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Synthesis of T4 Lysozyme Substrate

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Introduction

One of the most studied enzymes, T4 lysozyme, is produced by T4 bacteriophage and destroys cell walls of bacteria. It works by cleaving bacterial cell walls to release virions (individual viral particles) which leads to infection of other cells. The bacterial cell wall consists of long chains of alternating N-Acetylmuramic acid (NAM) and N-Acetylmuramic acid (NAM) units (Scheme 1).

Along with being attached to NAG units, the NAM units are also attached to sugars. It is difficult to clearly define the NAG-NAM bonds. Although T4 lysozyme is one of the most thoroughly studied enzymes structurally, there are hundreds of mutants made and crystal structures determined. The kinetic and mechanisms of this enzyme in greater detail. This also allows the ability to modify the substrate synthetically to explore the role of various parts of the substrate in binding and cleaving chains to help us study and understand the kinetics and mechanism of T4 lysozyme.

The substrate we propose is the muramic acid derivative. T4 lysozyme requires the N-acetylmuramic acid (NAM) to be substituted with a peptide side chain in order for cleavage to occur. Specifically, binding studies on cell wall fragments indicate that muramic acids substituted with Ala-Glu(Lys) tripeptide are cross-linked to other NAG-NAM chains, forming a covalent net around the cell.

In this project, we propose to synthesize a simple, convenient substrate. There is little kinetic data due to the lack of a simple substrate. Our goal is to synthesize a simple, convenient substrate.

Synthesis

Figure 1. Schematic of bacterial cell wall—the natural substrate for T4 lysozyme

Coupling of the Boc-D-Glu-OBn (8) and H-Lys(Fmoc)-OCH$_3$·HCl (9) with 1,3-dicyclohexylcarbodiimide (DCC) was straightforward in a one-pot reaction. Purification by flash chromatography of the protected tripeptide 18 afforded a yield of 78%.

In our initial peptide coupling attempt we obtained only 36% of the desired dippiti product and it still contained a significant amount of the solvent N,N-dimethylformamide (DMF). We found that using the DCC by distillation in vacuo prior to workup led to higher yields and pure product. This strategy was also applied to the synthesis of the tripeptide 12.

Current Work

Addition of (S)-2,4-dibromophenyl-acetic acid to (2-nitrophenyl)-2-acetamido-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranoside (6) is currently being worked out. When this reaction was previously carried out, cleavage of the nitrophenyl group was occurring and the dibromopropionic acid was not being added. This project is being carried out. To work out these problems, we have been using a similar, simpler sugar, Diacetone-D-glucose.

Future Work

We’ve also been working on the synthesis of the muramic acid with the 4-nitrophenyl group.

Future Work

Scheme 4 shows the target molecule, T4 lysozyme substrate, of the project. Once the muramic acid derivative and the peptide side chain are synthesized, DCC will be used to couple them together to produce the molecule of interest, 2-nitrophenyl muramic acid with Ala-D-Glu-Lysine 1.

Bibliography


Acknowledgements

University of Puget Sound
Dr. John Hanson