Study of PEGylated model protein reveals porous structure based on PEG size

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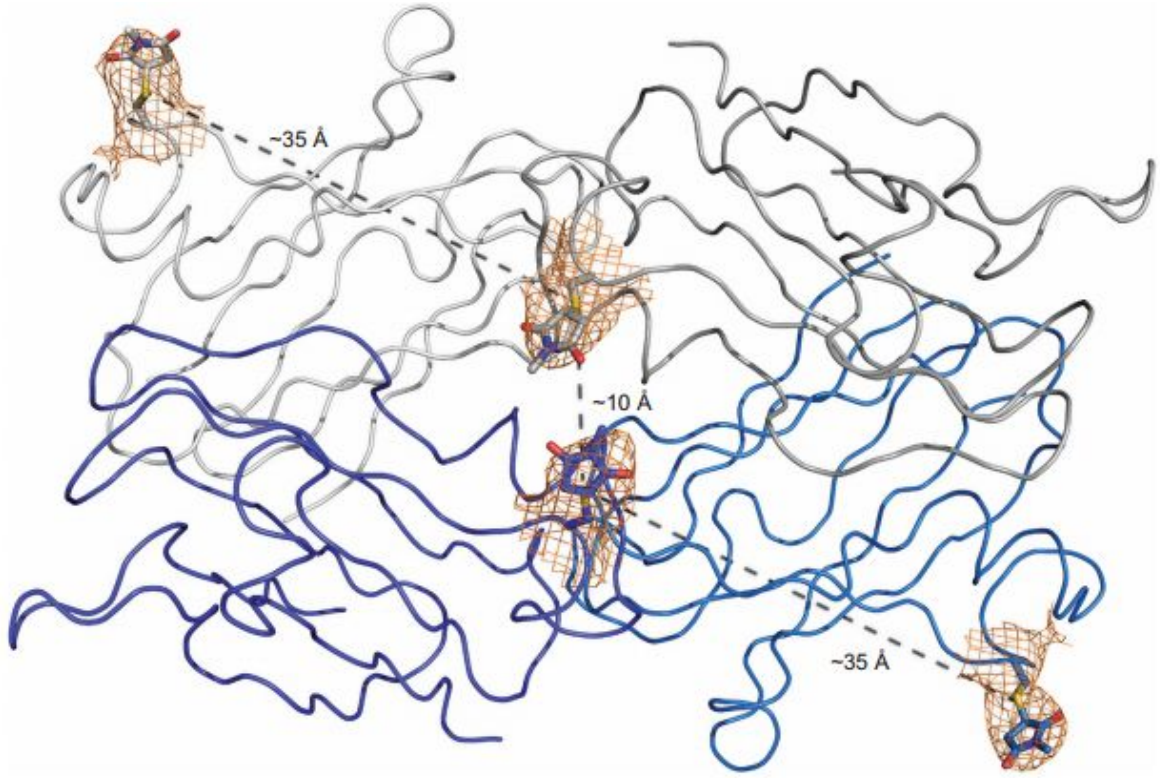

Crystal structure of PEG–Pc. Credit: (c) 2015 *Nature Chemistry*, DOI: 10.1038/NCHEM.2342

(Phys.org)—Biopharmaceutical macromolecules are often functionalized with polyethylene glycol (PEG), an inert chain of $-(CH_2CH_2O)-$ repeating units. PEG can help make a biomolecule more

soluble in water, or increase its circulatory half-life or cause the biomolecule to be "invisible" to the immune system. The assumption is that PEG does not affect the functionality of the biomolecule, but prior studies have shown that there is likely some interaction between PEG and proteins.

Giada Cattani, Lutz Vogeley, and Peter Crowley from the NUI Galway and Trinity College Dublin have characterized crystals of a PEGylated protein and shown that PEG maintains a random coil structure that minimally interacts with the protein. Interestingly, the crystalline assembly of the protein contained large pores that accommodate the PEG molecule. This suggests a possible way to control higher-order protein architecture. Their work is reported in a recent issue of *Nature Chemistry*.

Cattani, et al. used plastocyanin (Pc) as a model protein system. It is a copper-containing protein found in photosynthetic organisms. They changed a surface-exposed aspartic acid residue to cysteine that was then functionalized with methoxypolyethylene glycol maleimide 5,000. The product was confirmed using size-exclusion chromatography and mass spectrometry.

NMR studies using ^{1}H-^{15}N heteronuclear single-quantum correlation spectroscopy showed that the PEG-Pc had well-dispersed resonances that indicated the protein remained folded in a stable conformation. Chemical shift perturbations were only observed in and around the functionalized cysteine residue suggesting little interaction with the PEG chain. Peak splitting in the same region indicated the presence of both chiral products from the Michael Addition reaction of maleimide with the cysteine thiol. The NMR results provide further confirmation that the product was made and evidence that the PEG chain does not change the protein conformation in an appreciable way.

Cattani, et al. were able to grow crystals of Pc-PEG, and report this as the first instance of characterizing crystals of a PEGylated protein. PEG-Pc formed a right-handed double helix with PEG protrusions that the authors describe as a helical protein backbone decorated with a spiral arrangement of PEG chains. The porous crystal structure arose from the orthogonal packing of these helices. The X-ray data indicated that the PEG chains were disordered and likely remained as a random coil conformation.

To date, no other crystals of a PEGylated protein have been reported, and the PEG-Pc crystal structure may help explain why. Such crystals may be difficult to grow because the crystal assembly has large pores that accommodate the PEG chain. This may also imply that different-sized PEG chains could be used to tailor pore size.

Cattani, et al. also looked at the volume of a PEG molecule in the crystal. PEG 5,000 takes up a maximum valome of ~5.8 x 10^4 $Å^3$ which is within 10% of the calculated random coil value. Comparatively, PEG takes up a volume that is six-fold larger than the protein itself. If this behavior translates to other PEGylated proteins, the large spaces to accommodate the randomly coiled PEG chain may preclude the necessary protein interactions to form a crystal structure, making it difficult to grow crystals of PEGylated proteins.

This research verified that PEG does not make an appreciable difference to the protein structure, but it does seem to change the higher-order packing of the protein assembly. Further research is necessary to show whether the size of the PEG chain can generally be used to control crystal pore size in protein engineering.

More information: "Structure of a PEGylated protein reveals a highly porous double-helical assembly" *Nature Chemistry*, DOI: 10.1038/NCHEM.2342

Abstract

PEGylated proteins are a mainstay of the biopharmaceutical industry. Although the use of poly(ethylene glycol) (PEG) to increase particle size, stability and solubility is well-established, questions remain as to the structure of PEG–protein conjugates. Here we report the structural characterization of a model β-sheet protein (plastocyanin, 11.5 kDa) modified with a single PEG 5,000. An NMR spectroscopy study of the PEGylated conjugate indicated that the protein and PEG behaved as independent domains. A crystal structure revealed an extraordinary double-helical assembly of the conjugate, with the helices arranged orthogonally to yield a highly porous architecture. Electron density was not observed for the PEG chain, which indicates that it was disordered. The volume available per PEG chain in the crystal was within 10% of the calculated random coil volume. Together, these data support a minimal interaction between the protein and the synthetic polymer. Our work provides new possibilities for understanding this important class of protein–polymer hybrids and suggests a novel approach to engineering protein assemblies.