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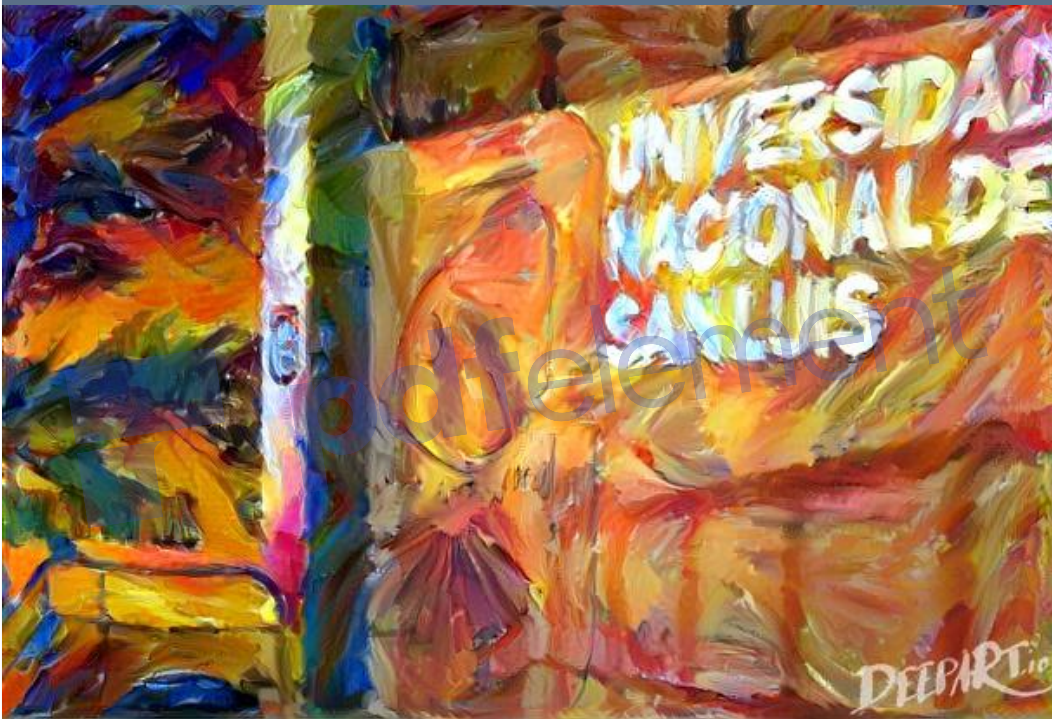

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Effect of PEG-induced molecular crowding on β-Gal thermal stability.

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The yeast β-galactosidase or lactase [EC 3.2.1.23] (β-Gal) is a soluble enzyme capable of catalyzing lactose hydrolysis into its constitutive monosaccharides: glucose and galactose. This enzyme has a commercial application for lactose hydrolysis in dairy products. Milk processing with b-Gal before milk is commercialized is important to solve nutritional (lactose intolerance) and technological (crystallization of dairy products) problems. In this context, it is important that the activity of β-Gal be evaluated in crowding media systems.

In this work we investigate the effect that molecular crowding induces on thermal stability of β-galactosidase from Kluyveromices lactis. PEG^{6000}, a non-charged highly water-soluble polymer with well-known effects on water dynamics was used to produce the crowded environment.

The effect of PEG on β-Gal thermal stability was studied with two different approaches. In the first one, β-Gal samples both in the absence or in the presence of PEG^{6000} were pre-incubated at different temperatures in a range from 37 to 75 °C. After that, the system was returned to optimal conditions and enzymatic activity was tested. Results obtained showed that β-Gal stability was enhanced in molecular crowded environment. The enzyme maintained its activity when it was pre-incubated at temperatures 5 degrees higher in the presence than in the absence of molecular crowding agent.

In the second approach, the inactivation kinetic was studied: in this type of experiments, the enzyme was pre-incubated at 37 or at 50 °C during different periods of time and after that, the enzymatic activity was measured in optimal conditions. Results obtained show again that molecular crowding conditions protect the enzyme from heat denaturation. In this case, it was observed that the enzyme maintains its activity even when it is subjected for a considerable period of time at high temperature when it is in the presence of the molecular crowding agent.

In both cases, the enzymatic reaction was evaluated by measuring kinetic parameters of β-Gal against an artificial substrate (ONPG).

Changes in protein compactness could be the responsible for the qualitative change behavior observed at the molecular crowding conditions assayed.

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