JMS Letters

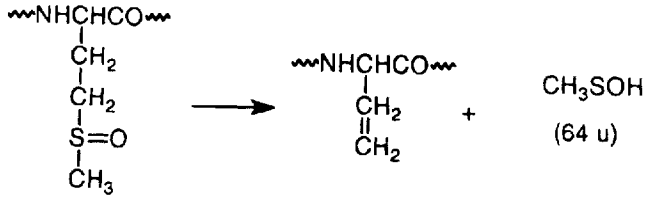
Dear Sir,

Identification of a MS–MS Fragment Diagnostic for Methionine Sulfoxide

The damage to proteins caused by reaction with free radicals was proposed as a mechanism of aging over 40 years ago.[1] This hypothesis has recently received widespread acceptance with supporting data from many investigations.[2,3] Mass spectrometry has the potential to be a particularly valuable tool in these investigations because it is capable of elucidating the structure of the reaction products, including the specific amino acid residues at which the modifications have occurred.

We have recently used mass spectrometry to identify the reaction products of lens α-crystallins with H_2O_2 and $FeCl_3$.[4] Because the structural proteins of the lens, including α-crystallins, have an extremely low turnover rate, they are particularly appropriate for investigating age-related protein modifications.[5] The solution of H_2O_2 and $FeCl_3$ forms a metal catalyzed oxidation system which is thought to cause oxidation by the formation of hydroxyl radicals via the Fenton reaction. The bovine lens α-crystallins were incubated for 24 h with 1 mM H_2O_2 and 0.1 mM $FeCl_3$, following a previously published procedure,[6] separated by reversed phase into αA- and αB-crystallins, and the molecular weights of the oxidized proteins determined by ESIMS. The oxidized proteins showed an increase of 32 u for both αA- and αB-crystallins, indicating that the oxidation had added two oxygen atoms to each protein. The oxidized proteins were enzymatically digested with trypsin or Asp-N, and the molecular weights of the resulting peptides determined by on-line HPLC–ESIMS. These data demonstrated that the oxidations were located in peptides 1–11 and 136–150 of αA-crystallin and peptides 1–11 and 57–69 of αB-crystallin. To determine the specific residue that was oxidized in each peptide, the modified peptides were analyzed by MS–MS (Micromass Auto Spec equipped with an oaTOF analyzer). A representative spectrum is shown in Fig. 1.

All four of the modifications were identified as oxidation of methionine to methionine sulfoxide, an addition of 16 u. In addition to the expected fragments due to cleavage along the backbone of the peptide, each spectrum contained peaks corresponding to the b-series–64. These peaks are attributed to loss of neutral methanesulfenic acid CH_3SOH (64 u) from the side chain of the oxidized methionine as demonstrated in the following scheme:



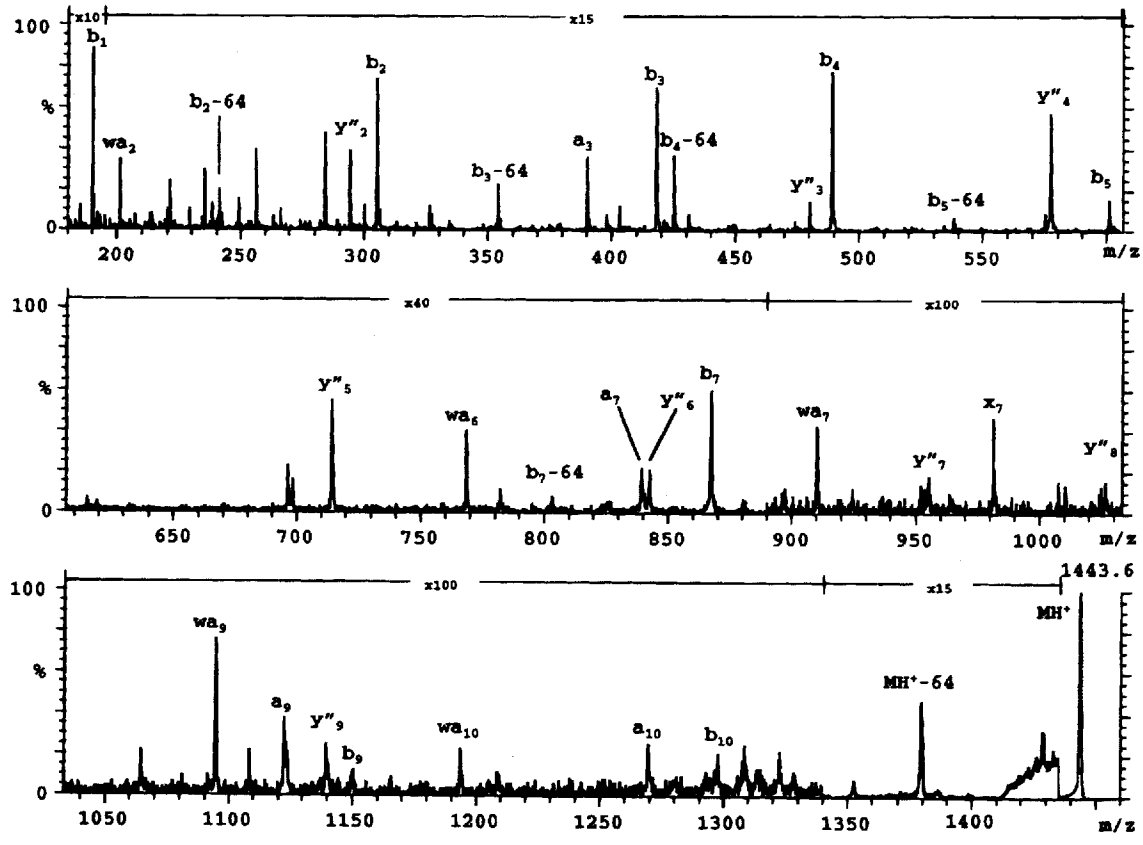


Figure 1. MS–MS spectrum of peptide 1-11 of αA-crystallin. The peptide has the sequence MDIAIQHPWFK with the methionine acetylated (+42 u) at the *N*-terminus and oxidized (+16 u) at the sulfur. Lettering of the fragment peaks is according to Roepstorff and Fohlman[10] and Biemann.[11] Note the peaks due to loss of 64 u from the side chain of the methionine sulfoxide.

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Methanesulfenic acid is well established as a product of thermal decomposition of organic sulfoxides;[7,8] its structure has been determined from microwave spectra.[9] Our results suggest that a similar decomposition is induced by collisional activation. This fragmentation pattern showing loss of 64 u is unique, in proteins, to methionine sulfoxide, and should prove useful for detecting oxidized methionine residues in proteins modified by a variety of oxidation systems.

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Yours

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