**s8a.m5.p1** **Structural Studies on the Proteins of Myelin**

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Compact myelin, the insulating material of axons, is essential for saltatory conduction of nerve impulses. Its loss or malformation has devastating consequences, as seen in diseases such as Multiple Sclerosis, Acute Disseminating Encephalitis and Pelizaeus-Merzbacher disease.

Myelin contains a high proportion of lipid and several specific proteins. Of especial note are the highly hydrophobic, transmembrane proteolipid protein (PLP) and the extrinsic, hydrophilic and highly charged Myelin Basic Protein (MBP), which account for approximately 50% and 30% of the total myelin protein in the central nervous system[1]. A third protein of interest is the extrinsic, basic lipid-binding P2 protein, a member of the superfamily of hydrophobic molecule transporters[2].

We have used a sequential detergent extraction to purify these three proteins under non-denaturing conditions from equine myelin, with a view to their crystallisation and structure determination. Essentially, a low concentration of the detergent LDAO is used to liberate the extrinsic MBP, which is then further purified by cation exchange chromatography. This procedure produces pure P2 as a byproduct. A second extraction at higher LDAO concentration releases PLP and its isoform DM20, which lacks residues 116-150, which are separated using molecular exclusion and ion exchange chromatographies.

Pure equine P2 protein was crystallised in the space group $P3_221$ and the structure was solved by molecular replacement using the bovine P2 structure[2] to a resolution of 2.1 Å. Refinement of this structure is currently in progress. Crystallisation trials are continuing for MBP and PLP. The former has proved difficult to crystallise since it occurs in several charge isoforms which are difficult to separate in sufficient quantity for crystallisation. The latter co-purifies with DM20; we are investigating the aggregation state of PLP, since its behaviour on molecular exclusion chromatography suggests a multimer, as noted previously[3], together with the possibility of a co-complex between the PLP and DM20.

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**s8a.m5.p2** **X-ray structure of the catalytic domain of human complement protease C1s: a trypsin-like domain modulated by a CCP module handle.**

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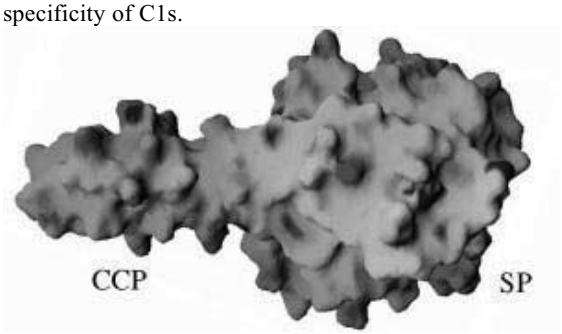
C1s is the highly specific modular serine protease that mediates the proteolytic activity of the C1 complex and thereby triggers activation of the complement classical pathway. The crystal structure of a catalytic C-terminal fragment from human C1s comprising the second Complement Control Protein (CCP2) module and the trypsin-like serine protease (SP) domain has been determined [1]. The structure was solved with the combined use of molecular replacement and automatic building and refinement at 1.7 Å resolution (WARP, [2]). In the areas surrounding the active site entrance, the SP structure reveals a restricted access to subsidiary substrate binding sites that could be responsible for the very narrow specificity of C1s.




The ellipsoidal CCP2 module is maintained perpendicularly to the surface of the SP domain through a rigid module-domain interface involving intertwined proline- and tyrosine-rich polypeptide segments. The relative orientation of the SP domain and the CCP2 module is fully consistent with the fact that both CCP1 and CCP2 modules are known to provide additional substrate recognition sites for C4. This structure provides a first template of a CCP-SP assembly that is conserved in diverse extracellular proteins [3].

In contrast with the rigid interaction between the SP domain and the CCP2 module, the CCP1-CCP2 interface is expected to be flexible. Consequently, it may function as a hinge mediating the movement of the catalytic domain from its initial location inside C1, where it is activated by C1r, to a more exposed position suitable for the cleavage of the C2 and C4 substrates.

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