Experimental Report

Proposal: **8-04-668** **Council:** 4/2012

Title: Biological behavior of bacterial spores

This proposal is a new proposal

Researh Area: Biology

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Samples: Bacillus subtilis cells and the mutants PS 4150 strain and FB 108 strain

Instrument	Req. Days	All. Days	From	To
IN13	11	8	22/11/2012	30/11/2012
IN5	2	2	29/11/2012	01/12/2012

Abstract:

Spores are very different from vegetative cells. They have many well separated compartments and it is difficult to get information on them separately. By investigating wild type spores and two mutants, we would like to get insights into hydration and water behavior of the different compartments and shed light on possible correlations between dynamics, the thermal transitions and the high resistance of the spores against stress. For that we will use elastic and quasi-elastic neutron scattering on IN13 and IN5.

Report experiment 8-04-668 on IN13 and IN5
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Biological behavior of bacterial spores

Bacillus subtilis cells have the capacity to form spores which are metabolically dormant in response to starvation. In this form, bacterial spores are highly resistant to stress and can survive for millions years [1]. They even represent the most resistant forms of life. Interestingly, when the conditions become favorable, bacterial spores can readily break their dormancy and return to their previous state of vegetative cells, the so-called germination process [2,3].

The structure of a bacterial spore is very different from that of a vegetative cell. It is a complex form with many layers, each layer having its own properties and participating in spore resistance to stress. It seems that the various components are behaving very differently, what makes it complicate to investigate them (as most of the techniques can only give average information for the whole sample) and to conclude about their properties individually. Some ideas of their characteristics are still controversial and thus the study of the biophysical properties and the mobility of bacterial spore components could probably answer some part of this question and explain the strong resistance of bacterial spores to stress.

One of the main specificity of bacterial spore is its ability to maintain the spore core in a relatively constant low hydrated state whatever the external conditions are (25-50% wet weight). This low, but constant hydration seems essential to maintain the functionality of vital biomolecules (ribosomes, enzymes, membrane receptors…) which are required for spore germinations. Quantity, localization, mobility and state of core water are up to now poorly known. Recent studies suggest that core water content and mobility could not explain solely spore resistance. The core is supposed to be hydrated at a level of 0,6 *h* (h = g water/g of dry matter), whereas a level of 0,1 *h* is required to allow a 40°C higher resistance of binary protein/water system as it is observed for bacterial spore compared to their vegetative form. They suggest that proteins would be immobilized, impeding complete unfolding and subsequent denaturation of the protein [4]. This finding had already been proposed by measuring the mobility of a GFP (Green Fluorescent Protein) associated to small acid soluble proteins (SASP) of the core using FRAP method (Fluorescence Recovery After Photobleaching) [5].

The coat is the outer layer of spores of a number of Bacillus species and it is composed mainly of proteins. It is only semi-permeable and it allows the passage of small molecules (<5kDa) to the spore's interior layers, in particular to the spores inner membrane where nutrient receptors are located. It also protects bacterial spores from lytic enzyme such as lysozyme, and other chemical agents [6]. The coat represents nearly 55% of the spore's proteins [4] shared between a soluble (70%) and an insoluble fraction [7]. For the study of water in the different compartments and the dynamics of proteins in the core, PS 4150, a mutant strain deleted for *CotE* and *GerE* (genes implicated in the assembly and the synthesis of spore's coat proteins) is a model of interest. It lacks most of the outer and inner coat proteins [6] and may allow isolating more accurately motions of proteins from the core.

Dipicolinic acid (DPA) is a specific component of bacterial spores accumulated during the last stage (IV to VI) of the sporulation process, concomitant to Ca2+ uptake, and would be implicated in wet heat resistance, probably because of its role in protoplasm dehydration [8]. It could account for up to 25% of the core dry weight [9]. Its precise role is still controversial because core proteins seem to remain somewhat immobile even after the leakage of DPA during germination of a mutant strain lacking the enzyme responsible for the lysis of the cortex. However, the present experiments will not allow to see potential differences in the mobility of core proteins with and without DPA because of the time scale considered here [5]. FB 108 is a mutant strain that cannot accumulate DPA during the sporulation process (modified on spoVF operon) [8]. It has also been used in our experiment in order to precise the role of this compound on the mobility of bacterial spore core water and proteins.

	Sample	Sample mass (g)	Dry weight (g_{dry}/g_{tot})	gD_2O/g dry weight
IN13 1	PS533-Spz-H2O	0,5411	0,27	2,71
2	PS533-2*SG liquid (slow kinetics)	0,4829	0,12	7,16
3	PS533-Spz	0,6587	0,15	5,52
4	PS533-2*SG agar	0,4991	0,14	6,08
5	PS4150-Spz	0,4956	0,17	4,73
6	PS533-2*SG liquid (fast kinetics)	0,5463	0,23	3,32

IN5	1	PS4150-Spz(2)	0,3569	0,19	4,17
	2	PS533-2*SG liquid	0,4981	0,1	8,71
	3	PS4150-Spz germinated on IN13	0,4956	0,17	4,73
	4	PS533-2*SG agar	0,5432	0,14	6,26
	5	PS533-Spz	0,4599	0,1	8,87
	6	FB108-Spz	0,61	0,12	7,25

Table 1: List of the samples measured on both instruments, including their masses and hydration states. (Spz and 2*SG correspond to the growth media used for sporulation of *Bacillus subtilis* : Spz = Spizizen Minimal Medium modified, 2*SG agar/liquid = 2*Schaeffer Glucose medium , Agar or liquid as specified)

We did first experiments on IN13 in June 2012 (CRG report 1910), where we concentrated on elastic measurements only and succeeded to see changes in the dynamics at temperatures corresponding to transitions in the samples. This time we investigated the dynamics of the wild type sample and several mutants on IN13 and IN5.

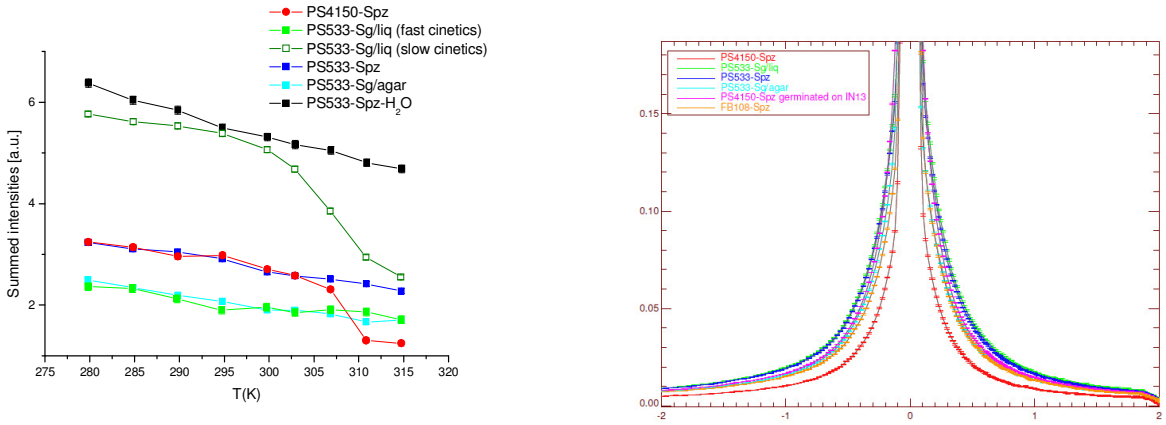

Figure 1: Summed intensities measured on IN13 (left side) and QENS curves summed over the whole Q range measured on IN5 at 75 µeV resolution and 290 K (right side).

Figure 1 shows interesting results obtained on both instruments. The summed elastic intensities are inversely proportional to the flexibility of the sample as function of temperature. Four of the samples present very similar slopes, but two of them germinated (as verified by phase contrast or DIC microscopy) during the experiment and show a clear jump around 307 K. However, the same sample (PS533-2Sg/liq) measured slowly (3.5h/T) or quickly (1h/T) undergoes or not the germination process. The quasi-elastic intensities are the largest for the most flexible samples. The data demonstrate clearly the tendency that the wild type samples are the most flexible and the mutants are more stable. The mutant germinated in advance on IN13 is the most flexible among the mutant samples. A detailed analysis of the data is still under progress.

References

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