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Screening and Purification of Metal Chelating Peptides in Hydrolysates for their Antioxidant Properties


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Societal context

Reactive Oxygen Species (ROS) induced oxidation
- Ageing and various pathologies in human related to oxidative stress, in particular inflammatory bowel diseases with iron anemia complication
- Degradation of consumption products (food, cosmetic matrix)

Living organisms developed their own antioxidant defenses against these ROS

Oxidative stress

Importance to discover natural antioxidants and evaluation of their bioactivity

Introduction

Metal-chelating peptides obtained after protein hydrolysis
- Antioxidant properties related to the inhibition of Fenton reaction, responsible of ROS production
- Used in pharmaceutics, foods and cosmetic

Novel strategy for the screening and purification of the peptides to target their antioxidant properties. Novel approach developed in order to save time.

Currently developed methods for the screening and separation of metal-chelating peptides

An interdisciplinairy approach

Screening of metal-chelating peptides by mass spectrometry (MS): identification of the peptide(s) of interest present in the complex mixture

Co-ordination chemistry

Understanding of peptide-metal complexation, SPR and MS results

Bioactivity tests

Determination of metal chelation capacity using UV-visible spectrophotometry

Pharmacology study of metal chelating peptides in cellulo: determination of their intestinal permeability

Nanoparticles for bioimaging

Some results

Conclusion and Perspectives

Screening of complex mixture of peptides by SPR

Identification by MS of the peptide(s) of interest present in the complex mixture. MS (Orbitrap, ESI, Direct infusion) solution. (A) without Fe²⁺. (B) with Fe²⁺.

Mass shift of +56 u and isotopic pattern = iron-containing ion.

Screening of peptide-metal complexes&&

SPR using a M²⁺-chip:
- a miniaturized approach of IMAC-type affinity chromatography
- screening of the most interesting sources of metal-chelating peptides.

Positive correlations evidenced between SPR screening and metal chelation tests using UV-Vis spectrometry on five sources of peptides.

The affinity parameters are determined by SPR on some synthetic model peptides and then used to predict the separation of peptides on IMAC chromatographic column.

Novel chromatography-MS method currently developed:
- Screening of metal-chelating peptides sequences in a given complex mixture
- Methodology successfully set-up on a mixture of synthetic peptides
- Further tests on real hydrolysates

Identified peptides currently assessed for their antioxidant activity by chemical metal chelation and by in cellulo tests.

Potential health application
- Treatment of ferriprive anemia related to MICI, or inversely to hemachromatosis
- Anchoring of these chelating peptides on nanoparticles for bioimaging or therapeutic applications.