Experimental Report

Proposal: **8-04-681** **Council:** 4/2012

Title: Dynamics of Human Acetylcholinesterase complexed with the covalent Soman inhibitor

This proposal is a new proposal

Researh Area: Biology

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Samples: human acetylcholinesterase complexed with soman

Instrument	Req. Days	All. Days	From	To
IN16	3	3	30/11/2012	03/12/2012
IN6	4	4	15/11/2012	21/11/2012
IN13	2	2	07/12/2012	09/12/2012

Abstract:

Human Acetylcholinesterase (hAChE) is an enzyme, which actually attracts much attention, because it is fundamental for nervous system function being involved in various diseases. In 2009, we measured the dynamics of the sample, complexed or un-complexed with the non-covalent Huperzine A inhibitor as a function of temperature on three different spectrometers (IN6, IN13 and IN16). As result, we found almost no differences in the mean square displacements (MSD's) of the inhibited enzyme with respect to the native form, but significant variations between both concerning the vibrational density of states (DOS) on IN6 at 80K and QENS measurements taken on IN16.

The aim of the present proposal is to repeat the same measurements with hAChE complexed now with the covalent inhibitor Soman, because we are expecting more dramatic effects on the motions in this case. The results will help us to better understand possible relations between enzymatic activities and molecular dynamics.

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Dynamics of Human Acetylcholinesterase complexed with the covalent Soman Inhibitor

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Human Acetylcholinesterase (hAChE) is an enzyme, which actually attracts much attention, because it is fundamental for nervous system function being involved in various diseases. Overwhelming inhibition of hAChE is invariably lethal. However, moderate reversible inhibition of this enzyme is effective in the treatment of a number of diseases as in the case of Alzheimer's disease (1), or in pre-treatment of organophosphorus poisoning. Having recently succeeded to purify hAChE in sufficient quantities for neutron experiments, we characterized extensively its dynamics as function of temperature and pressure during the last few years (2). We measured first the dynamics of the sample, complexed or un-complexed with the non-covalent Huperzine A (HupA) inhibitor as a function of temperature on three different spectrometers (IN6, IN13 and IN16) (3). As result, we found almost no differences in the mean square displacements (MSD's) of the inhibited enzyme with respect to the native form. Just at the denaturation temperature (350 K) on IN13, we extracted a very little decrease of the MSD's of the inhibited enzyme with respect to the wild form. This observation is in accordance with results from Gabel et al. (4) for tetrameric human butyrylcholinesterase (hBChE), both native and covalently inhibited by the organophosphate nerve agent Soman, and with results of molecular dynamics simulations (5).

Furthermore, we probed the vibrational density of states (DOS) on IN6 at 80K for two resolutions (50 and 100 μeV). It revealed a significant softening of the vibrations relative to the unbound enzyme, meaning that the complexed form is more flexible, in accordance with results of Balog et al. (6) on DHFR. The elastic results on IN16 were also compared with the data of Gabel et al. (4) on hBChE. Noteworthy, hAChE, which has a catalytic activity about ten times higher than hBChE, seemed to be much more flexible than hBChE (7) or mouse AChE (8). While we concentrated our investigations so far on the action of a non-covalent inhibitor (3), F. Gabel et al. (4,9) did a comparative neutron scattering study of native hBChE and its conjugate with the covalent inhibitor Soman, known to dramatically increase the thermostability of the enzyme. Their results suggested that the stabilization of hBChE phosphorylated by Soman was due to an increase in free energy of the unfolded state related to a decrease in entropy.

The aim of the present proposal was thus to measure the dynamics of hAChE/Soman conjugates as a function of temperature to get as complete insights as possible in comparison with the hAChE/HupA complex and hBChE/Soman conjugate. The sample preparation and the experiments were almost identical to the previous ones, as described

in (2, 3 and 7). We extracted mean square displacements from the elastic data collected on IN13 and IN16 (see Figure 1).

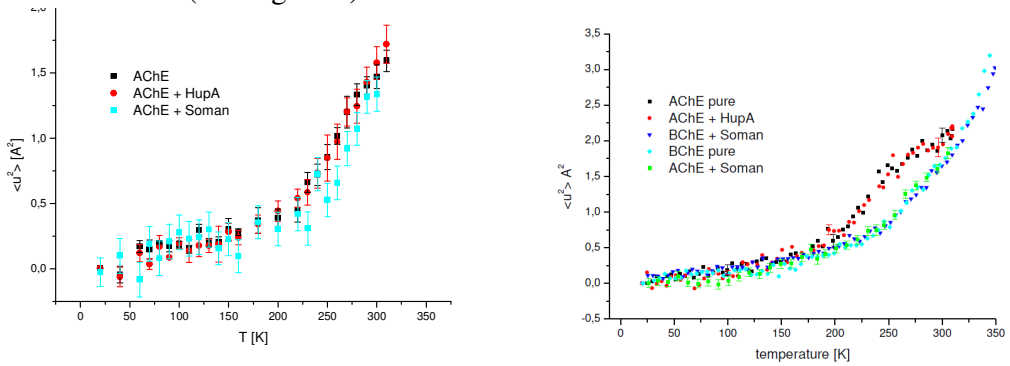

Figure 1: Mean square displacements (MSD) extracted from elastic data collected on IN13 (left) and on IN16 (right).

Surprisingly, the effect of Soman on AChE dynamics is much more dramatic than that of HupA, reducing it to the same level as the dynamics of BChE or BChE+Soman on IN16. The decrease of the MSD in presence of Soman is also confirmed on IN13. The neutron experiments results will now be compared with enzyme kinetics, thermal stability measurements, and molecular dynamics simulations to identify putative correlations between global dynamics properties and atomic-level events.

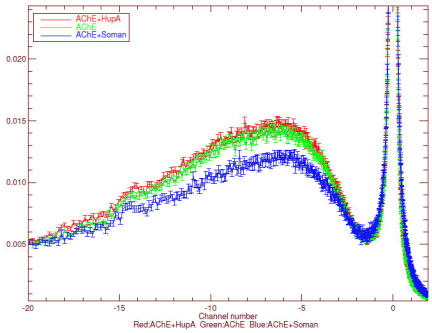

Figure 2: Density of states extracted from IN6 data for AChE (in green), AChE+HupA (in red) and AChE+Soman (in blue).

Figure 2 shows a preliminary analysis for the DOS measured on IN6. Whereas a softening of the vibrations was found in presence of HupA relative to the unbound enzyme, what means that the complexed form is more flexible, Soman seems to significantly strengthen the enzyme, in accordance with the elastic data. The analysis of the QENS measurements on IN6 and on IN16 is still under progress.

1. H. Soreq et al., Nat. Rev. Neurosci, 2, 294-302, 2001
2. ILL exp. reports CRG-IN13 1501, 8-04-497, 8-04-547, CRG-IN13 1791
3. M. Trapp et al., J. Phys. Chem. B. **116** (2012) 14744 – 14753.
4. F. Gabel et al., Biophys. J., 89, 3303-3011, 2005
5. Tara, S., Straatsma, T.P. and McCammon J.A. (1999) Biopolymers, 50, 35-43.
6. Balog, E. et al., (2004) Phys. Rev. Lett., 93, 028103,1-4.
7. J. Peters et al., Phys. Chem. Chem. Phys. **14** (2012), 6764 – 6770.
8. M. Trovaslet et al., Chem Biol Int (2012), DOI: 10.1016/j.cbi.2012.08.004.
9. F. Gabel et al., Biophys. J., 96, 1489-1494, 2009