Experimental report

25/04/2017

Proposal: INTER-348 **Council:** 4/2016

Title: Internal time on IN13

Research area:

This proposal is a new proposal

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Samples: Ca2+
calmodulin-D2O
trifluoperazine (C21H24F3N3S)
peptide (C, H, O, N)

Instrument	Requested days	Allocated days	From	To
IN13	12	12	17/11/2016	28/11/2016

Abstract:

Experimental Report INTER-348

Effect of calmodulin-ligand binding on the sub-nanosecond protein dynamics

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Calmodulin (CaM) is a small Ca^{2+} binding protein and is involved in numerous signaling pathways in eukaryotic cells. Due to its marked conformational flexibility it also binds hundreds of different target molecules. In this project, we have explored the CaM dynamics underlying the conformational sub-states of CaM upon binding two different ligands. The binding of ligands is largely electrostatic in nature and sensitive to pressure. Thus, the ligand-calmodulin binding strength will be lowered gradually by the application of moderate pressures up to 4 kbar. holo-CaM is binding 4 Ca^{2+} ions. Initially, it has an open, dumbbell-shaped conformation. Bound to a target molecule it has a closed, globular conformation. When holo-CaM binds the antagonist trifluoperazine (TFP), the radius of gyration decreases from 21.9 Å to 17.6 Å, and the maximum diameter decreases from 70 Å to 45 Å [1]. In addition to TFP, we have also studied the binding of a short peptide, which represents the hypervariable region of the signaling peptide K-Ras4B, a key regulator in cells [2].

Here, we have used elastic incoherent neutron scattering (EINS) to reveal the sub-nanosecond dynamics of holo-CaM before and after binding the two ligands, TFP and the K-Ras4B peptide, using the instrument IN13. EINS at proteins probes averaged motions of hydrogen atoms on length scales of a few Å and time scales ranging from ps to ns, because hydrogen has a large incoherent neutron scattering cross section. The analysis yields values of the mean squared displacement (MSD) of atomic motions, $\langle u^2 \rangle$, reflecting a variety of hydrogen motions (vibration, rotation, translational diffusion) on a time scale faster than about 1 ns (which is defined by the energy resolution of the instrument). Moreover, the EINS experiments are carried out as a function of pressure up to 4000 bar (400 MPa) to gradually tune the CaM-ligand binding strength. According to le Châtelier's principle, pressure prefers any state that has a lower volume. When ion bonds are broken in aqueous solution, the concomitant hydration of the separated anion and cation leads to a denser packing of water molecules in the hydration shells, which will be a source for volume reduction [3].

In Fig. 1, the MSD of holo-CaM without and with TFP as ligand is plotted as a function of pressure. It has been obtained using a simple Gaussian approximation for the scattered neutron intensity, $I(Q)$, at small wavevector transfers, Q, within the energy resolution of the instrument, ΔE:

$$I(Q,0 \pm \Delta E) \approx I_0 \exp(-Q^2 \langle u^2 \rangle / 3)$$

Apparently, there is a pressure-induced decrease of the MSD for both samples reflecting the reduction in accessible volume, i.e., $\langle u^2 \rangle(p) \propto [V(p)/V(0)]^{2/3}$, with $V(0)$ being the

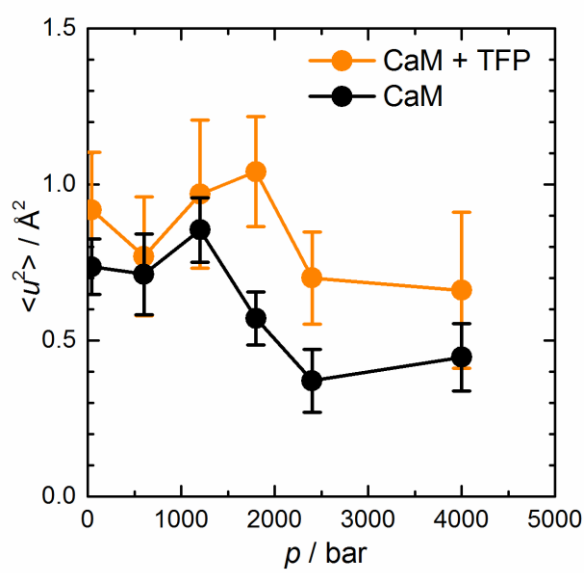

Fig. 1. Atomic MSD of CaM without and with bound TFP.

intrinsic volume of the protein at ambient pressure. However, when CaM binds TFP, the pressure-induced decrease of the MSD is retarded to higher pressures. It is suggested that the TFP ligand is stabilizing CaM against pressure perturbation. From SAXS data (not shown here), it is known that the closed, globular conformation of CaM with TFP is pressure stable up to a few kbar, whereas the open, dumbbell-shaped conformation of CaM is partially unfolding over the pressure range from 500 to 2000 bar. Moreover, pressurization of holo-CaM above 3000 bar will lead to a Ca^{2+} release [4]. Of course, the EINS intensity is also affected by the translational diffusion of the protein. The closed, globular conformation of CaM with bound TFP has a larger diffusion coefficient than the open, dumbbell-shaped conformation, which contributes to the larger $<u^2>$ value.

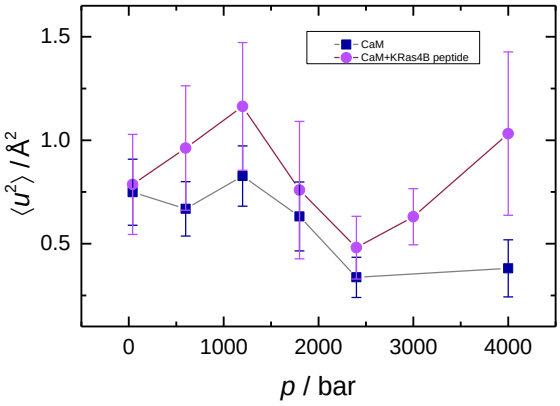

Fig. 2. Atomic MSD of CaM without and with bound K-Ras4B peptide.

In Fig. 2, corresponding data are shown, where the K-Ras4B peptide is binding to CaM. Again, the binding of the ligand induces a compact protein shape and will increase the diffusion constant of CaM, thus enhancing the observed MSD. However, the peptide is interacting with CaM much weaker than TFP. The pressure-induced reductions of the MSD are very similar for CaM without and CaM with bound peptide (the 4 kbar data point with peptide has a large scatter). Thus, pressure-dependent studies of the MSD of hydrogen atoms, as performed in this project, provide a sensitive means to measure the interaction of proteins with target drugs.

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[3] Q. Luong, S. Kapoor, R. Winter, *ChemPhysChem* **16** (2015) 3555.
[4] N. Erwin, S. Patra, R. Winter, *Phys. Chem. Chem. Phys.* **18** (2016) 30020.