# TOWARDS AN UNDERSTANDING OF THE INTRAMOLECULAR SIGNAL TRANSDUCTION THROUGH ENZYMES 3D STRUCTURE

**G. Hervé[1], J.-M. Zanotti[2] and M.-C. Bellissent-Funel[2]**

[1]Biochimie des Signaux Régulateurs Cellulaires et Moléculaires, Université Pierre et Marie Curie, Paris
[2]Laboratoire Léon Brillouin (CEA-CNRS), CEA-Saclay, 91191 Gif-sur-Yvette cedex

*E. coli* aspartate transcarbamylase (ATCase) is an allosteric enzyme which catalyses the first committed step in pyrimidine biosynthesis. As shown by X-ray crystallography, the binding of the substrates into the protein induces significant conformational changes. The protein switches from a T form towards a R form which results in an increase of 5% of the radius of gyration of the protein. We study the effect of substrate binding on the local picosecond dynamics of ATCase by incoherent quasi-elastic neutron scattering. The implication of these results in the field of signal transduction within enzymes 3D structure is tentatively discussed.

## Introduction

In the field of biomolecular regulation, ATCase is an interesting molecular machinery. This 310 kDa enzyme, whose structure is reversibly and co-operatively modified by the presence of its substrates, presents all the known regulation modes of catalytic activity. The latter is in particular strongly activated by ATP, a puric base, but inhibited by CTP and UTP, the two pyrimidic bases that are the final products of the reaction catalysed by ATCase. This interplay of activation and retro-inhibition that ensures the intracellular balance of puric and pyrimidic bases for DNA and RNA synthesis in healthy organisms is seen as a promising powerful tool to be used in cancer therapy.

*At the molecular level, how does the fixation of a first substrate on one of the six catalytic sites influence the affinity for the substrate of the other five sites, distant of as far as 70 Å?* This is the central problem of "signal transduction". The enzymatic activity of ATCase is modulated by large structural changes (Fig. 1). As a consequence of this structural modification, the radius of gyration of protein, as measured by small angle X-ray scattering increases by 5% [1].

*Is enzymatic activity modulated by internal dynamics?* The inspection of high resolution structures only enables to estimate enthalpic contributions. Entropic contributions, originating partly from dynamic contributions, are difficult to deduce from structures. Inelastic neutron scattering can help to solve the problem.

## Material and method

ATCase was purified from overproducing strains [2]. The protein concentration of the solution was determined by absorbance measurement and found to be 18 mg/ml (0.06 mM). The switch from the T form towards the R form has been obtained by dissolution of exchanged N-(phosphonacetyl)-L-aspartate (PALA) in the solution. The corresponding buffer solution was prepared by addition of same amount of exchanged PALA to buffer solution. Each sample was sealed in a 3.0*4.0*0.8 $mm^3$ aluminium cell.

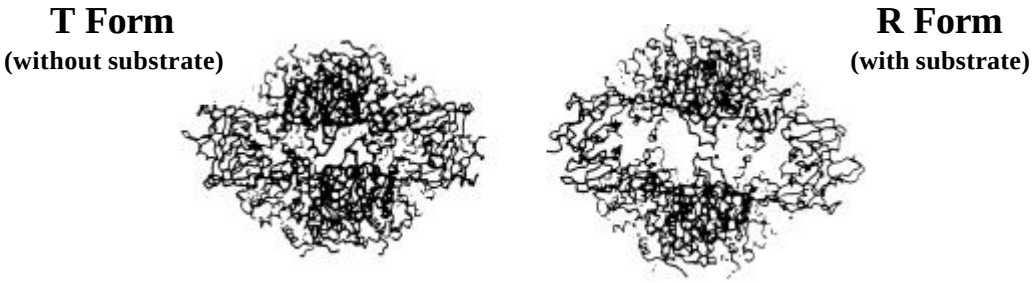


**Figure 1:** ATCase conformation without substrate (T form) and in liganded state (R form). View along the $C_2$ axis from [1].

**Inelastic neutron scattering**

Incoherent quasi-elastic neutron scattering is a unique tool to get experimental information on protein dynamics in the 0.1 to several hundreds ps range. It takes advantage of the large incoherent scattering cross-section of hydrogen atoms ($\sigma_{inc}$ = 80 barn) with respect to that of other atoms (C, O, N) in proteins. In the present case, we have recorded the difference induced by a slight modification of the system by stabilising the protein in the R state with a non-metabolisable substrate analogue: PALA.

**Experiments**

The experiments have been performed on MIBEMOL time-of-flight spectrometer of LLB using two different energy resolutions (96 μeV, λ = 6 Å and 410 μeV, λ = 4 Å). Figure 2 presents a quasi-elastic spectrum obtained at a resolution of 96 μeV.

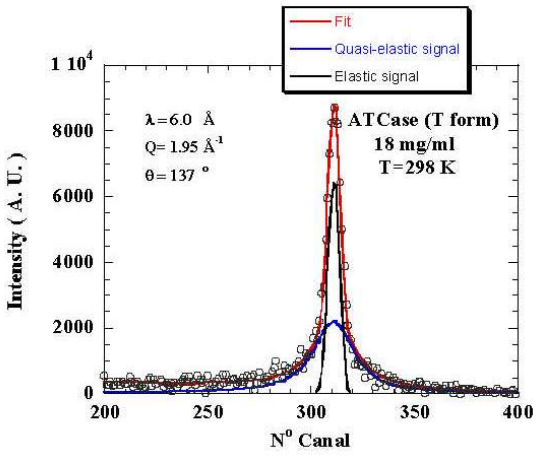


**Figure 2.** Incoherent quasi-elastic spectrum of T form of ATCase 18 mg/ml at T=298 K. The buffer signal has been subtracted. The resolution is 96 μeV. Circles are the experimental points, the red solid line is the model fit using an elastic peak and a single Lorentzian line. The elastic and quasi-elastic contributions are shown as black and blue lines respectively.

**Results**

From the Q dependence of the integrated intensity of the quasi-elastic region, the averaged mean-square vibrations of protons $\langle u^2 \rangle$in the protein are obtained. There is no significant effect of PALA on their values given below:

$\langle u^2 \rangle_{ATCase}$ /3=0.09 ±0.05 Å$^2$ and $\langle u^2 \rangle_{ATCase+PALA}$/3=0.07 ±0.05 Å$^2$.

The spectra are well described by an elastic peak (correlation times longer than the time resolution) and a quasi-elastic component with a Lorentzian line shape (Fig. 2) [3]. The self-diffusion coefficient of the protein in solution has been taken into account in the analysis of the spectra. From the Elastic Incoherent Structure Factor (EISF) we access the geometry of motions. Due to the narrow time window of the instrument, the dynamics of the system is well described by a single Lorentzian line providing with a single correlation time, even if a broad distribution of correlation times exists in the system. Due to the complexity of the system, we have chosen to describe the EISF in terms of a simple model: a model of a particle diffusing inside a sphere of radius a allows to fit properly the data and to show that the diffusive motions are experienced by surface residues (Fig. 3).

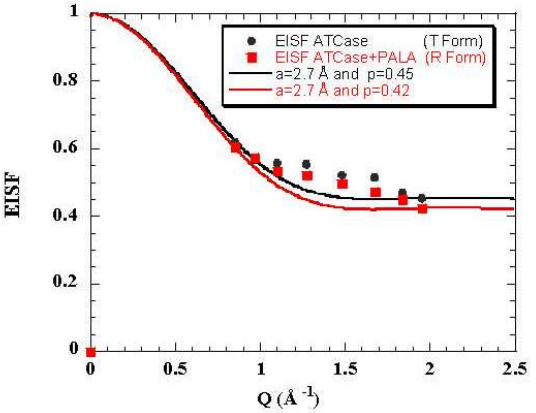


**Figure 3.** Experimental Elastic Incoherent Structure Factor of T and R forms of ATCase. Error bars are of the size of the points. Fit using the model from ref. 3 (red and black full lines). The extra broad contribution around 1.4 Å is due to a coherent contribution, not taken into account by the model.

**Conclusion**

Short-time dynamics of ATCase has been studied by quasi-elastic neutron scattering. At room temperature, about 55% of protons experience diffusive motions inside a sphere of radius 2.7 Å, on a time-scale of 15 picoseconds. The fraction of mobile protons essentially accounts for protons of the polar and charged side-chains at the surface of the protein. A slight, but significant difference in the dynamics of T and R forms of ATCase, is revealed by an increase of 3% of the fraction of protons experiencing diffusive motions upon ligand binding (see EISF). The obtained difference is not due to a modification of the whole protein dynamics**.** It is a consequence of the modification of the structure protein upon ligand binding. The protein accessible surface

area is increased by 16000 Å$^2$. This large increase of surface accessibility is consistent with the take up of 200 water molecules as measured by Li Cata et al. [4] by osmotic stress technique. This important finding shows how much careful comparison between neutron scattering data from different proteins has to be made. This is particularly the case for homologous proteins (extremophiles as an example) that have the same function but different sequences and so slight difference between accessible surface areas or conformations.

[1] L. Fetler, P. Tauc, G. Hervé, M. Moody and P. Vachette, J. Mol. Biol., 251 (1995) 243.
[2] S. F. Nowlan and E. R. Kantrowitz, J. Biol. Chem., 260 (1985) 14712-14716.
[3] J.-M. Zanotti, M.-C. Bellissent-Funel and J. Parello, Biophys. J., 76 (1999) 2390;
J. Perez, J.-M. Zanotti and D. Durand. Biophys. J., 77 (1999) 454.
[4] V. J. Li Cata and N. M. Allewell, Biochemistry, 36 (1997) 10161-10167