MODULATION OF *Bacillus subtilis* LEVANSUCRASE ELONGATION MECHANISM BY ENZYME CONCENTRATION

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Introduction. *B. subtilis* SacB levansucrase (EC 2.4.1.10) catalyzes fructosyl transfer from sucrose to synthesize levan, a fructose polymer with *β*2-6 linkages with many applications in the food, pharmaceutical and cosmetic industries [1]. Two types of levans are produced by SacB: a high molecular weight levan (LevanA) with an average of 3500 kDa and a low molecular weight levan (LevanB) with 8.3 kDa. Reaction conditions such as ionic strength, temperature and substrate concentration affect the distribution of levan molecular weight in reactions catalyzed by glycosyltransferases [2] [3]. However, there is no clear understanding of which structural elements determine the action of these enzymes and particularly the product molecular weight spectrum.
In this work we investigate the molecular weight distribution of the levan synthesized by SacB as a function of substrate and enzyme concentration. Based on the resulting product profile, hypotheses concerning the enzyme elongation mechanism are discussed.

Methods. Size-exclusion chromatography (SEC) was carried out on a Waters 717 system including two Ultrahydrogel SEC columns in series (Ultrahydrogel Linear, 7.8 x 300 mm, and Ultrahydrogel 500, 7.8 x 300mm). Ion exchange chromatography (HPAEC-PAD) of oligosaccharides was achieved on a Carbopac PA200 column (Dionex; 3 x 250 mm).

Results. SacB reactions were carried out in experiments with three enzymatic activities (0.1, 1 and 10 U/mL) and two sucrose concentrations (100 and 400 g/L). The experiments were performed at 37°C, pH 6 and 350 rpm. While sucrose concentration did not modify SacB product profile, the enzyme concentration significantly influenced enzyme specificity. The synthesis and the amount of LevanA and/or LevanB was a strong function of the enzymatic activity employed; actually, the molecular weight of synthesized levan was found as inverse function of enzyme concentration in the reaction (Fig. 1). In particular, at 10 U/mL only LevanB was synthesized by a non-processive elongation mechanism with different oligomers involved as intermediates, as observed when the evolution of the reaction was followed (Fig. 2).
In order to explore if this behavior was produced by a kinetic effect, enzyme concentration was also varied increasing total enzyme concentration (0.55, 5.5 and 55 µg/mL) while keeping enzymatic activity constant (0.1 U/mL). This was achieved by addition of the inactive mutant SacB E342A. Surprisingly, these experiments result in the same product profile already shown in Fig. 1, demonstrating that specificity modification depends on structural elements related to enzyme conformation and not to enzyme activity (kinetic elements).

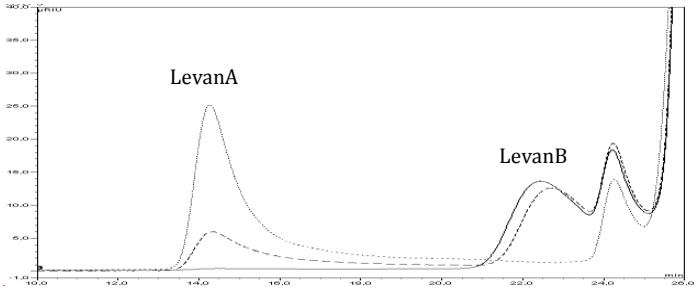

Fig. 1 Comparison of levan synthesis by different SacB enzymatic activities: 0.1 U/mL (•••), 1 U/mL (– –), 10 U/mL (—). Reaction conditions: 100 g/L sucrose, pH 6, 37°C (SEC).

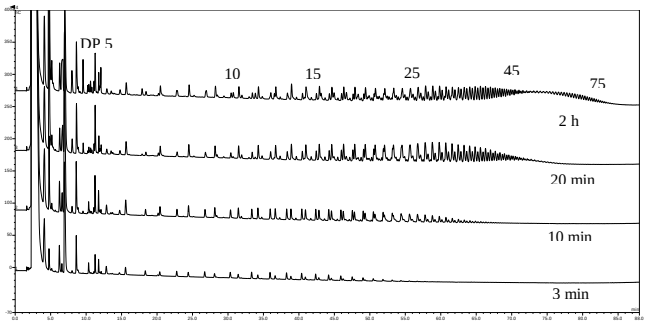

Fig. 2 Evolution of LevanB synthesis by SacB levansucrase. Reaction conditions: 10 U/mL, 400 g/L sucrose, pH 6, 37°C (HPAEC-PAD). DP=Degree of polymerization.

Conclusions. Our results reveal that synthesis of LevanB and LevanA may take place through different processes. We clearly demonstrate LevanB synthesis involves a non-processive mechanism. In addition, enzyme concentration modulates the two processes and is therefore strongly related to the type of elongation mechanism. We suggest that, depending on enzyme concentration (active or not) SacB may associate in forms that define the elongation mechanism or specificity which leads LevanB or LevanA production. This could explain why other factors also affect levan molecular weight distribution.

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