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Determinants of tyrosylprotein sulfation coding and substrate specificity of tyrosylprotein sulfotransferases in metazoans

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ABSTRACT

This short review likes to give a historical view on the discovery of metazoan Tyrosylprotein Sulfotransferases (TPSTs) setting its focus on the determinants of substrate specificity of these enzymes and on the hitherto knowledge of the sulfation coding mechanism. Weak points of the to-date models of sulfation coding will be outlined and a more detailed and complex view on tyrosylprotein-sulfation coding will be presented with respect to recent cellular investigations on TPSTs.

1. Introduction

Tyrosine sulfation is one of the most common post-translational modifications found in metazoan organisms. The importance of tyrosine sulfated proteins and peptides is reflected by their various functions in autoimmunity [1], blood clotting [2], hormone and chemokine receptor signaling [3,4] and fertility [5,6], pathogen recognition and invasion [7–9], stress induced apoptosis [10] as well as viability [11]. Details of tyrosylprotein sulfation have been reviewed in a series of excellent papers [12–16]. All these papers mainly focus on TPSTs with respect to the importance of sulfated peptides and proteins in various cellular events, on the catalytic mechanism and substrate specificity. After a historical view on TPST discovery this review will focus on the pro and cons of the hitherto defined determinants of sulfation and will show approaches to a more detailed understanding of sulfation coding and patterning in peptides and proteins comprising multiple tyrosine sulfation sites.

2. A brief history of metazoan TPSTs

After the first description of a tyrosine sulfated peptide derived from fibrinogen by Bettelheim [17] the scientific research on tyrosine sulfation expanded in the late 80th of the last century. Tyrosine sulfation was promptly proofed to occur in metazoans as well as in the green algae *Volvox caterii* [18,19]. It was predominantly detected in secretory and plasma membrane proteins in all vertebrate tissues, blood plasma and leukocytes with exception of erythrocytes [18]. A first description of an enzymatic activity responsible for tyrosine sulfation was detected in a cell free lysate of PC12 cells [20]. This activity depended on the ubiquitous sulfate carrier 3'-phospho-adenosine-5'-phospho-sulfate (PAPS) and was inhibited by addition of the metal chelating agent EDTA. The sulfation was attributed to a vertebrate tyrosylprotein sulfotransferase (TPST). Two years later TPST activity was detected in the Golgi fraction of bovine adrenal medulla cells and was tested for its specificity by using synthetic non-sulfated peptides [21]. The catalytic active region of the tyrosine sulfating enzyme was found to reside in the lumen of Golgi apparatus. In a subsequent study, the localization of TPST was narrowed down to the *trans*-Golgi network [22]. Decisive was the observation that Immunoglobulin M (IgM) was non-sulfated and lacking final sialyl and galactosyl moieties when

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extracted from the *pre-trans*-Golgi fraction of hybridoma cells, while *trans*-Golgi IgM fractions containing these moieties were already sulfated. Beginning of the 90th scientists succeeded in purifying and characterizing an O-glycosylated tyrosylprotein sulfotransferase from bovine adrenal medulla [23] which turned out to be an integral membrane protein [24]. Finally, in 1998 the groups of Huttner in Heidelberg and Moore in Oklahoma simultaneously succeeded in molecular cloning and expressing two isoforms of human TPSTs, TPST-1 and TPST-2 as well as homolog enzymes from other species [25–27]. Cloning of these enzymes now enabled investigations in their cellular environment and of their catalytic properties. TPST-1 turned out to be less efficient in catalyzing the model substrates than TPST-2. Mutational studies as well as FRET and photo-bleaching experiments provided evidence that O-glycosylation appeared at two asparagine residues in TPST and that Golgi targeting and retention as well as dimerization of TPSTs in cells is determined by their transmembrane domains [28]. In 2013 Japanese scientists succeeded in solving the first crystal structure of a TPST-2 dimer in complex with a sulfated peptide and APS [29]. The structural model allowed proposing a SN2 mechanism for catalysis with the sulfated peptide residing in an L-shaped conformation within a deep catalytic cleft. The structural arrangement and mode of peptide recognition resembled that of receptor tyrosine kinases.

3. The acidic sulfation motif – a general determinant of tyrosylprotein sulfation

Shortly after the discovery of the enzymatic activity of TPSTs the question arose what determinants help decide whether a certain tyrosine sidechain in a protein sequence is going to be sulfated or not. The first evidence of a certain sulfation motif came from the observation that a decrease within the catalysis rate of TPST occurred upon exchange of amino acids in close sequential proximity of the sulfated tyrosine [30]. In this precompetitive study, TPST enriched homogenate from bovine adrenal medulla was used to sulfate tyrosine moieties in synthetic peptides derived from either known tyrosine sulfated or tyrosine phosphorylated proteins. The enzyme substrate interaction became substantially stronger with the presence of a growing number of tyrosine sulfation sites within the peptides. Acidic amino acids adjacent to the sulfation sites were important for site recognition. The rate of sulfation decreased when acidic residues were substituted by their neutral homologs. TPST was even able to sulfate tyrosines residing in peptides derived from prominent tyrosine kinase sites which are prone to be phosphorylated in the cytoplasm of cells. All sequences accepted for sulfation by TPST shared a common acidic feature. The dependency of TPST activity on the presence of such an acidic motif was further investigated in a series of ongoing research studies using synthetic peptides.

Target site specificity was investigated in more detail by determining the kinetics of rat liver TPST using peptides related to the sequence of complement C4 component [31]. By eliminating negative charges within these peptides the minimal TPST interacting segment was confined to a size of about 4–5 amino acids. Moreover, substitution of charges located next to the tyrosine moiety at −1 and +1 positions showed the most dramatic increase in affinity pointing towards a special importance of the presence of these acidic residues. New determinants defining the acidic motif were further discovered when demonstrating a basic residue in the −2 position enhanced sulfation [32]. A deeper understanding for the special arrangement of amino acids within the acidic motif was finally given by the high resolution structure of TPST-2 [29]. For instance, the −1 and +1 positions are specifically recognized by either the backbone nitrogen of T198 (−1) or by the side chain of R105 (+1) within the enzyme. Peptides that do not have the corresponding negative residues in those positions are either sulfated with less efficiency (higher Km) when conserving other relevant features of the motif or not modified at all. An electrostatic and energetic analysis of the catalytic center of TPST-2 revealed a strong positive patch indicating that only acidic (or neutral) peptides may be substrates of TPST-2 in complex with a substrate peptide and that tyrosine containing peptides or stretches within proteins that are prone to be sulfated may undergo a local unfolding to fit into the catalytic cleft of the enzyme [33].

Shortly after the acidic motif has been investigated in detail, Seibert et al. [34] demonstrated the sequential order of sulfation in multi-tyrosine containing peptides. The scientists used recombinant TPST-1/2 and synthetic peptides derived from the CCR5-chemokine receptor, the major co-receptor for the entry of macrophage-trophic HIV-1 strains, to demonstrate that both isoenzymes are capable of sulfating their substrates in a descending step-wise order from C- to N-terminal tyrosine positions.

With these studies the tyrosine sulfation site in proteins was tracked down to defined acidic motifs which are going to be modified in a linear order when occurring in sequential arrangement within a peptide sequence. As each TPST protomer carries a single catalytic center, this model implied that peptides might be thread through the catalytic cleft from the C- to the N-terminal site. Alternatively, sulfation may start at the N-terminal tyrosine and after charge neutralization by sulfation the next C-terminal unmodified tyrosine may be sulfated and so on. This might occur within the same protomer or by a ping-pong-like mechanism between protomers of the dimer. Either way the question arises of what determinant justifies to make the very N-terminal tyrosine to be the first bound and sulfated one – especially when all residues are not residing in a short peptide but in a longer protein chain. If this simple linear model is accepted and both TPSTs accept the same substrates and sulfate them in a similar way, why have two isoforms evolved - not only in vertebrates but also in many invertebrates, too?

4. Sulfation determinants – a jigsaw puzzle

First doubts that the acidic motif found is a sufficient determinant for sulfation of proteins *in vivo* came from the early observation that some oligopeptides lack the features of the described motif (reviewed in [12]), but are nonetheless sulfated *in vivo*. These observations led to a reevaluation of the determinants of sulfation. A computational comparison of hitherto known sulfation sites characterized peptides that lack the typical acidic motif but got sulfated *in vivo* [35]. In this study the secondary structure was found to be a major determinant. In line with the later released structure of TPST-2 the authors found that the −1 position need not necessarily be occupied by an acidic residue within sulfated peptides. It is possible that hydrophobic residues or even histidine and cysteine are at this position, but only if other residues in close proximity are acidic and are thus compensating for the unfavorable sidechain at −1 position. Still unexplainable was the facts that horse gastrin and turtle antral peptide are predicted to be sulfated at the site of an acidic motif but are not found sulfated *in vivo*. However, they are sulfated when expressed in mammalian HIT cells. Moreover, different sulfation states of proteins had already been recognized upon expression of proteins in different cell lines [12]. To hypothesize, one might come up with revising statements that can give reasonable explanations for these observations. A, either there are hidden determinants for sulfation that still await their discovery (e.g. allosteric modulators, cofactors etc.) b, the substrate specificity of the described TPST homologs and/or isoforms as well as the acidic motifs recognized by the enzymes are