Studying the interaction of membrane enzyme PglB with substrate and inhibitory peptide

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Abstract

During the catalytic cycle of enzymatic reactions, the recognition of a specific substrate by the enzyme is one of the most important steps, and the analysis of the affinity between enzyme and substrates and/or inhibitors is an important aspect in mechanistic biochemistry. Here, we have performed an analysis of the binding affinity between two different peptides and the bacterial oligosaccharyltransferase, PglB. Using labeled peptides, we have performed MicroScale Themophoresis experiments to determine the K_d values for the interaction between PglB and these peptides. The results show a high concordance with the values previously reported for the same interaction determined by fluorescence anisotropy, showing that MST is a suitable tool for the study of membrane protein-substrate interactions in detergent solutions.

Introduction

N-glycosylation is a protein modification that occurs in all three kingdoms of life. It consists of the transfer of a glycan from a lipid-linked carrier to an asparagine residue within a glycosylation sequon (D/E-x-N-X-S/T for bacteria, N-X-S/T for archaea and eukaryotes) [1]. In bacteria, the reaction is catalyzed by the oligosaccharyl-transferase, PglB. Recently, the crystal structure of PglB was solved [2], and various studies at functional level have allowed a better understanding of the mechanism of N-glycosylation (Figure 1). One of the most important aspects that have been studied is the interaction of PglB with the acceptor peptide. Recently, it was reported how modifications in the asparagine that is glycosylated by PglB, have an influence in the peptide binding and activity of PglB [3]. In those studies, the K_d values for the interactions of PglB with different peptides were determined by fluorescence anisotropy, using synthetic, labeled peptides. Here, we show the use of MicroScale Themophoresis (MST) to analyze the interaction of two different labeled peptides with PglB. One of them containing the wild type version of the glycosylation sequon, while in the other peptide the asparagine residue of the sequon has been replaced by 2,4-diaminobutanoic acid.

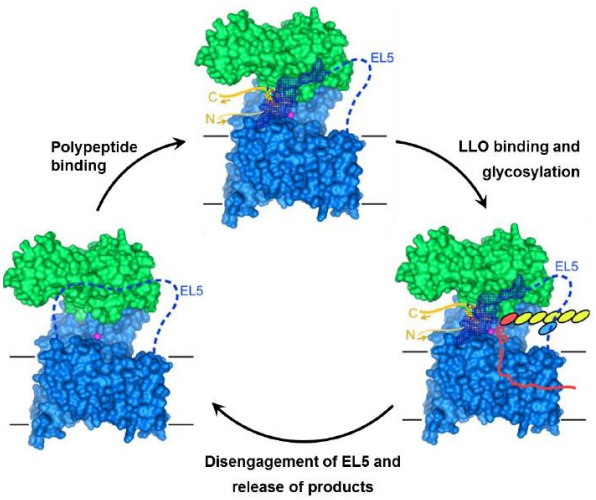

Figure 1. Reaction cycle of N-glycosylation by PglB. PglB first binds to a substrate peptide. In the next step, a glycan from a lipid-linked carrier is engaged and transfered to an asparagine residue in the peptide. The glycosylated peptide is released from PglB which is then free to start a new reaction cycle.

Results

We first analyzed the binding of an acceptor peptide to PglB. This acceptor peptide contains an asparagine residue within a consensus sequon, and it is fluorescently labeled with 5-Carboxyfluorescein (Table 1). The concentrations of labeled peptide and Mn^{2+}, an essential PglB cofactor, were kept constant in all samples at 50 nM and 10 mM respectively, whilst the concentration of PglB was varied. The samples were incubated at 4 °C for 10 minutes, before loading into MST standard treated capillaries. The MST measurements were then performed at 10 °C. For the interaction of PglB with the acceptor peptide, a K_d of 0.42 +/- 0.17 µM was determined (Figure 2). This value is in the same range of the K_d value previously reported for the interaction of PglB with the same acceptor peptide. In that case the determination was performed using fluorescence anisotropy and the value was 1.02 +/- 0.06 µM [3].

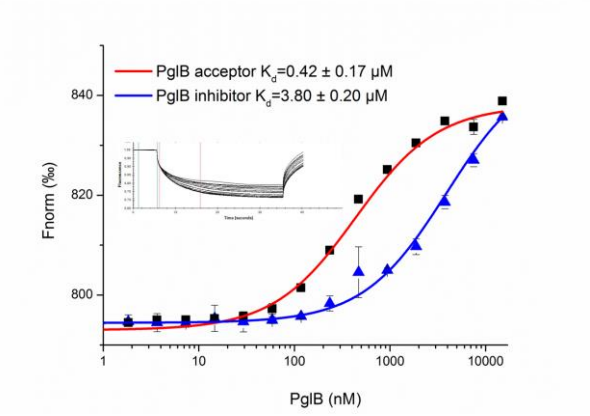

Figure 2: Binding of the acceptor and inhibitor peptides to PglB. Both 5-FAM fluorescently labeled peptides were used at a constant concentration of 50 nM with varying concentrations of PgB. The acceptor peptide binds with a K_d *of* 0.42 +/- 0.17 µM to PglB (squares), while the binding affinity of the inhibitor peptide (triangles) to PglB was 3.80 +/- 0.20 µM. The error bars reflect standard deviation (SD) from 2 independent measurements.

In a second experiment, we analyzed the binding of PglB to a peptide in which the asparagine residue within the glycosylation sequon, was replaced by 2,4-diaminobutanoic acid (Dab). This exchange has been shown to have inhibitory effects in some eukaryotic OST [4]. The inhibitory peptide, as well as the acceptor peptide, is labeled with 5-Carboxyfluorescein. The concentrations of labeled peptide and Mn^{2+} were kept constant, while the concentration of PglB was varied. In this case, a K_d of 3.80 +/- 0.2 µM was determined for the interaction between PglB and the inhibitory peptide. This value is again in the same range that the one previously reported for the same interaction, 10.15 +/- 0.25 µM. Furthermore, we observed that the affinity between PglB and the inhibitory peptide is ~ 10-fold lower than for the acceptor peptide, which is completely in concordance with our previous results, as shown in Table 1 (Lizak et al, 2013).

The lower affinity observed for the interaction of PglB with the inhibitory peptide has been explained before by two different aspects: In the acceptor peptide the carbonyl of the asparagineresidue is likely interacting with PglB, whereas the positive charge of the primary amine group in the inhibitory peptide could be weakening the binding to PglB.

Conclusions

Our experiments show that MicroScale Themophoresis (MST) is a suitable tool for the analysis of interactions between detergent-solubilized membrane proteins and their substrates, as demonstrated here by analyzing the interaction of labeled peptides and the membrane enzyme PglB. The K_d values that were determined for the interactions between PglB and the two different labeled peptides were in concordance with the values obtained using fluorescence anisotropy.

Peptide	Sequence	K_d (MST)	K_d (Fluorescence anisotropy)
Acceptor	5-FAM-GSDQNATF	0.42 +/- 0.17 µM	1.02 +/- 0.06 µM
Inhibitor	5-FAM-GSDQ(Dab)ATF	3.80 +/- 0.2 µM	10.15 +/- 0.25 µM

Table 1: Comparison of the K_d values determined for the interactions of PglB with different peptide, determined by using MicroScale Thermophoresis and Fluorescence anisotropy.

Material and Methods

Labeled peptides: Synthetic peptides, fluorescently labeled with 5-Carboxyfluorescein, were reported by Gerber, Lizak [5].

Protein Preparation: PglB carrying a C-terminal decahistidine tag, was purified as previously described by Lizak, Gerber [2]. The protein was purified in desalting buffer (10 mM MES pH 6.5, 100 mM NaCl, 3 % glycerol, 0.016 % DDM) and concentrated to 35 µM in an Amicon Ultra-15 concentrator (Millipore) with a molecular mass cutoff of 100 kDa.

Binding of labeled peptides to PglB: The concentrations of labeled peptide and $MnCl_2$ were kept constant at 50 nM and 10 mM respectively. For the interaction analysis of PglB with the peptides, PglB was titrated in 1:1 dilutions. The highest concentration of PglB was about 20-fold above the expected K_d for the interaction PglB-acceptor peptide, which was previously determined as 1 µM by fluorescence anisotropy [3]. The samples were incubated for 10 min at 4 °C, and then filled into standard treated capillaries (Cat# K002, NanoTemper Technologies, Germany).

Instrumentation: The measurements were performed in a NanoTemper Monolith NT.115 instrument, at 30 % LED power and 90 % MST power.

References

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