Intestinal cell targeting of a stable recombinant Cu–Zn SOD from Cucumis melo fused to a gliadin peptide

The mRNA encoding full length chloroplastic Cu–Zn SOD (superoxide dismutase) of Cucumis melo (Cantaloupe melon) was cloned. This sequence was then used to generate a mature recombinant SOD by deleting the first 64 codons expected to encode a chloroplastic peptide signal. A second hybrid SOD was created by inserting ten codons to encode a gliadin peptide at the N-terminal end of the mature SOD. Taking account of codon bias, both recombinant proteins were successfully expressed and produced in Escherichia coli. Both recombinant SODs display an enzymatic activity of ~5000 U mg−1 and were shown to be stable for at least 4 h at 37 °C in biological fluids mimicking the conditions of intestinal transit. These recombinant proteins were capable in vitro, albeit at different levels, of reducing ROS-induced-apoptosis of human epithelial cells. They also stimulated production and release in a time-dependent manner of an autologous SOD activity from cells located into jejunum biopsies. Nevertheless, the fused gliadin peptide enable the recombinant Cu–Zn SOD to maintain a sufficiently sustained interaction with the intestinal cells membrane in vivo rather than being eliminated with the flow. According to these observations, the new hybrid Cu–Zn SOD should show promise in applications for managing inflammatory bowel diseases.
Liens

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