RNASE-S PROTEIN-PEPTIDE COMPLEX STUDIED USING A RADICAL PROBE AND ELECTROSPRAY IONISATION MASS SPECTROMETRY

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The reaction of proteins with radicals in the minds of most scientists is irrecoverably linked with structural damage. We have shown, however, in a series of published reports (1-3) that the treatment of proteins with a high flux of oxygen-containing radicals on extremely short (millisecond and sub-millisecond) timescales confers minimal, if any, degradation or damage to their structure. Furthermore, the degree to which specific amino acid side chains react with solution-based radicals is strongly influenced by the extent to which these residues are exposed to the bulk solvent.

Radicals have been generated on microsecond timescales using both synchrotron radiolysis and electrical discharges on an electrospray ion source. The latter approach can be conveniently implemented on an ESI source with minimal modification. The technique has been developed to study the equilibrium unfolding of proteins (2) and protein-protein interactions (3). In preliminary work, we have investigated the association between actin and the actin-regulatory protein gelsolin that plays a key role in cellular motility and differentiation (3).

In this work, we describe the application of the discharge-based method to study the interaction between RNase S-protein and S-peptide [4]. The protection of methionine at position 13 in S-peptide in the presence of S-protein, over oxidisable residues in several other peptides, indicated that S-peptide was exclusively bound. The binding domain of S-protein was localized to the tryptic fragment comprising residues 85-104. Tandem mass spectrometry revealed that the C-terminal portion of this segment underwent less oxidation in the presence of S-peptide. Further proteolysis generated two segments 85-95 and 96-104 both containing histidine, proline, and either tyrosine or phenylalanine. Despite the presence of these common residues in both segments, oxidation was affected exclusively at these residues in the later segment (96-104) upon binding to S-peptide. These observations are consistent with X-ray crystallographic data for the RNase-S Complex and the disulphide bond to cysteine at position 90 that prevents this region of the protein from accommodating the S-peptide.

The presentation will illustrate how the electrical-discharge experiments were performed for studies of protein complexes and demonstrate how the interaction of S-protein to S-peptide was investigated using MS and MS/MS data for each of the binding partners.

References:

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