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POST-TRANSLATIONAL MODIFICATIONS: IDENTIFYING THE WHEAT AND SORTING THE CHAFF

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Introduction

Signal transduction, particularly in eukaryotic cells, involves the processing of extracellular information carried by molecular messengers into intracellular responses. This is usually mitigated through the agency of plasma membrane receptors that, when occupied by the messenger molecules, produce chemical modifications of intracellular molecules. These alterations are usually reversible and, hence, transient, which allows modulation of the signal (and the response) and importantly the reuse of the participating moieties. However, irreversible changes are also physiologically essential and impart vectoral signals leading to terminal responses, such as differentiation or apoptosis. There is a wide variety of chemical modifications known and most are introduced into proteins as essential steps for the regulation of their activity, cellular translocation and/or turnover. However, certainly not all are induced during signal transduction. Most cellular-mediated modifications arise enzymatically in a well-controlled manner but some can occur non-enzymatically, essentially randomly, such as N-glycation and methionine oxidation. Those of importance to signaling processes are clearly part of the former group. Additionally, modifications can be introduced during experimental manipulations, both deliberately, e.g., as in producing stable cysteine derivatives, and inadvertently. The latter can arise from a variety of sources, but usually result from exposing the protein (or derived peptides) to chemicals used in purification steps.

The identification of post-translational modifications (PTMs) can be accomplished in a number of ways, such as by using chemical and immunological reagents, but is increasingly being accomplished with mass spectrometry (MS) because of the unbiased nature of the methodology. However, in order for PTMs to be correctly characterized with MS techniques the derivative has to survive the ionization and the activation required for MS/MS measurements. In addition, some isobaric modifications cannot be readily distinguished, e.g., persulfide and double oxidation derivatives of cysteine (1). This can be particularly vexing if the modification in question is new, i.e. has not been described before. Moreover, it is often difficult to distinguish artifactual modifications from genuine ones. Formylation of lysine can occur both intracellularly or from experimental manipulation (2). Thus, one of the major challenges of proteomics will be to design protocols that can resolve these ambiguities and insure not only the correct chemical identification but also whether or not it results from a physiologically relevant action.

Results and Discussion

The problems of making definitive identification of covalent modifications and determining their physiological relevance is particularly well illustrated by the sulfation of serine and threonine residues (3). Phosphorylation of these same residue types is a major part of eukaryotic signaling pathways and the identification of these sites in various cellular paradigms that have been stimulated in a variety of fashions has been a major focus of proteomic studies (4). It has been predicted that approximately a third of all proteins are phopshorylated that most of these are modified on multiple sites, and that threonine and serine residues are about 2000 times more likely than tyrosine to be altered. This reflects the fact that the human genome, for example, contains some 500 protein kinases (and about 20% as many phosphatases) with varying degrees of specificity. There are other residues that can be phosphorylated but these are of relatively minor importance in eukaryotes. Phosphorylation is of wide-ranging importance and plays a role in almost every type of cellular processes. It is involved in cytoskeletal rearrangements, transcription/translation and molecular transport to name only a few. Protein sulfation, on the other hand, is of far more limited occurrence. Sulfation, in general, is the product of 15 sulfotransferases (in humans) that are subdivided into two categories. One group occurs in the cytosol (10 members) while the other is found in the Golgi (5 members) (5). Both types utilize only 3'-phosphoadenosine 5'-phosphosulfate (PAPS) as the sulfate donor. In fact, PAPS is the only known donor of sulfate in biological systems. The Golgi class of transferases participates in the synthesis of sulfated proteoglycans and polysaccharides and generates sulfotyrosine residues on a limited number of exported proteins. The cytosolic isoforms modify a number of small molecules such as steroids, catecholamines and thyroid hormone but are not known to modify any protein substrates. The sequence and crystal structure of all the ten of these enzymes are known.

The first report of O-sulfoserine and threonine in proteins was made by Medzihradszky et al (3). This study demonstrated, on three different types of proteins (from three different sources), the presence of this modification. It was distinguished from the more common phosphorylation by differences in the CID spectra, and was confirmed using synthetic peptides containing either the sulfo - or phospho substitution: Sulfopeptides undergo a gas-phase rearrangement reaction that results in complete SO_3 elimination. None of the peptide fragments retains the

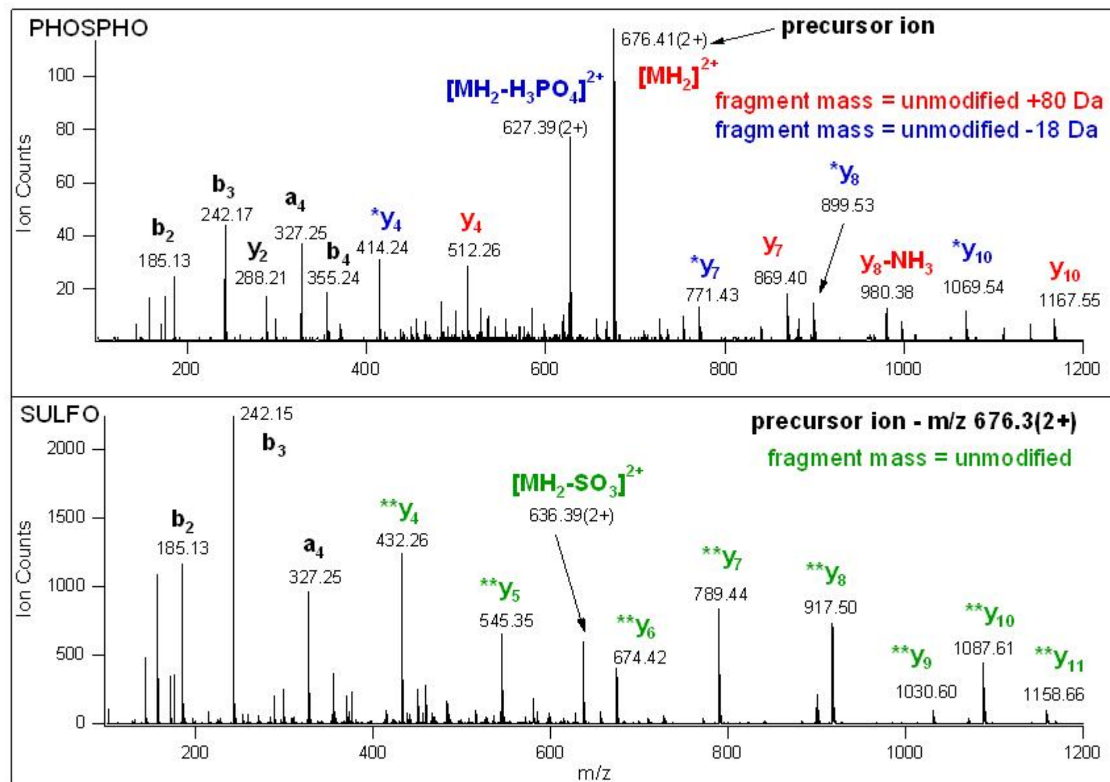

Figure 1. Comparison of the CID spectra of phosphorylated and sulfated LAGLQDEIGSLR. The phosphopeptide (upper panel) features fragments that underwent β-elimination (in blue), or display the phosphorylation (in red). The corresponding sulfopeptide fragments (in green) do not reflect any modification because of the gas-phase elimination of SO_3.

sulfate modification. Thus, the modification site cannot be assigned (see Figure 1). Using the synthetic peptides it was also ascertained that β–elimination products clearly marked the location of the substitution. The sulfation of serine and threonine was also confirmed with accurate mass analyses.

Attempts have been made to determine which, if any, of the known cytosolic sulfotransferases might be involved in forming the serine/threonine derivatives. Experiments using synthetic peptides that were identical to sequences found to be sulfated in intact proteins, carried out in collaboration with the research group of Dr. Cheryl Arrowsmith of the University of Toronto, have so far proven to be inconclusive. Thus, it is as yet unclear what the origin of these modifications is.

Serine and threonine sulfation has been observed in several samples and cell types. These modifications are highly selective and of apparent high occupancy. This argues against any non-specific reagent as the causative agent, although an artifactual origin cannot at this point be ruled out. If these modifications have a physiological origin, they may arise as a product of a new class of sulfotransferases that may utilize a different donor other than PAPS. However, a role for the known cytosolic enzymes has not bee rigorously excluded; they may require intact properly folded proteins as substrates, the synthetic peptides having too low affinity (too high Km).

Regardless of the source of this modification, it is clear that it occurs and is found, admittedly sparingly, in cell extracts. Since it is so easily overlooked (mistaken for phosphorylation), it must be concluded that at least some phosphorylations that have been reported are actual sulfations. Thus, the modification takes its place on the list of derivatives that can confound correct identification of PTMs. Whether it also has significant biological functions as well remains to be determined.

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