effect of different divalent cations on the synthesis of α-lactalbumin nanotubes

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α-Lactalbumin (α-La) is the second most prevalent whey protein in cow milk, at about 20% total protein content, thus contributing for its physical, biological and nutritional characteristics. Partial hydrolysis of α-La by a serine endoprotease from Bacillus licheniformis (BLP) will result in the formation of nanotubes in the presence of Ca\(^{2+}\) [1, 2]. Calcium ions act as salt-bridge between two negatively charged carboxylic groups formed after partial hydrolysis of α-La [3] stabilizing the nanotube structure. α-Lactalbumin can also promote binding with other positive charged ions, like Mn\(^{2+}\), Zn\(^{2+}\), Cu\(^{2+}\), Al\(^{3+}\), Mg\(^{2+}\) and Ba\(^{2+}\) [1].

In this work we investigated nanotubes formation from α-La employing two different divalent cations: Ca\(^{2+}\) and Mn\(^{2+}\), at different molar ratios (R) respect to α-La concentration (R = 1, 2 or 3). First we evaluated self-assembly by the light scattered with increasing sizes during nanotubes synthesis. We could see how in both cases, with Ca\(^{2+}\) and Mn\(^{2+}\), increasing the amount of the cation resulted in a faster peaking of the scattered light, i.e., increasing the self-assembly rate and therefore the nanotube formation rate. In the same way the self-assembly was increased when we used Mn\(^{2+}\) instead of Ca\(^{2+}\) for all the values of R assayed.

In order to follow the structure of the aggregates and the aggregation kinetics we used the same method previously described by Graveland-Bikker [4], measuring the optical density (OD) at 633 nm as function of the incubation time at 50 °C. In general the aggregation in presence of Ca\(^{2+}\) was slower than in presence of Mn\(^{2+}\). In addition the gels obtained with Ca\(^{2+}\) were weak for all of the relations studied and turbid for R = 2 and R = 3. In contrast the gels obtained with Mn\(^{2+}\) were stronger and more transparent. Graveland [1] appointed that the ions concentration has no effect on the hydrolysis kinetics and these differences could be attributed to an increase of self-assembly (i.e., nucleation) in presence of Mn\(^{2+}\), probably because this ion fits better in the specific bridge due to its size and/or preferred ligands orientation.

As the latency in the OD measurements is related with both hydrolysis and nucleation we studied the hydrolysis of α-La by RP-HPLC. The degree of hydrolysis (DH) was evaluated in a similar way to that described by Welderufael et al. [5]. Hydrolysis rate increased when we used Mn\(^{2+}\) instead of Ca\(^{2+}\) in the reaction. However, as Graveland [1] appointed for increasing Ca\(^{2+}\) ratios, this behaviour could not be due to an effect of the ion in the hydrolysis. Thus, in presence of Mn\(^{2+}\) self-assembly of partially hydrolyzed α-La was faster incorporating inside hydrolysis products and, thus, hardly accessible for the enzyme. However self-assembly in presence of Ca\(^{2+}\) was slower and therefore the enzyme had more substrate longer and continue further degradation of the hydrolyzate.

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References: