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A Powder Diffraction Study of the Binding of N-acetylglucosamine Oligomers to Chicken Egg Lysozyme

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Beamline(s): X3B1

Introduction: The experimental verification of the binding mode for small molecule ligands to proteins under a wide variety of conditions is needed to understand the mechanisms of protein action and inhibition. This work is an attempt to examine the interaction between a series of small oligosaccharides and lysozyme by high-resolution x-ray powder diffraction.

Methods and Materials: Powder diffraction samples were prepared by combining 10-25mg chicken egg lysozyme with 1-3mg N-acetylglucosamine oligomers (NAG₂, NAG₃, NAG₄, NAG₅ or NAG₆) in 200 μ l pH 6.0 1.0M NaCl buffer in an agate mortar. The resulting slurry for each case was loaded into a 1.5mm diameter glass capillary, centrifuged and the sealed to prevent evaporation. Powder patterns were collected from 1-14 $^{\circ}$ 2 θ at $\lambda=0.70\text{\AA}$ in 0.002 $^{\circ}$ steps over 10-12h on line X3B1. Analysis of the diffraction data from the NAG_n/lysozyme complexes were done with GSAS and ΔF maps generated from extracted structure factors generated during preliminary Rietveld refinements revealed the location of the bound NAG_n ligand in each case (Fig. 1).

Results: Preliminary examination of the ΔF maps showed clear indication for the location of the NAG_n ligand bound to the A-F cleft of chicken egg lysozyme. The NAG₂/lysozyme structure has been further analyzed to give a detailed view of the complex (Fig. 2); the NAG₂ is bound to the C-D sites of lysozyme and straddles the presumed active site.

Conclusions: Clearly high-resolution x-ray powder diffraction can be used to investigate protein/ligand complexes and useful structural results can be obtained from this technique.

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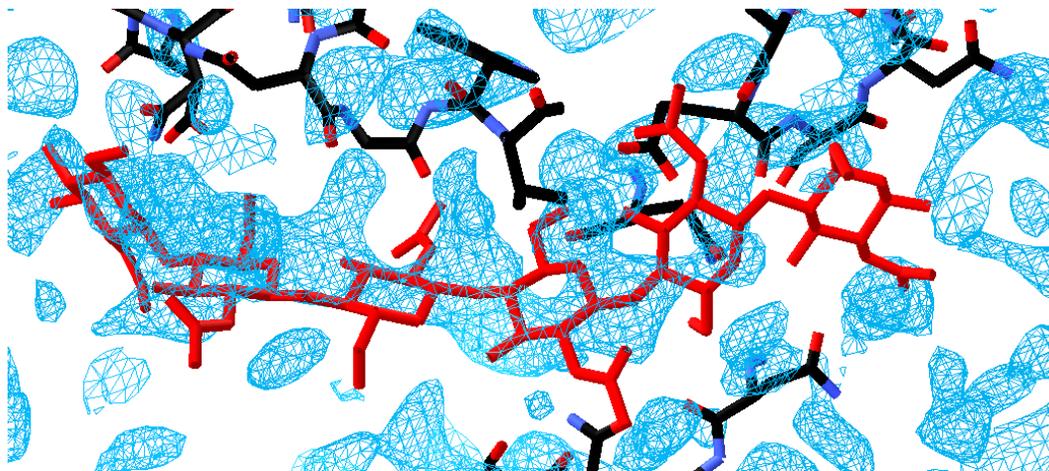


Fig.1 ΔF map with extracted structure factors from a preliminary Rietveld refinement with high resolution X-ray powder diffraction data from the NAG₆/lysozyme complex. NAG₆ molecule (in red) placement is not optimized with respect to the map density but only to indicate the extent of the structure in the map.

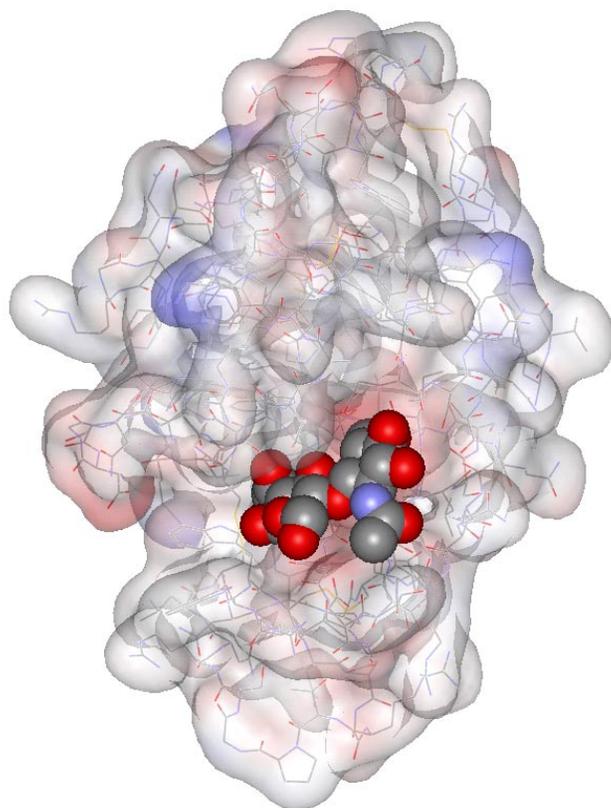


Fig. 2 Structure of the NAG₂/lysozyme complex as determined from high-resolution x-ray powder diffraction. The NAG₂ ligand spans the C-D binding site of lysozyme.