Crystal Structure of Human Tyrosylprotein Sulfotransferase: Insights into Substrate-Binding and Catalysis of Post-Translational Protein Tyrosine Sulfation

Post-translational protein modification by tyrosine sulfation plays an important role in extracellular protein–protein interactions, with implications for immune response, inflammation, hemostasis, and viral infection including that of the human immunodeficiency virus (HIV). The sulfation reaction is catalyzed by the Golgi enzyme called tyrosylprotein sulfotransferase (TPST). We reported the first crystal structure of the human TPST complexed with a substrate peptide (designated C4P5Y3) derived from a tyrosine-sulfated protein, complement C4, and 3'-phosphoadenosine-5'-phosphate (PAP), a degradation product of the sulfate donor, 3'-phosphoadenosine-5'-phosphosulfate (PAPS). The structural information, in conjunction with the mutational analysis data, provides a molecular basis for substrate-binding and catalysis, and explains how TPST can accommodate a variety of substrate proteins.

Protein tyrosine sulfation is a ubiquitous posttranslational modification that occurs in multicellular eukaryotic organisms. This unique modification has been demonstrated in a variety of secretory and membrane-bound proteins, such as complement factor C4, immunoglobulins, hirudin, P-selectin glycoprotein ligand-1, secreted signal peptides, and chemokine receptors, which are involved in a variety of physiological processes including immune response, hemostasis, development, and inflammation. In many cases, the sulfated tyrosine residues contribute to extracellular protein-protein interactions such as the antigen recognition of antibodies, inflammatory leukocyte adhesion to blood vessel walls, and the binding between chemokine receptors and their ligands. In the case of the chemokine receptor CCR5, its N-terminal extracellular region carrying the sulfated tyrosine residues has been shown to be crucial in mediating HIV binding/infection.

The protein tyrosine sulfation is catalyzed by the tyrosylprotein sulfotransferase (TPST) located in the Golgi apparatus. However, since no TPST structure is currently available, the mechanisms underlying the catalysis of the tyrosine sulfation reaction and the recognition of substrate proteins remain unclear.

We reported the first crystal structure of the human TPST (hTPST), complexed with a substrate peptide derived from complement C4 protein and PAP, a degradation product of the sulfate donor, PAPS, at 1.9 angstrom resolution [1] (Fig. 1). The structure revealed, for the first time, a detailed atomic view of the catalytic mechanism and substrate binding. The TPST is similar in catalytic mechanism to other types of sulfotransferases, but differ substantially in substrate recognition mode.

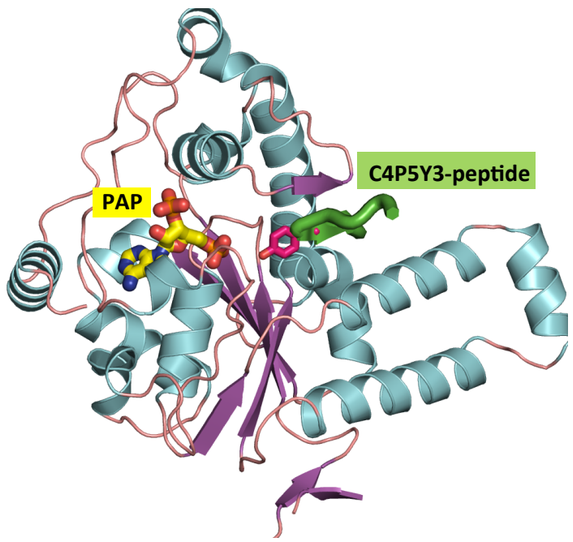

Figure 1: Overall structure of hTPST.

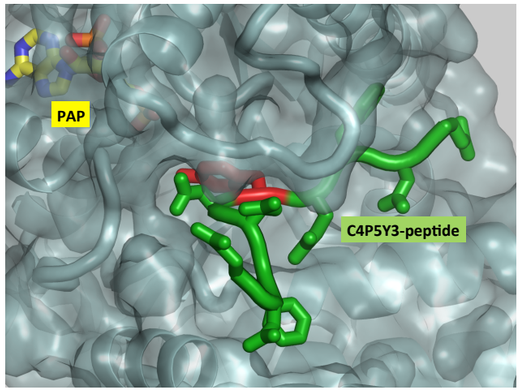

Figure 2: Substrate binding site of hTPST

The ternary structure of hTPST-PAP-C4P5Y3 provides atomic details concerning the reaction mechanism of TPST. When PAPS is superimposed onto PAP, the hydroxyl group of $Y^{acceptor}$ is located 2.6 Å from the sulfur atom of PAPS. The location of the sulfonate group is consistent with a S_N2-like in-line displacement mechanism as previously proposed for other sulfotransferases. The residues Arg78, Glu99, Lys158 and Ser285 of hTPST are in proper positions to play a catalytic role in the sulfonate transfer reaction. Superposition of hTPST with the active site of SULT1D1 with PAPS and *p*-nitrophenol and that of HS3OST3 with PAP and tetrasaccharide also indicate the catalytic functions of the above-mentioned residues of hTPST. Data from the mutational studies provide further support for the roles of Arg78, Glu99, Lys158, and Ser285 as, respectively, catalytic acid, catalytic base, stabilization of transition state, and stabilization of transition state.

The substrate peptide binds in the deep cleft of the hTPST2 and forms an L-shaped structure by using a short parallel β-sheet type interaction (Fig. 2). The structural, mutational, and other information reveal that an essential requirement for the substrate is to have only one acceptor tyrosine residue located in an intrinsically flexible region in order to fit into the deep cleft of hTPST. Other observed specific interactions may just serve to strengthen the affinity of the substrate and therefore are not essential for substrate binding. Probably because the aspartic acid residue located at the -1 position most highly strengthens the affinity by hydrogen bonding with the main chain of hTPST, this residue is most commonly found among the target sequences. Many other hydrophobic and electrostatic interactions are also utilized in diverse substrate amino acid sequences around tyrosine sulfation sites of proteins.

Surprisingly and interestingly, the mode of recognition for substrate peptide seems to resemble that observed for the receptor type tyrosine kinases. Because both these two types of enzymes are involved in post-translational modification on Tyr residue, the similarity might be the result of convergent evolution regardless of their distinct intracellular locations and substrate proteins.

To summarize, the molecular description of the hTPST crystal structure provides a framework for understanding the reaction mechanisms of protein tyrosine sulfation, which is one of the major posttranslational protein modifications. The information should be useful for the structure-based design of specific inhibitors of TPST.

REFERENCE

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