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## Protein Surface Mapping by Mass Spectrometry to Monitor pH-Dependent Structural Transitions in Beta-Lactoglobulin A\*

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Novel Aspect: First demonstration of protein surface mapping with MS to probe pH-induced cavity opening in beta-lactoglobulin A.

### Introduction

Beta-lactoglobulin is a major protein found in the milk of ruminants. In addition to its nutritional function, it is a key transporter of hydrophobic molecules via its hydrophobic cavity. In the acidic environment of the stomach, the hydrophobic cavity is held closed by pH-sensitive amino acids loops. When the protein encounters the more basic pH intestinal region, the pH-sensitive loops expose the cavity and release the hydrophobic cargo. The pH-dependent conformation of this protein has been worked out by detailed NMR studies. The objective of this report is to demonstrate a rapid surface mapping technique based on hydroxyl radical adduction and MS detection to characterize the conformational changes that occur in a protein as a function of pH change.

### Methods

The pH of beta-lactoglobulin solutions (0.5 mg/ml) was adjusted to 2, 4, 6, 8, and 10 with either HCl or NaOH with PBS present. Each sample then had 15% H<sub>2</sub>O<sub>2</sub> added, and was exposed to UV irradiation (UV Stratalinker 2400) for five minutes to generate the hydroxyl radicals *in situ*. The reaction was quenched by isolating the proteins by SepPak clean-up. The oxidized proteins were characterized by high resolution ES-FTICRMS to determine the level of oxidation. Following tryptic digestion, each sample then was examined by HPLC-MS/MS on a quadrupole ion trap MS to verify the site(s) of oxidation. Sequest was used to analyze the data for identification of normal and oxidized peptides. All tentative oxidation assignments were validated manually.

### Preliminary results

A urea-denatured beta-lactoglobulin A sample was oxidized and characterized by MS as a control sample to investigate the maximum oxidation under the most extended conformational condition. This provided a means to evaluate the residue specificity of oxidation. We found that the hydroxyl radical attachment reaction is most facile for the sulfur-containing residues (M, C) and the aromatic residues (W, F, Y), and is observed to a lesser extent for the alkyl residues of P, L, I, and H. The degree of oxidation and sites of attachment was found to be very sensitive to the protein conformation (i.e., closed vs. open cavity) under different pH ranges. At pH 2, the protein is a monomer with a closed hydrophobic cavity. Oxidation was observed to be limited to the N- and C-terminal tails. Even though there are other highly-reactive residues such as cysteine present, they are buried in the cavity and inaccessible. At pH 4, the protein begins to aggregate, with the cavity still primarily in the closed position. Oxidation at this level is very similar to pH 2. At pH 6, the major E-F loop begins to open over the cavity. Oxidation at this condition reveals more extensive hydroxyl attachment to characteristic residues on the upper inside rim of the cavity. At pH 8, the cavity is thought to be completely open. Oxidation now is quite extensive and quite similar to that observed for the denatured conditions. Not only are several characteristic residues within the hydrophobic cavity oxidized, but the alpha-helix on the outside of the cavity also begins to unravel, as evidenced by oxidation as well. Because the oxidation induced by hydroxyl radical reaction primarily targets the hydrophobic residues, this probe was found to be very informative about which of these residues are solvent accessible under different pH conditions.

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