NMR Structural studies of the starch binding domain from the alkaliphilic and thermophilic Bacillus sp. Strain TS-23

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In order to functionalize biomaterials based on starch, such as hydrogels or nanoparticles, a starch-binding module (SBM) was cloned and expressed using recombinant DNA techniques. The SBM, which belongs to an α-amylase from *Bacillus* sp. TS-23, tightly and specifically binds to starch based materials. Several applications can be envisaged for SBM fusion proteins as virtually any peptide/protein with biological activity can be linked to the SBM and subsequently be adsorbed to the starch biomaterial. For these types of approaches it is important to know the structure and mobility of the fusion proteins when bound to the biomaterials to predict accessibility and modes of interaction with cells.

Isotopic enrichment of SBM-TS23 in ^{15}N, and ^{13}C was achieved by using $^{15}NH_4Cl$ as the sole nitrogen source and U-$^{13}C_6$-glucose as the sole carbon source. Sample concentrations were ca. 1mM with 50 mM phosphate buffer at pH 7.0 and 100 mM KCl. Spectra were acquired on Bruker NMR spectrometers operating at a proton frequency of 500MHz and/or 600MHz with cryoprobe at 298K. Backbone assignment was carried out using standard procedures: the HNCO, HNCOCA (contains Cα *i-1*), HNCA (contains Cα *i* and *i-1*), HNCOCACB (contains Cβ *i-1*), and HNCACB (contains Cβ *i* and *i-1*) spectra allowed the backbone resonances to be assigned. The ^{15}N-NOESY spectrum was used to confirm spin system assignment using side chain patterns. Determination of the structure in solution is in progress and the residues that are affected when dextrin nanoparticles are present will be detected using chemical shift mapping. Analysis of backbone dynamics from ^{15}N relaxation measurements will also be used to determine dynamic behavior of SBD-TS23 with and without nanoparticles.

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