Exolytic products of alginate by the immobilized alginate lyase confer antioxidant and antiapoptotic bioactivities in human umbilical vein endothelial cells

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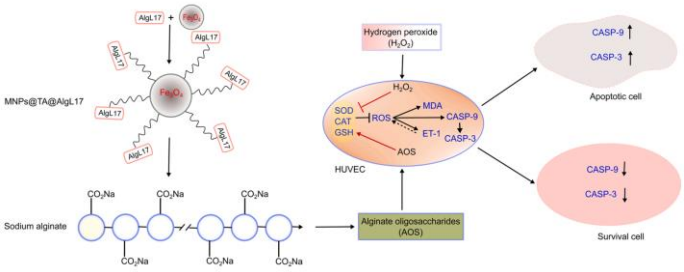
Abstract

Alginate is a natural polysaccharide resource abundant in brown algae and it can be cleaved into alginate oligosaccharides by alginate lyase. Alginate lyases and the bioactive alginate oligosaccharides have been applied in diverse fields such as pharmaceutical therapy and nutraceutical supplementation. Immobilized enzymes greatly facilitate their industrial application owing to their reusability, stability, and tunability. In this study, magnetic Fe3O4 nanoparticles were synthesized and used to immobilize an exolytic alginate lyase AlgL17 that was characterized previously. The immobilized AlgL17 demonstrated enhanced thermal and pH tolerance, extended storage stability, and moderate reusability. The mass spectrum indicated the specific activity of the immobilized AlgL17 to release alginate oligosaccharides (AOS) from alginate polysaccharide. The produced AOS exhibited their antioxidant and antiapoptotic activities in H2O2-stressed human umbilical vein endothelial cells by upregulation of reactive oxygen species scavenging activities and attenuation of the caspase-mediated apoptosis pathway.

Introduction



Purpose

This study provides a pilot exploration of production and application of immobilized alginate lyase AlgL17 on magnetic nanoparticles, and presents a better understanding of the antioxidant function of alginate oligosaccharides in human cells.

Materials & Methods

1. Preparation of AlgL17 crude enzyme and immobilization

➢The nanobeads werensurface functionalized with 2.5 % tannic acid (w/v) to obtain the MNPs@TA particles;

➢ To immobilize AlgL17 on MNPs@TA nanobeads, the beads were first treated with glutaraldehyde. MNPs@TA nanobeads (10 mg) were resuspended in 200 μL of 1.0 % glutaraldehyde solution (w/v) in 50 mM Tris-HCl buffer (pH 8.0) with ultrasonication treatment for 5 min and then incubated at 5 ◦C for 2 h for crosslinking of glutaraldehyde with MNPs@TA beads.

➢The morphology and distribution of the immobilized AlgL17 on MNPs@TA were observed under scanning electron microscope at the voltage of 15.0 kV (SEM; Quanta 250 FEG, FEI Company). the stored MNPs@TA@AlgL17 and MNPs@TA nanoparticles were used to prepare the thin plate using KBr as the matrix for infrared spectrum analysis using Fourier transform infrared spectrometer according to the manufacturer's instructions.

2. antioxidant and antiapoptotic bioactivities in human umbilical vein endothelial cells

➢100 μL of HUVECs cell culture (5 × 104 cells/mL) was transferred to each well of 96 well plate and incubated at 37 ◦C for 24 h. After aspirating the liquid medium, 100 μL o fresh growth medium containing different concentrations of AOS (0–1,000 μg/mL) wer added to the cells for additional incubation as specified. After the treatments, 20 μL o MTT (5 mg/mL in PBS buffer) was added to each well and incubated at 37 ◦C for 4 h.

➢To evaluate the oxidative stress in HUVECs, the content of malondialdehyde (MDA) i HUVECs and the secretion of endothelin-1 (ET-1) in the liquid medium were quantifie using the corresponding assay kits following the manufacture's instruction. The activitie of superoxide dismutase (SOD), catalase (CAT), and the levels of glutathione (GSH) wer measured using the corresponding assay kits following the manuals provided and were recorded as units per mg of total protein for SOD and CAT, and μmol per mg of total protein for GSH.

Conclusion

➢the immobilized AlgL17 on magnetic iron oxide nanoparticles was prepared and employed to produce alginate oligosaccharides through its exolytic degradation activity over sodium alginate.

➢ The advantages of developing Fe3O4-conjugated alginate lyase lie in the paramagnetic property of the iron oxide nanoparticles, its stability and reusability, and the avoidance of enzyme contamination in the products.

➢The produced AOS demonstrated effective antioxidant and antiapoptotic activities against hydrogen peroxide-induced oxidative stress in human umbilical vein endothelial cells, firmly supporting our hypothesis of the bioactivities of the exolytic products AOS.

➢The biochemical characterization of free radical scavengers and apoptosisinducing markers provided sound explanation for the AOS functioning against oxidative stress, building up a comprehensive image of the potential underlying mechanism of their functions in vivo.

➢ It opens up the possibility of further developing the immobilized AlgL17 for pharmaceutical and industrial purposes with more emphasis on its yield and stability

Results

1. Preparation of AlgL17 crude enzyme and immobilization

1.1 AlgL17 is immobilized on magnetic Fe3O4 nanoparticles

1.2 Enzyme catalytic properties and stability of immobilized AlgL17

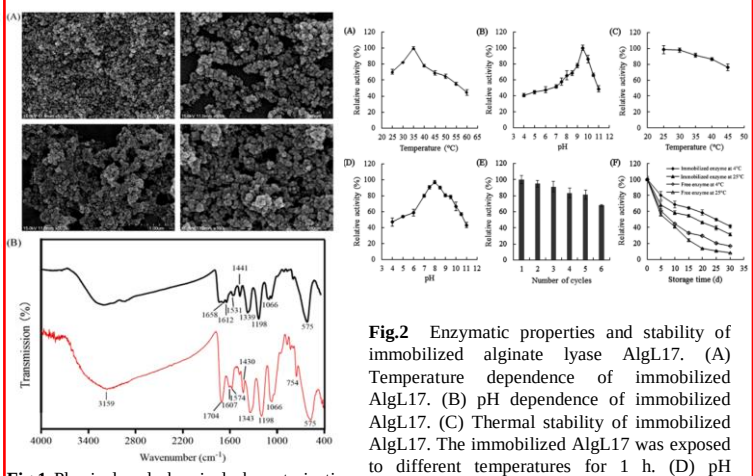


Fig.1 Physical and chemical characterizations of immobilized alginate lyase AlgL17. (A) Scanning electron microscopy (SEM) images of free MNPs@TA nanoparticles (top panel) and the immobilized AlgL17 (bottom panel). (B) Fourier transform infrared spectroscopy (FTIR) showing the spectra of free MNPs@TA (red) and the immobilized AlgL17 (black).

Fig.2 Enzymatic properties and stability of immobilized alginate lyase AlgL17. (A) Temperature dependence of immobilized AlgL17. (B) pH dependence of immobilized AlgL17. (C) Thermal stability of immobilized AlgL17. The immobilized AlgL17 was exposed to different temperatures for 1 h. (D) pH stability of immobilized AlgL17. The immobilized AlgL17 was incubated in buffers with varying pH values at 25 ◦C for 1 h. (E) Reusability of immobilized AlgL17. (F) Storage stability of immobilized AlgL17. The immobilized AlgL17 was stored at 4 ◦C and 25 ◦C over a certain period as indicated. Values are mean of three biological replicates. Error bars represent standard deviation.

1.3 The produced alginate oligosaccharides exhibit antioxidant activity

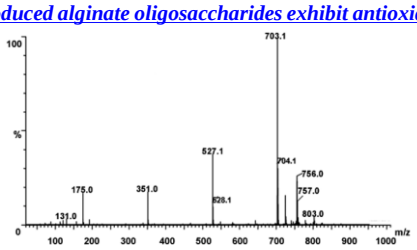


Fig.3 Identification of the enzymatic products of sodium alginate by immobilized alginate lyase AlgL17 using mass spectrometry. The mass over charge ratio (m/z) was shown for the major peaks.

2. antioxidant and antiapoptotic bioactivities in human umbilical vein endothelial cells

2.1 The produced alginate oligosaccharides exhibit antioxidant activity

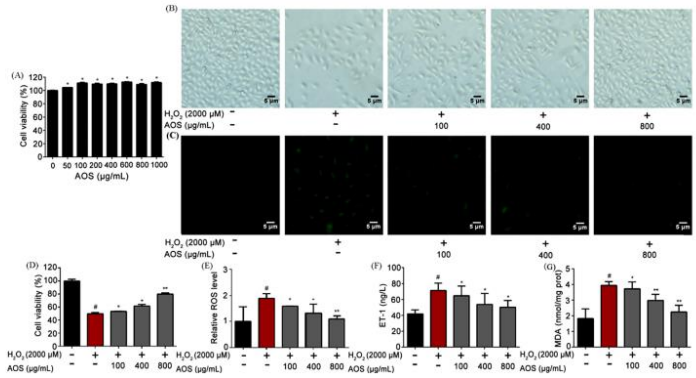


Fig.4 Antioxidant activity of alginate oligosaccharides (AOS) in human umbilical vein endothelial cells (HUVECs)

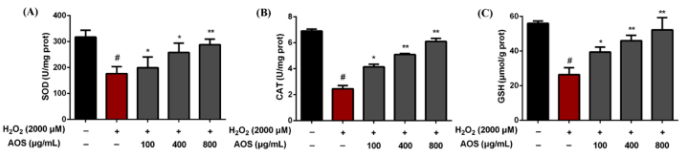


Fig.5 The stimulatory effects of alginate oligosaccharides (AOS) on the activity of free radical scavengers in human umbilical vein endothelial cells (HUVECs).

2.2 Effects of native- and desulfated-ascophyllan on RAW264.7 cells activation

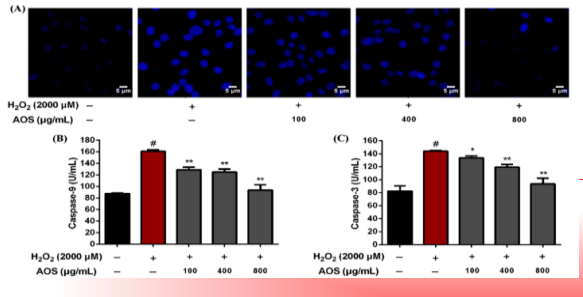


Fig.6 Anti-apoptotic activity of alginate oligosaccharides (AOS) in human umbilical vein endothelial cells (HUVECs).