Experimental Report

Proposal: **8-04-728** **Council:** 4/2014

Title: Molecular Dynamics of Serine Hydrolases

This proposal is continuation of: 8-04-708

Researh Area: Biology

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Samples: bacterial serine hydrolase SsEM28
human phospholipase A2 (hPLA2VII)

Instrument	Req. Days	All. Days	From	To
IN13	9	6	12/11/2014	18/11/2014
IN16B	2	2	19/11/2014	21/11/2014
IN6	5	5	28/11/2014	03/12/2014

Abstract:

The serine hydrolase family contains many pharmacologically important enzymes and drug targets. In our research, we use this protein family to investigate fundamental aspects of molecular recognition, mechanism of catalysis/inhibition and rational drug design. In this proposal we would like to investigate the molecular dynamics of two serine hydrolases (phospholipase A2 and bacterial serine hydrolase SsEM28) in the presence or absence of ligands (inhibitors). This study is a follow up on a study initiated 2013 on the serine hydrolase acetylcholinesterase (ILL exp. report 8-04-708). The results obtained by neutron scattering will be related to data from other computational and experimental techniques applied by us to this particular system.

Molecular dynamics of Chymotrypsin in Absence and Presence of Covalent Inhibitors

Experiment 8-04-728 on the instruments IN16B: 19 – 21/11/2014 (time scale up to 1 ns), IN13: 12 – 18/11/2014 (time scale up to 100 ps) and IN6: 28/11 – 3/12/2014 (time scale up to 10 ps)

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Chymotrypsin is a serine protease that selectively cleaves peptide bonds next to aromatic residues. The precursor of chymotrypsin (chymotrypsinogen) is produced in the pancreas, and the active form of chymotrypsin is generated when trypsin cleavage chymotrypsinogen. The mature enzyme has a molecular weight of approximately 25 kDa and the catalytic triad (His57, Asp102 and Ser195), is exposed to the bulk solvent. Chymotrypsin is a well-studied enzyme and several classes of both covalent and reversible inhibitors have been reported.

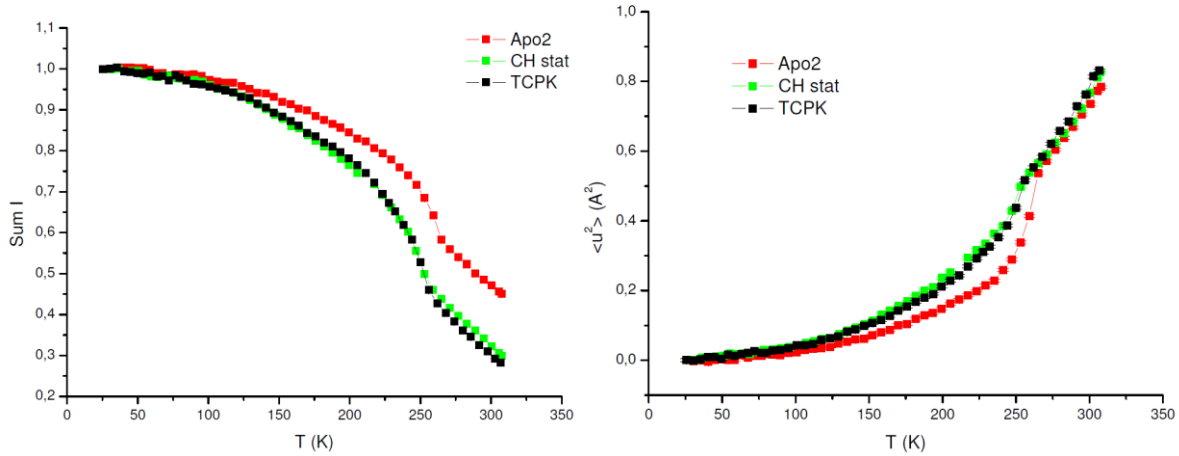
F. Gabel and co-workers [1] and J. Peters and co-workers [2, 3] have previously successfully used elastic, quasi-elastic and inelastic incoherent neutron scattering to investigate the temperature dependence of the dynamics of AChE and butyrylcholinesterase, in complex or not with an inhibitor. The current experimental team has complemented these previous studies by exploring the temperature dependent dynamics of mAChE, C5685(R)•mAChE and C5685(S)•mAChE in order to gain additional knowledge about protein-ligand interactions (experiment 8-04-708).

In the present study we include a different serine protease, chymotrypsin, in order to expand our studies to more proteins. We have studied the molecular dynamics of the protein in the absence (Apo1 and Apo2, two different preparations) and presence of two covalent inhibitors, chymostatin (CH stat) and N-p-Tosyl-L-phenylalanine chloromethyl ketone (TCPK). Again we used the same spectrometers IN6, IN13 and IN16 for a better comparison with the previous results and to have a complete dynamical picture within the time window up to 1 ns.

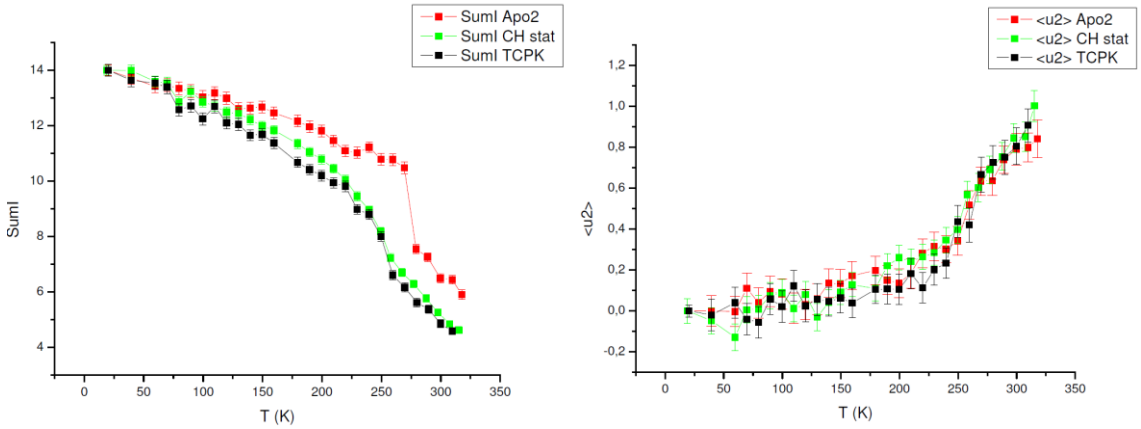
On all three instruments, it becomes clear that the enzyme was more flexible in presence of an inhibitor, maybe even slightly more in presence of TCPK (Figure 1). Apo1 was only measured on IN6 and it shows a similar behavior as Apo2, with the exception of a little bump for the summed intensities of Apo2 around 240 K. Interestingly, the maximum value of the MSD reached at high temperature is almost the same on IN13 and on IN16B, what would mean that no additional motions are coming up between 100 ps and 1 ns (no larger transitional motions), but there is a difference between the values found on IN6 and the values found on IN13. On IN6 we mainly see small local motions induced by the surrounding water molecules, whereas the motions that we see on IN13 correspond to the local dynamics of the protein side chains. Quasi-elastic measurements were also performed on IN6 and IN16B and analysis of the data is ongoing.

A multivariate analysis using principal component analysis of the intensities at different Q-values reveal that especially the data of IN13 clearly can differentiate between the different samples. The differences are most apparent in the temperature range between 110K and 250K. CH stat/Chymotrypsin is less flexible than TCPK/Chymotrypsin, and show more dynamics similarities to the apo structure, (although more flexible). TCPK/Chymotrypsin show a smoother transition when temperature goes up with increased motions at lower temperatures compared to the other two samples.

IN16B



IN13



IN6

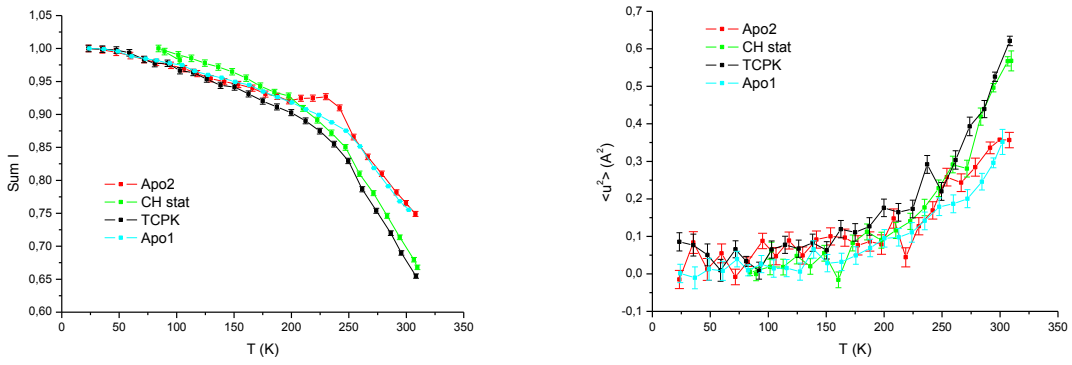


Figure 1. On the left side, we presented the intensities summed over all scattering angles or Q values, on the right side are the mean square displacements (MSD).

References

[1] Gabel, F.; Weik, M.; Masson, P.; Renault, F.; Fournier, D.; Brochier, L.; Doctor, B. P.; Saxena, A.; Silman I.; and Zaccai, G., *Biophys. J.,* 2005, 89, 3303–3311.

[2] Peters, J.; Trovaslet, M.; Trapp, M.; Nachon, F.; Hill, F.; Royer, E.; Gabel, F.; van Eijck, L.; Masson, P.; Tehei, M., *Phys. Chem. Chem. Phys.* (2012), 14, 6764–6770.

[3] Trovaslet, M.; Trapp, M.; Weik, M.; Nachon, F.; Masson, P.; Tehei, M.; Peters, J., *Chem Biol Int* 203 (2013) 14 – 18.