Improvement of the thermostability of a highly active GH16 glucanase without losing catalytic performance via alanine substitution

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Introduction

As an important industrial enzyme, glucanase is widely used in the fields of kitchen waste treatment, food processing and as feed additives. Improving the thermostability of glucanase through enzyme engineering methods to obtain heat-resistant glucanase is of great significance to its industrial application and cost saving. The substitution of alanine for charged amino acid residues on the protein surface can eliminate the challenges associated with protein rigidity loss caused by uneven charge distribution, thereby optimizing the protein surface charge and improving thermal stability.

Content

In this study, a structure-based rational design approach was used to improve thermostability with enhanced catalytic performance of a highly active glucanase from Bispora sp. MEY-1 via alanine substitution of charged residues on the protein surface. Through screening, three locations, D28A, D194A and D234A were identified as the contributors to thermostability and low-temperature catalytic performance. Single (D28A, D194A and D234A) and combined mutants (D28A/D194A/D234A) were produced and compared with the wild-type (WT). All mutants showed improved thermal properties, in the order D28A/D194A/D234A > D234A > D28A > D194A. In comparison with BisGlu16B, single and combined mutants showed pronounced shifts in T_m of approximately 3.8, 3.1, 5.6 and 6.8 ℃, and T_{50} of approximately 8, 5.5, 9.5 and 16 ℃ upward, with the half-life ($t_{1/2}$) prolonged by 10, 6, 13, and 25 min at 60 ℃, and 5, 2, 12 and 21 min at 70 ℃ upward, respectively (Table 1). Molecular dynamics simulations show that the mutation of the charged amino acid aspartate on the surface to alanine increases the overall rigidity of the protein molecule, reduces the energy of the protein molecule, increases the number of hydrogen bonds inside the protein molecule, and finally enhances the enzyme thermostability (Figure 1). The specific activity and catalytic efficiency of all mutants were the same as the wild type. The improvement of thermal stability under the premise of no loss of catalytic activity is realized (Table 2).

Table 1. Comparison of thermal stability between wild-type and its mutants for thermal inactivationa

Enzyme	$t_{1/2}$ (min) at		T_{50} (℃)	T_m (℃)	ΔT_m (℃)
	60 ℃	70 ℃			
BisGlu16B	18	4	48	41.3	
D28A	28	9	56	45.1	+ 3.8
D194A	24	6	53.5	44.4	+ 3.1
D234A	31	16	57.5	46.9	+ 5.6
D28A/D194A/D234A	43	25	64	48.1	+ 6.8

[a] The enzyme activity was assayed at each optimal conditions for 10 min

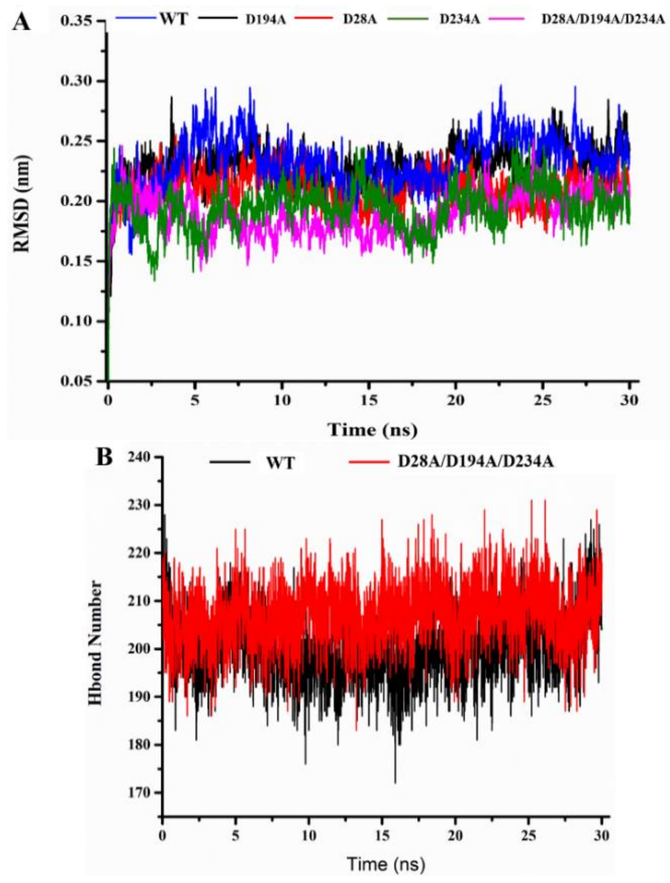

Figure 1. Molecular dynamics simulation analysis of thermal stability. A. Root mean square deviation (RMSD) values of the wild-type BisGlu16B and its mutants. B. The total number of hydrogen bonds within the enzyme molecule changes.

Table 2. Kinetic parameters and specific activity of BisGlu16B and its four mutants with different substrates at optimium temperature.

Substrate	Barley glucan			Lichenin		
Enzyme	k_m(mg/mL)	k_{cat}/k_m(mL/min·mg)	Specific activity(U/mg)	k_m(mg/mL)	k_{cat}/k_m(mL/min·mg)	Specific activity(U/mg)
BisGlu16B	3.2 ± 0.13	158 ± 6	34700 ± 665	2.6 ± 0.04	133 ± 8	23900 ± 442
D28A	3.6 ± 0.03	140 ± 9	37500 ± 337	2.1 ± 0.13	142 ± 9	24100 ± 331
D194A	3.1 ± 0.12	177 ± 11	44200 ± 734	1.9 ± 0.08	157 ± 5	24600 ± 379
D234A	3.3 ± 0.06	145 ± 7	36000 ± 794	3.1± 0.02	92 ± 2	22600 ± 189
D28A/D194A/D234A	4.2 ± 0.06	117 ± 5	38700 ± 698	2.9 ± 0.02	128 ± 5	29300 ± 543

Conclusion

This study effectively improved the thermal stability of the enzyme from the perspective of protein surface charge optimization. The mechanism of the three charged amino acids D28, D194 and D234 in the GH16 glucanase on the thermostability of the enzyme was confirmed. A glucanase with high thermal stability and high catalytic activity is obtained, eliciting conditions for the application of glucanase in waste treatment, food processing and feed addition.

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