Effects of N-glycosylation on thermal stability and catalytic efficiency of GH10 family xylanases

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Abstract

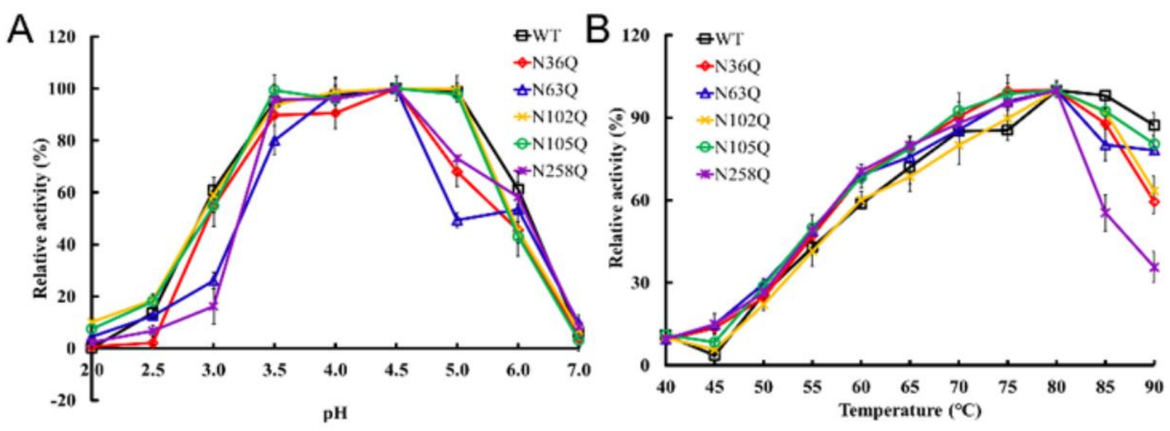
The improvement of the thermostability of the highly active xylanases is of great significance to the use of biomass materials to produce clean energy. N-glycosylation has important effects on enzyme thermostability and the mechanism is unclear. In this paper, the mechanism of the influence of different N-glycosylation sites on the thermostability of the enzyme was explored. XYL10CΔN and 4XX6 are high temperature resistant GH10 family xylanases. Sequence alignment showed that two enzymes both contained five N-glycosylation sites. Mutants N36Q, N63Q, N102Q, N105Q and N258Q were obtained after removing N-glycosylation of XYL10CΔN. Mutants N35Q, N72Q, N115Q, N147Q and N320Q were obtained after removing N-glycosylation of 4XX6. Among them, the thermostability of mutant XYL10CΔN-N258Q and 4XX6-N72Q were decreased sharply, $t_{1/2}$ were decreased by 3.5 min and 31 min, and T_{50} were decreased by 6 ℃ and 13 ℃. The thermostability of mutant XYL10CΔN-N105Q was increased sharply, $t_{1/2}$ was increased by 5.5 min, and T_{50} was increased by 0.5 ℃. This study provided a reference for further studies on the effect of N-glycosylation on enzymatic properties.

Methods

The enzyme XYL10CΔN and 4XX6 were expressed in Pichia pastoris GS115. Sequence alignment showed that they are both contained five N-glycosylation sites. The enzymatic properties and substrate kinetics of the mutant after removing N-glycosylation were analyzed to study the effect of N-glycosylation on the thermostability and catalytic efficiency of the enzyme. Its structure was analyzed by RMSD and RMSF simulation.

Results & Discussion

Fig.1 comprehensively compared the optimum pH, optimum temperature, $t_{1/2}$ value and T_{50} value of the XYL10CΔN and mutants, and it was found that the change trend of optimum temperature and optimum pH of wild type and mutant type is consistent. The optimum temperature and pH of all mutants were the same as those of wild type, and the relative activity of the mutant N258Q decreased rapidly at the range of 80 ℃ and 90 ℃. When the N-glycosylation site of the enzyme was removed, the thermal stability of N105Q became stronger and the thermal stability of other mutants became weaker. T_{50} values of wild-type and mutants were also measured. Among them, the thermal stability of N36Q and N258Q mutants decreased, and the data were consistent with $t_{1/2}$.



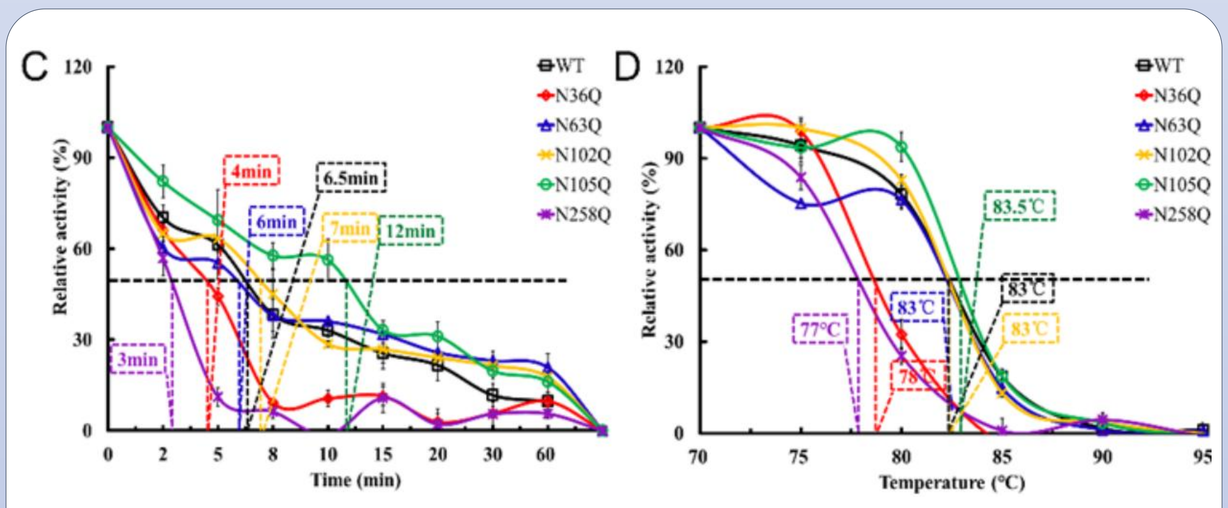


Fig.1 A: Optimum pH for XYL10CΔN and its mutants; B: Optimum temperature for XYL10CΔN and its mutants; C: $t_{1/2}$ of XYL10CΔN and mutants at 80 ℃; D: T_{50} values of XYL10CΔN and mutants.

Fig.2 shows the optimum pH, optimum temperature, $t_{1/2}$ value and T_{50} value of the 4XX6 and mutants, and it was found that the optimum temperature and pH of all mutants were the same as those of wild type, and the optimum pH range of mutant N72Q was decreased. T_{50} values showed that when the N-glycosylation sites were removed, the thermal stability of all mutants decreased. Among them, the thermal stability of mutant N72Q decreased most seriously. The experimental date of $t_{1/2}$ showed the same results.

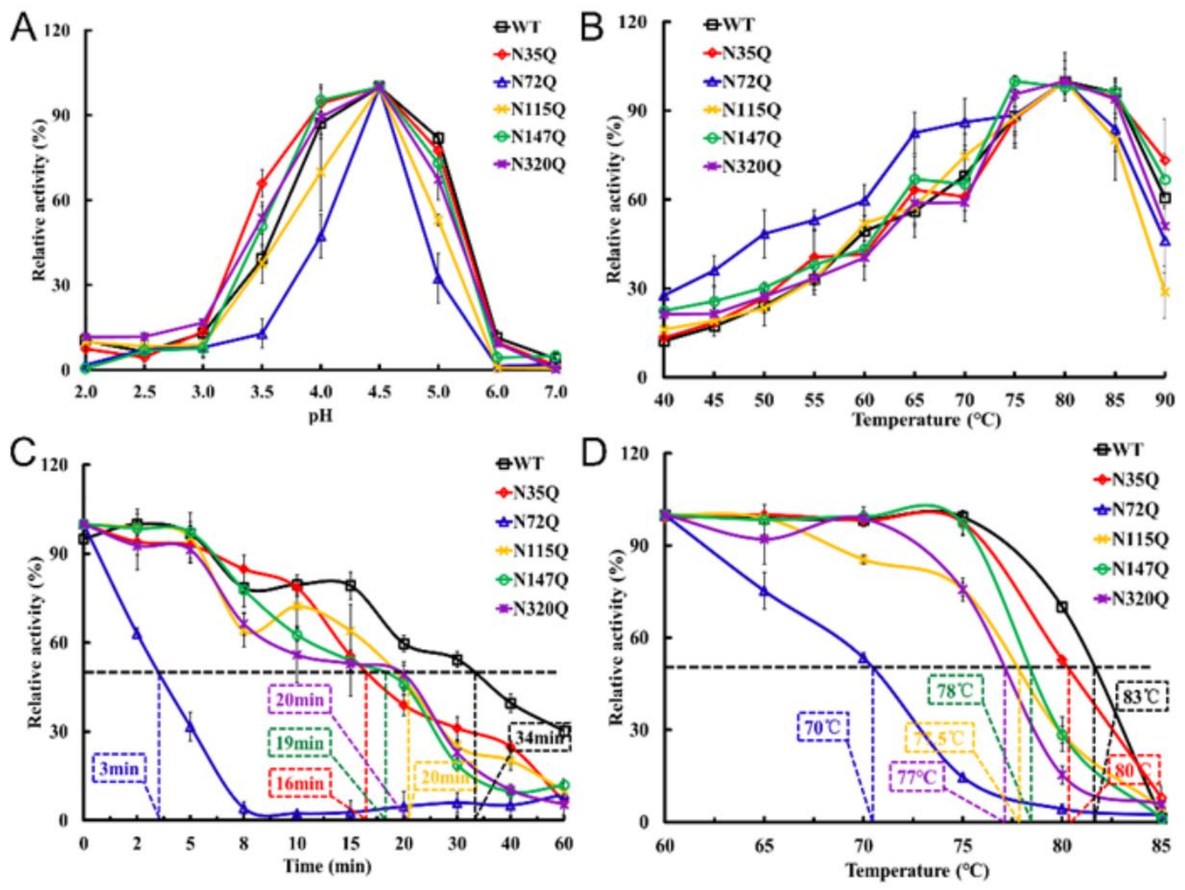


Fig.2. A: Optimum pH for 4XX6 and its mutants; B: Optimum temperature for 4XX6 and its mutants; C: $t_{1/2}$ of 4XX6 and mutants at 65 ℃; D: T_{50} values of 4XX6 and mutants.

Conclusion

This is the first study of the effects of N-glycosylation on thermal stability of GH10 family xylanases XYL10CΔN and 4XX6. The experimental results showed that the sugar chain at this site has an important influence on thermostability. This is of great help to the research on molecular modification of the GH10 family xylanases. It also has considerable potential in feed and biorefinery applications.

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