Experimental report

24/08/2016

Proposal: 9-11-1771 **Council:** 4/2016

Title: Dynamics of hydration water of Hyaluronic Acid

Research area: Soft condensed matter

This proposal is a continuation of CRG-2280

Main proposer: Isabelle MORFIN

Experimental team: Isabelle GRILLO
Dominik ZELLER
Judith PETERS
Isabelle MORFIN
Sylvie SPAGNOLI

Local contacts: Judith PETERS

Samples: hyaluronic acid C28H44N2O23

Instrument	Requested days	Allocated days	From	To
IN13	7	7	24/06/2016	01/07/2016

Abstract:

Hyaluronic acid (HA) is one of the most important polysaccharide in biology. It can associate with proteins by specific interactions but also via electrostatic interactions, hydrogen bounding and hydrophobic forces. One important properties of the HA is that its biological functions can be opposite depending on its molecular weight. Like any biological system, its functionality is related to the dynamics of hydration water. With the goal of understanding the mechanisms followed by this versatile biopolymer and in particular the effect of the chain length, we propose to continue the characterization of the dynamics of its hydration water by elastic incoherent neutron scattering. We already investigated 2 hydration rates for a long chain HA sample, in H2O, in D2O and in the dry state. The characterization of the influence water has on the dynamical transition is under investigation. We would like with this proposal to complete our data by measuring another (a second) HA mass in the same hydration conditions, in order to study the possible relation between their biological opposite functions and the hydration properties. The time required for such an experiment will be 7 days on IN13.

Report of experiment 9-11-1771 on IN13

I. Morfin, M. Plazanet, S. Spagnoli, J. Peters (LiPhy), J. Combet (ICS Strasbourg), I. Grillo (ILL)

Dynamics of hydration water of Hyaluronic acid

Biopolymers, in particular polysaccharides, have a great importance in biology. Among these polymers, hyaluronic acid (Ha) has an important role in various biological processes, being largely present in the human body. It can associate with proteins by specific interactions but also via electrostatic interactions [Moss]; its biological functions can be opposite depending on its molecular weight [Stern].

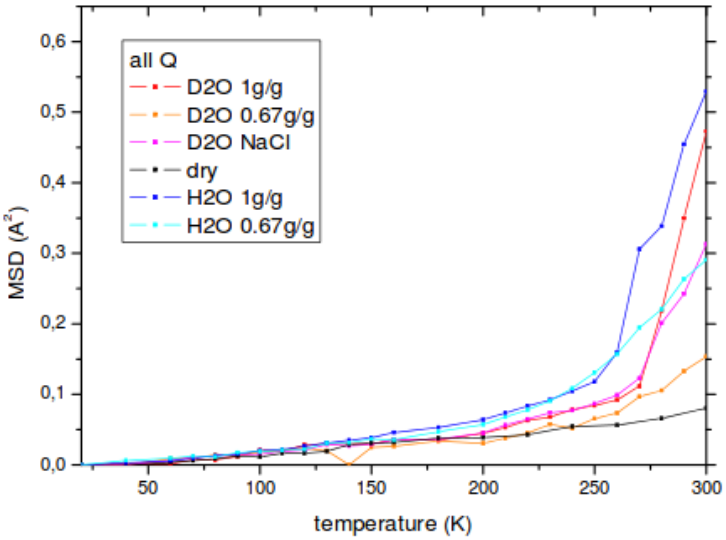
From the group of substances known as glygoaminoglycan (GAG), Ha is a linear polyelectrolyte with the repeating disaccharide structure poly((1→3)-β-D-GlcNAc-(1→4)-β-DGlcA). In addition, and in spite of its natural origin, Ha is an excellent model for polyelectrolyte of the semi-rigid class [Buhler] that is able to associate via electrostatic forces but also eventually via H-bonds. The understanding of the properties of this polysaccharide with complex behavior remains, nowadays, a soft matter subject that has to consider electrostatic, H-bond, and hydrophobic forces.

It is now well established that hydration water has a large importance in biological systems, being necessary for the system to function. Protein fluctuations are indeed locked to water or solvent dynamics [Frauenfelder]. Very different situations occur: depending on the polymer surface, the water mobility can be either slowed down or enhanced. In most cases, the water at the protein surface presents the characteristics of confined water, with reduced mobility with respect to the bulk one [Bellissent-Funel]. Indeed, in some situations, due to a subtle balance of hydrophilic and hydrophobic sites at the polymer surface, the water can be hyper-mobile [Kabir, Fichou]. Several of the very important physiological roles that Ha plays in living organisms are functions directly linked to the hydration water (maintenance of viscoelasticity, in synovial fluid in the joints, or eye vitreous humor, control of the tissue hydration, water transport...). With the goal of understanding the mechanisms associated with Ha in the physiological conditions, complexed with other proteins or free in solution, it is therefore necessary to characterize the water dynamics at its surface and understand the role of the chain length. Having previously observed Ha hydration on a large molecular weight HA (Mw>100000g/mol, [Morfin2]), the current experiment was done with Ha small chains (molecular weight 16000 > Mw > 6500 g/mol).

The experiment was undertaken on IN13, in June 2016. We prepared 6 samples :

- a dry sample obtained after lyophilization
- 2 samples containing approximately 1 g H_2O for 1g of HA and another sample of 1.1 g D_2O for 1g of HA to obtain the same hydration level (called 1g/1g later on)
- 2 samples containing approximately 0.67g H_2O for 1 g HA and 0.74 g D_2O for 1g HA, called 1g/0.67 g hereafter.
- 1 sample of 1.1 g D_2O for 1g of HA with [NaCl]=150mM.

All samples were scanned elastically as function of temperature in the range 20 to 310 K and treated further with LAMP. From there we extracted mean square displacements for all samples in the Q range from 0.2 to 4.5 A^{-1} (see figure):



At a first glance, the differences due to chain lengths seem to be minor, however, addition of NaCl clearly reduces dynamics at the same level of hydration.
A further analysis of these data compared to the previously obtained ones with large HA chains are under progress.

References:

[Buhler] Buhler, E. ; Boué, F. *Macromolecules* 37 *(*2004), 1600-1610.
[Bellissent-Funel] S. Dellerue and M.-C. Bellissent-Funel, *Chem. Phys.* 253 (2000) 315-325.
[Fichou] Y. Fichou et al., *Proc. Nat. Acad. Sci. USA* 112 (2015) 6365 - 6370
[Frauenfelder] H. Frauenfelder, G. Chena, J. Berendzena, P.W. Fenimore, H. Jansson, B.H. McMahon, I.R. Stroe, J.
Swenson and R.D. Young, *Proc. Nat. Acad. Sci. USA* 106 (2009), 5129-5134.
[Kabir] Kabir *et al.*, *Biophys. J.* 85 (2003), 3154-3161.
[Kogan] Kogan, G; Soltes, L; Stern, R; Mendichi, R, chap. 31 of Handbook of Polymer research (2007) Nova Science Publishers, Inc., Editors: Perhrick et al.
[Morfin] Morfin, I.; Grillo, I.;Cousin, F.; Buhler, E., Boué, *Biomacromolecules* 12 (2011), 859-870.
[Morfin2] Morfin, I. et al., ILL report CRG 2280.
[Moss] Moss, J; Van Damme, M.-P.; Murphy, W.; Preston, *B. Arch. Biochem. Biophys.* 348 *(*1997), 49-55.
[Stern] Stern, R.; sari, A.A.; Sugahara, K.N. *Eur. J. of Cell Biol.* 85 *(*2006), 699-715.
[Zaccai] Zaccai, G., *Science* 288 (2000), 1604–1607.
[Zang] Zhang, R; Shklovskii, B. I. *Phys. Rev. E* 69 *(*2004), 021909.