Elucidating mechanisms of peroxidase induced cross-linking of proteins

by

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Aim

Elucidate the mechanism of peroxidase induced peptide cross-linking, as a model of protein cross-linking.

Introduction

Proteins are main contributors of the structural properties in many foods why controlled modification of proteins is a mean of optimising functionality and sensory perception of foods. One way of modifying proteins is to promote controlled cross-linking and hereby build new structural entities of importance for textural properties of processed foods. However, before such an approach can be further elaborated a basic understanding of the underlying mechanisms for controlled cross-linking are needed.

Conclusion

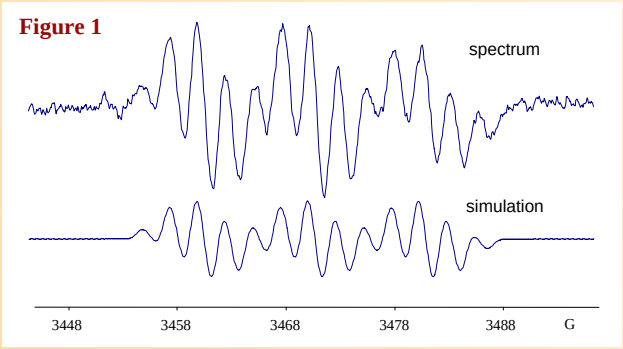
•Peroxidase induced cross-linking using a model peptide was proven using MALDI-TOF, hereby rendering that similar reactions occur in proteins.

•The reaction involves a carbon centered radical intermediate located on the tyrosine ring, which most probably is the precursor to a subsequent dityrosine formation.

Results and discussion

A spin-trapped radical intermediate of the cross-linking reaction is seen in the ESR spectrum of figure 1. The spin- trapped radical with the hyperfine splittings; a^N =10.31, $2a^H$ =2.48, and $2a^H$ =2.4 is most likely a carbon centred radical located on C^γ of tyrosine.

Figure 1

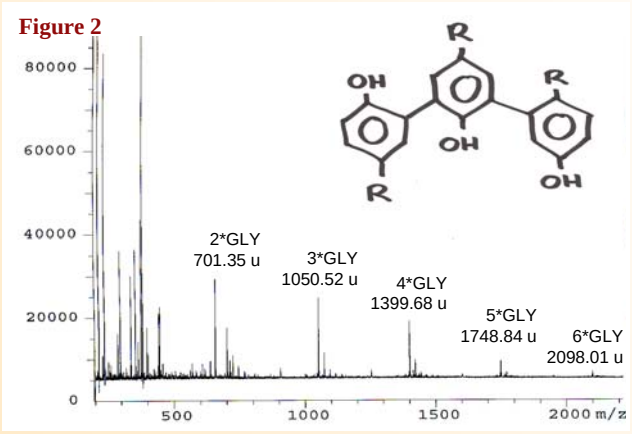


Reaction mechanism hypothesis: It is possible to oxidize tyrosine to the tyrosyl radical by abstraction of hydrogen from the alcohol group. Tyrosyl radicals are able to react and form dityrosine bonds1. As one tyrosine can form dityrosin bonds to two other tyrosines, a polymer can be created in this way.

The formation of polymers with masses close to a multiplum of the tripeptide mass is seen in the mass spectrum of figure 2.

Calculated masses of GLY polymers assumed to be cross-linked by tyrosine bonds corresponds with the observed masses within 0.2 mass unit, confirming that the cross links could be dityrosine bonds forming dityrosin polymers as shown in the legend of figure 2.

Figure 2



The reaction was found to proceed through a radical intermediate, which is likely to be located on the tyrosyl ring. The calculated masses of GLY polymers assumed to be formed by dityrosin bonds suggest that the cross links could be dityrosine bonds.

Methods

The tripeptide Glycine –Leucine-Tyrosine (GLY) shown in figure 3 was chosen as the model substrate for cross-linking studies.
A reaction mixture containing 3 mM GLY, 20 mM of the spin trap MNP, and 0.3 mg/ml HRP is activated by 1 mM hydrogen peroxide, and electron spin resonance spectroscopy (**ESR**) studies, is performed in order to detect intermediate radical formation.
For matrix assisted laser desorption ionization time of flight mass spectroscopy (**MALDI-TOF**) studies of reaction products, a reaction mixture containing 3 mM GLY, and Horseradish peroxidase immobilized on sephadex beads, is activated by 1 mM hydrogen peroxide.

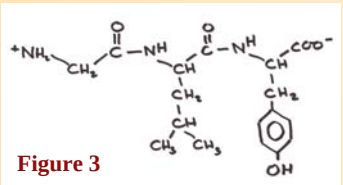


Figure 3

Reference

[1]Heinecke, J. W., Li, W., Daehnke III, H. L., and Goldstein, J. A. (1993). Dityrosin, a specific marker of oxidation, is synthesized by the myeloperoxidase-hydrogen peroxide system of human neutrophils and macrophages. *The Journal of Biological Chemistry*, *268*(6), 4069-4077.

Acknowledgment

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