (F1). **b** ^{1}H rows showing the amide region at the water frequency extracted from ^{15}N-edited/^{14}N,^{12}C-filtered NOESY (*top*) and ^{15}N-edited/^{14}N,^{12}C-filtered ROESY (*bottom*). The mixing time was 60 ms in all experiments

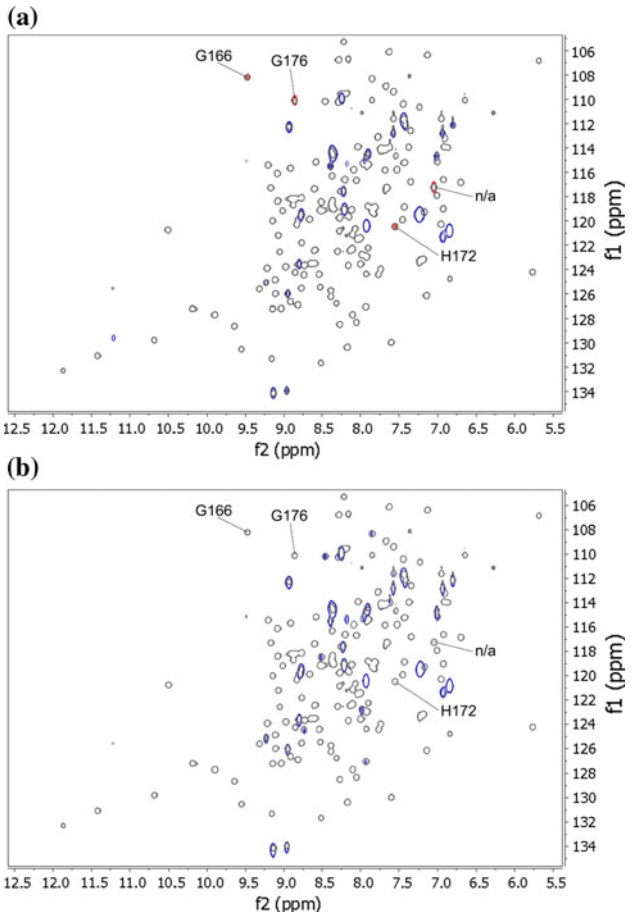

Fig. 2 Comparison of the 2D projection plane of ^{15}N-edited/^{14}N,^{12}C-filtered ROESY (**a**) and 2D CLEANEX (**b**), both recorded with 100 ms mixing time, superimposed on the ^{15}N-HSQC map (*grey*). The exchange peaks are shown as positive (*blue*) peaks in both spectra, while the ROESY also displays negative (*red*) peaks originating from dipolar cross relaxation between slowed down water molecules and protein amide protons

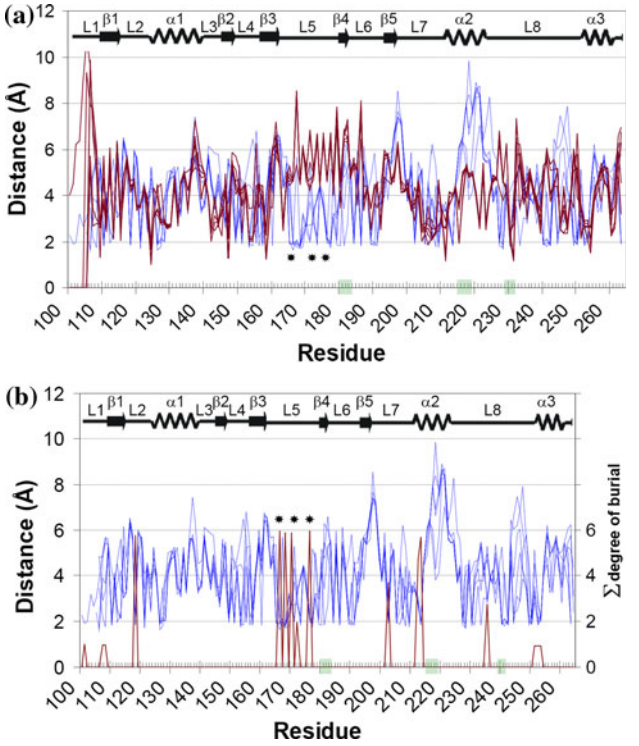

Fig. 3 Structural analysis of an ensemble of six crystal structures of the catalytic subunit of MMP-12, where every amide proton is plotted against the nearest water molecules (*blue thin lines*), and residues G166, H172 and G176 that show a negative ROESY crosspeaks have been highlighted with *stars*. Residues making up the active site are shaded in *green* on the x-axis. **a** The distance from each amide to the nearest exchangeable proton, defined as either a labile side chain proton or a neighboring amide proton that gives rise to a detectable CLEANEX peak (*red lines*). **b** The sum of the degree of burial for nearest water of each amide proton in every crystal structure satisfying the criterion: within 3 Å of the amide and with a burial degree above 90 % (*red lines*). Hence, a 100 % buried water molecule present within 3 Å in all six crystal structures would reach the maximum value of six on the y-axis in this plot

protein surface that are merely slowed down but still tumble fast enough in order to not fully satisfy the $\tau_c\omega_0 > > \sqrt{5/2}$ approximation of a molecule tightly bound to a protein, where τ_c is the rotational correlation time and ω_0 is the Larmor frequency. Therefore, water molecules *tightly* embedded into the protein structure

would be expected to be characterized by positive NOESY peaks, negative ROESY peaks and no CLEANEX peak.

In order to confirm the above findings, a series of 2D ^{15}N-correlated CLEANEX (Hwang et al. 1997) spectra were recorded. These spectra contained a number of strong positive signals (Fig. 2b) originating from backbone amides in fast exchange with water correlating perfectly with the positive ROESY peaks in the ^{1}H-^{15}N-plane of ^{15}N-edited/^{14}N,^{12}C-filtered ROESY spectra (Fig. 2a), and only a few extremely weak negative signals near the noise level. The presence of these very weak negative CLEANEX peaks, corresponding to the strong negative ROESY peaks that are not fully suppressed, indicates that the water molecules giving rise to the correlation are only weakly associated to the protein. These observations fit well with the more and more established view that hydration waters are merely slowed down by the topology of protein cavities rather than associating tightly through highly favourable enthalpic binding (Halle 2004).

Structural analysis

As an independent proof, the absence of any relayed contribution to the observed negative ROESY peaks has been ascertained by structural analysis using an ensemble of six previously published crystal structures of the catalytic subunit of MMP-12 in complex with different inhibitors, denoted: 1JIZ (Nar et al. 2001), 2W0D (Isaksson et al. 2009), 2W09 (Holmes et al. 2009), 3LJG (Devel et al. 2006), 3NX7 (Bertini et al. 2007) and 1Y93 (Bertini et al. 2005). An in-house script was used to extract the distances from each amide proton in the backbone to the nearest water molecule in each structure, plotted in blue in Fig. 3. Assigned residues giving rise to negative ROESY peaks are marked with stars. Nearly all amide protons are within 5 Å of a water molecule, with the exception of three dry patches consisting of residues 136-139, 194-199 and 215-224. Labile protons that could possibly be participating in relay of water exchange to the amides were surveyed by plotting the distance from each amide to the nearest labile side chain OH or NH, or nearest backbone NH that in turn has a positive CLEANEX peak (Fig. 3a, red lines). The result clearly shows that there are no available relay points within 4 Å of any of the assigned amide protons showing negative ROESY peaks (G166, H172 and G176, stars) to water. Thus, major contributions from relay effects are highly unlikely and the distance analysis strongly suggests that direct cross relaxation between structurally significant water molecules and amide protons is responsible for the observed ROEs.

Examination of the crystal structure ensemble reveals that there are indeed two completely buried water molecules, conserved in all crystal structures in the ensemble, only 1.9 Å from G166 and G176, and 3.2 Å from H172. The position of these two water molecules in the crystal structure of the MMP-12 + CGS27023A complex [accession code 2W0D (Isaksson et al. 2009)] is shown in Fig. 4a, b, in cartoon and surface stereo views, respectively. In order to visualize the correlation between the structural ensemble and the observed cross relaxation, the sum of the normalized degree of burial for the nearest water molecule within 3 Å from in each structure was plotted (red) into the nearest-water plot (blue) (Fig. 3b). For extra clarity, only water molecules more than 90 % buried into the surface of the protein were accepted. The resulting graph shows that there are indeed water molecules present in all crystal structures that satisfy this criterion for the three identified negative ROESY peaks, but it also reveals additional buried water molecules that satisfy the same criteria without leading to detectable negative ROESY peaks, namely for residues: H168, D170, I118, L114 and F115. The first two, residues H168 and D170, can unfortunately not be assigned. Bertini et al. (2005) attributes this to slow motions on the millisecond scale leading to line broadening beyond detection for residues 167-170. Residues I118, L212 and F213 give no detectable CLEANEX peaks, indicating that the exchange contribution is negligible. However, close examination reveals the presence of weak negative ROESY peaks (very weak in the case of L212). If we assume that the crystal structures are not subject to extensive crystal packing effects, the significantly lower cross relaxation efficiency can be attributed to shorter residence times of these water molecules in solution compared to the deeply buried waters giving strong negative ROESY peaks. The position of these amide protons, in the hinge regions between β1/L2 and L7/α2, respectively, could give rise to dynamics that would accommodate such an increase in water mobility at these sites (see Figs 3b and 4a). The quenching of the residual dipolar couplings for these regions that has been reported by (Bertini et al. 2005) is in line with our observations.

There are several residues that show strong positive CLEANEX peaks, but only very weak positive ROESY peaks, suggesting that there is also a substantial ROE contribution of reversed sign. Even though it is tempting to ascribe this to waters participating in cross relaxation, relay through neighbouring labile protons would produce the same net result. Indeed, cross referencing to the crystal structure ensemble reveals many such cases, wherefore we will refrain from drawing any conclusions based on the discrepancies in the intensities between the CLEANEX peaks and the weaker positive ROESY peaks for these residues.

In addition, the 2D ^{1}H-^{15}N-plane of ^{15}N-edited/^{14}N,^{12}C-filtered ROESY and CLEANEX on the MMP-12 protein without any inhibitor were also acquired (spectra not shown). These two spectra were essentially identical to the ones of the protein-inhibitor complex in terms of water

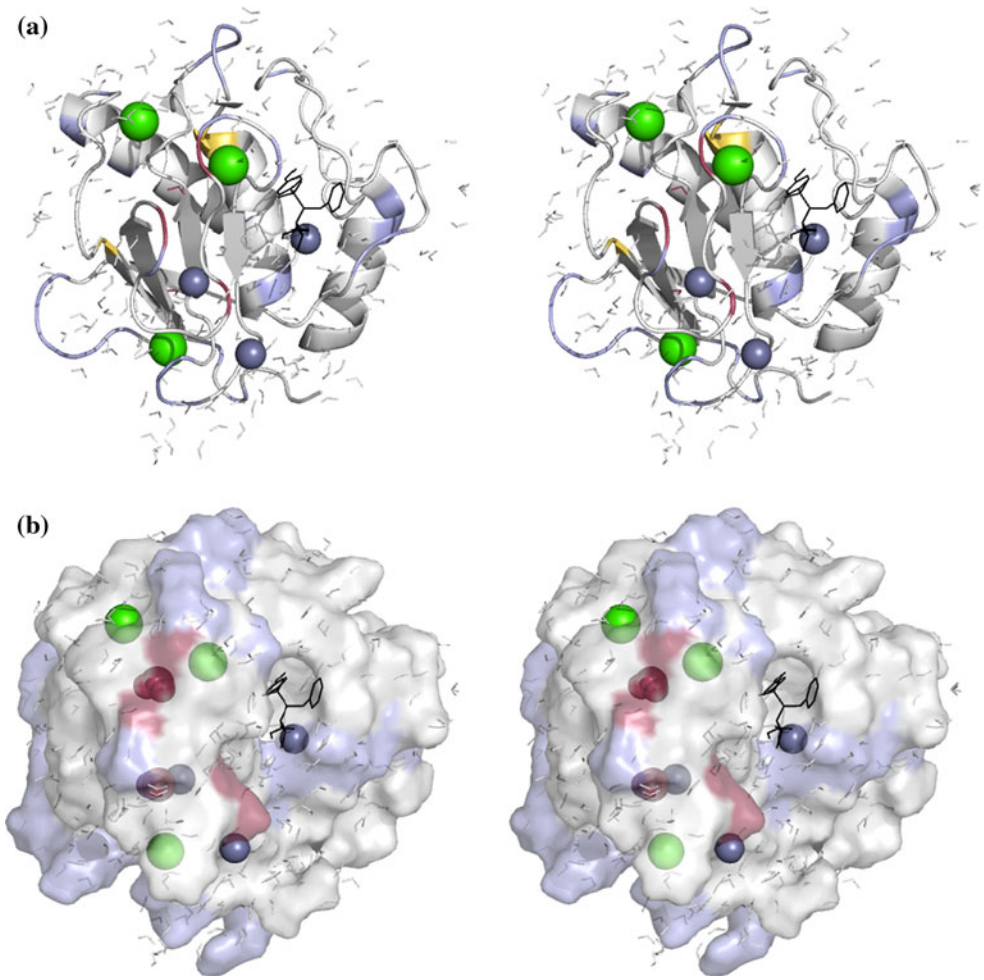

Fig. 4 Cartoon (**a**) and surface (**b**) stereo views showing the two conserved completely buried water molecules (*red sticks/spheres*) in the crystal structure of the MMP-12 + CGS27023A complex [accession code 2W0D (Isaksson et al. 2009)] that correlate with the negative amide-water ROESY contacts in the NMR sample (Fig. 2). Amino acids with negative ROESY peaks are displayed in *red*, amino acids with CLEANEX exchange peaks are shaded in *light blue*, the CGS27023A inhibitor is shown as *black sticks* and the metal ions are represented by *spheres*: Zn^{2+} in grey, Ca^{2+} in *green*. The hinges discussed above, β1/L2 and L7/α2, are highlighted in *yellow* in the cartoon

contacts. Even though the crystal structure of MMP-12 + HAE (1Y93) (Bertini et al. 2005) reports water molecules trapped in the active site, we did not find any evidence for this in the current spectra. This is not surprising given the relatively open topology that is not expected to significantly restrict water mobility.

Water molecules are expected to fill the void of the empty active site in the apo form of the protein, but the absence of detectable water molecules indicates that the residence times of such water molecules are shorter than 1 ns. Thus, the activation energy involved in replacing one water molecule by another is very frequently overcome at biologically relevant temperatures. Borsi et al. (2010) have recently estimated the entropy cost of the binding of two such water molecules in the active site of MMP-12, in complex with PMA + AHA (pdb:3LKA) and AHA (pdb:1Y93) respectively, to be much smaller than 1 kcal/mol at 298 K and they report respective occupancies of 0.40 and 0.30 for these water molecules. Detectable but low occupancy suggests that the enthalpy gain and entropy cost of water immobilization are of comparable magnitude at 298 K. This is also in agreement with Halle (2004) who argues that the vast majority of water-protein interactions are of approximately the same strength as water–water interactions. These observations, together with the findings in this work and the relatively open topology of the MMP-12 active site, let us conclude that these water molecules are not likely to significantly affect the binding of nanomolar inhibitors.

Conclusions

This work demonstrates that the ROESY version of the well established ^{15}N-edited/^{14}N,^{12}C-filtered NOESY can

be straightforwardly used to extract information about water molecules interacting with isotope labeled proteins, without the need of selective pulses or advanced relaxation schemes. In this experiment, the $^{14}N,^{12}C$-filter removes any contributions from protein alpha protons under the water signal. For a reasonably sized protein (in the present case 18 kDa), transverse relaxation during the ROESY spinlock suppresses practically all contributions from the protein–ligand interactions for longer spinlock durations, allowing us to simply analyze the 2D projections of the ^{15}N-edited/$^{14}N,^{12}C$-filtered ROESY. For MMP-12, no water contacts could be identified inside the active site, neither in the complex with the CGS27023A inhibitor nor in the apo form of the protein. The absence of water interactions detectable by NMR at the binding site of the apo form of MMP-12 implies that the water residence times there are less than 1 ns. Two water molecules embedded into cavities in the surface of MMP-12 were identified in the current work. Their residence times are expected to be in the low nanosecond regime.

Supplementary material

Supplementary material comprises a schematic drawing of pulse program for the 3D ^{15}N-edited/$^{14}N,^{12}C$-filtered ROESY experiment and the build-up curves (cross peak intensity vs. increasing mixing time) of the CLEANEX and 3D ^{15}N-edited/$^{14}N,^{12}C$-filtered ROESY cross peaks.

References

Badger J (1997) Modeling and refinement of water molecules and disordered solvent. Methods Enzymol 277B:344–352

Bertini I, Calderone V, Cosenza M, Fragai M, Lee Y-M, Luchinat C, Mangani S, Terni B, Turano P (2005) Conformational variability of matrix metalloproteinases: beyond a single 3D structure. Proc Natl Acad Sci USA 102:5334–5339

Bertini I, Calderone V, Fragai M, Giachetti A, Loconte M, Luchinat C, Maletta M, Nativi C, Yeo KJ (2007) Exploring the subtleties of drug-receptor interactions: the case of matrix metalloproteinases. J Am Chem Soc 129:2466–2475

Böckmann A, Guittet E (1996) Suppression of radiation damping during selective excitation of the water signal: the WANTED sequence. J Biomol NMR 8:87–92

Borsi V, Calderone V, Fragai M, Luchinat C, Sarti N (2010) Entropic contribution to the linking coefficient in fragment based drug design: a case study. J Med Chem 53:4285–4289

Clore GM, Bax A, Wingfield PT, Gronenborn AM (1990) Identification and localization of bound internal water in the solution structure of interleukin 1.beta. by heteronuclear three-dimensional proton rotating-frame Overhauser nitrogen-15-proton multiple quantum coherence NMR spectroscopy. Biochemistry 29:5671–5676

Dalvit C, Hommel U (1995) Sensitivity-improved detection of protein hydration and its extension to the assignment of fast-exchanging resonances. J Magn Reson B 109:334–338

Devel L, Garcia S, Czarny B, Beau F, Lajeunesse E, Vera L, Georgiadis D, Stura E, Dive V (2006) Insights from selective non-phosphinic inhibitors of MMP-12 tailored to fit with an S1 loop canonical conformation. J Biol Chem 285:35900–35909

Grzesiek S, Bax A (1993) Measurement of amide proton exchange rates and NOEs with water in 13C/15 N-enriched calcineurin B. J Biomol NMR 3:627–638

Halle B (2002) Flexibility and packing in proteins. Proc Natl Acad Sci USA 99:1274–1279

Halle B (2003) Cross-relaxation between macromolecular and solvent spins: the role of long-range dipole couplings. J Chem Phys 119: 12372–12385

Halle B (2004) Protein hydration dynamics in solution: a critical survey. Philos Trans R Soc London, B 359:1323–1328

Holmes IP, Gaines S, Watson SP, Lorthioir O, Walker A, Baddeley SJ, Herbert S, Egan D, Convery MA, Singh OMP, Gross JW, Strelow JM, Smith RH, Amour AJ, Brown D, Martin SL (2009) The identification of [beta]-hydroxy carboxylic acids as selective MMP-12 inhibitors. Bioorg Med Chem Lett 19:5760–5763

Hwang T-L, Mori S, Shaka AJ, van Zijl PCM (1997) Application of phase-modulated CLEAN chemical EXchange spectroscopy (CLEANEX-PM) to detect water-protein proton exchange and intermolecular NOEs. J Am Chem Soc 119:6203–6204

Isaksson J, Nyström S, Derbyshire D, Agback T, Kovacs H, Bertini I, Giachetti A, Luchinat C (2009) Does a fast nuclear magnetic resonance spectroscopy- and X-ray crystallography hybrid approach provide reliable structural information of ligand-Protein complexes? A case study of metalloproteinases. J Med Chem 52:1712–1722

Islam SA, Weaver DL (1990) Molecular interactions in protein crystals: solvent accessible surface and stability. Proteins: Struct, Funct, Bioinf 8:1–5

MacPherson LJ, Bayburt EK, Capparelli MP, Carroll BJ, Goldstein R, Justice MR, Zhu L, Hu S, Melton RA, Fryer L, Goldberg RL, Doughty JR, Spirito S, Blancuzzi V, Wilson D, O'Byrne EM, Ganu V, Parker DT (1997) Discovery of CGS 27023A, a non-peptidic, potent, and orally active stromelysin inhibitor that blocks cartilage degradation in rabbits. J Med Chem 40:2525–2532

Modig K, Liepinsh E, Otting G, Halle B (2004) Dynamics of protein and peptide hydration. J Am Chem Soc 126:102–114

Morales R, Perrier S, Florent J-M, Beltra J, Dufour S, De Mendez I, Manceau P, Tertre A, Moreau F, Compere D, Dublanchet A-C, O'Gara M (2004) Crystal structures of novel non-peptidic, non-zinc chelating inhibitors Bound to MMP-12. J Mol Biol 341:1063–1076

Mori S, Berg JM, Zijl PCM (1996) Separation of intramolecular NOE and exchange peaks in water exchange spectroscopy using spin-echo filters. J Biomol NMR 7:77–82

Nar H, Werle K, Bauer MMT, Dollinger H, Jung B (2001) Crystal structure of human macrophage elastase (MMP-12) in complex with a hydroxamic acid inhibitor. J Mol Biol 312:743–751

Newby MI, Greenbaum NL (2002) Investigation of Overhauser effects between pseudouridine and water protons in RNA helices. Proc Natl Acad Sci USA 99:12697–12702

Nucci NV, Pometun MS, Wand AJ (2011) Site-resolved measurement of water-protein interactions by solution NMR. Nat Struct Mol Biol 18:245–249

Ohlendorf D (1994) Accuracy of refined protein structures. II. Comparison of four independently refined models of human interleukin 1[beta]. Acta Crystallogr Sect D: Biol Crystallogr 50:808–812

Otting G, Liepinsh E (1995) Selective excitation of intense solvent signals in the presence of radiation dampening. J Biomol NMR 5:420–426

Otting G, Liepinsh E, Farmer BT, Wüthrich K (1991a) Protein hydration studied with homonuclear 3D 1H NMR experiments. J Biomol NMR 1:209–215

Otting G, Liepinsh E, Wuthrich K (1991b) Protein hydration in aqueous solution. Science 254:974–980

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, vol 1, 2, 3. Cold Spring Harbor Laboratory Press, New York

Zwahlen C, Legault P, Vincent SJF, Greenblatt J, Konrat R, Kay LE (1997) Methods for measurement of intermolecular NOEs by multinuclear NMR spectroscopy: application to a bacteriophage N-peptide/boxB RNA complex. J Am Chem Soc 119:6711–6721