

Protein cross-links are formed in regulated biochemical processes in many biological systems, but they are also generated inadvertently via the reactions of exogenous or endogenous oxidants. Site-specific identification and characterization of such cross-links is challenging, and the goal was, therefore, to develop mass-spectrometry-based approaches tailored for proteins subjected to oxidative challenges that also are applicable for the analysis of complex samples. Using trypsin-mediated O-18 isotopic labeling, different types of data acquisition workflows, and designated database software tools, we successfully identified tyrosine-tyrosine, tyrosine-tryptophan, tyrosine-lysine, and histidine-lysine cross-links in proteins subjected to sensitizer-mediated photo-oxidation with rose bengal or chemical oxidation with peroxyl radicals generated from the water-soluble compound 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH). Subsequently, AAPH was also applied to a protein extract from the Gram-positive bacterium Lactococcus lactis, demonstrating the feasibility to identify tyrosine-tyrosine, tyrosine-tryptophan, and tryptophan-tryptophan cross-linked peptides in a complex system. Different fragmentation techniques were evaluated, and it was observed that higher-energy collisional dissociation (HCD) resulted in a higher number of identified cross-link peptides, while electron transfer dissociation supplemented with HCD (EThcD) generally provides higher fragment ion coverage of the cross-linked peptides.

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