Thermal stability of FAD-dependent oxidoreductase *Ct*AO from thermophilic fungus *Chaetomium thermophilum*

Leona Švecová[1, 2], Tereza Skálová[2], Tomáš Kovaľ[2], Lars Henrik Østergaard[3], Petr Kolenko[1, 2], Martin Malý[1, 2], Jan Dohnálek[2]

[1]Departement of Solid State Engineering, Faculty of Nuclear Sciences and Physical Engineering, Czech Technical University in Prague
[2]Institute of Biotechnology of the Czech Academy of Sciences, v.v.i., Biocev center, Průmyslová 595, Vestec, 252 50, Czech Republic
[3]Novozymes A/S, Brudelysvej 26, DK-2880 Bagsværd, Denmark
leona.svecova@ibt.cas.cz

Abstract

Chaetomium thermophilum is a soil-borne thermophilic fungus, which is able to grow in temperatures up to 60 °C. Its thermostable proteins have great potential for usage in high-temperature industry. The *Chaetomium thermophilum* FAD-dependent oxidoreductase (*Ct*AO) studied here participates in the lignin degradation process, producing hydrogen peroxide for ligninolytic peroxidases. Here we present a study of *Ct*AO thermal stability. The thermal stability was tested in a variety of buffers with pH in range 3.0–9.0. The best results were obtained in the buffer containing 50 mM NaCl and 50 mM Tris-HCl, pH 7.0. In this buffer, *Ct*AO is stable in temperatures up to 65 °C.

Keywords: FAD-dependent oxidoreductase; *Chaetomium thermophilum*; nano differential scanning fluorimetry.

Introduction

Cheatomium thermophilum (*Ct*) is a cellulolytic thermophilic fungus found in soil, dung or compost heaps. It is a member of a small group of eukaryotes that can survive a long-standing exposure to temperatures higher than 45 °C. Since *Ct* is able to grow at temperatures up to 60 °C, it appears to be a rich source of new thermostable proteins for high-temperature industrial processes [1, 2].

Novel *Chaetomium thermophilum* FAD-dependent oxidoreductase *Ct*AO is an extracellular protein from the glucose-methanol-choline oxidoreductase (GMC) family. As it is typical for the GMC family members, it consists of two domains – an FAD binding domain and a substrate binding domain. *Ct*AO participates in the lignin degradation process, where it catalyses an oxido-reductive reaction yielding product H_2O_2 for ligninolytic peroxidases. Therefore, *Ct*AO has a potential in lignocellulose decay processes or biorefinery applications (e.g. biopulping, biobleaching, etc.). Since *Ct*AO comes from a thermophilic fungus, the *Ct*AO temperature stability is the subject of the study.

Nano differential scanning fluorimetry (nanoDSF) is a method for determination of protein thermal stability. It utilizes fluorescence of tryptophan (Trp) and tyrosine (Tyr), i. e. amino acids naturally presented in proteins. Fluorescence of Trp and Tyr strongly depends on their close surroundings. Therefore, their emission spectrum reflects protein structural changes connected with increasing temperature and finally with protein denaturation [3].

Experimental details

The *Ct*AO melting temperature T_m and dependency of temperature stability on pH was studied by the nanoDSF technology using a Prometheus NT.48 instrument (NanoTemper). The heating rate was set to 2.5 °C per min starting at temperate 20 °C and ending at 95 °C. The initial buffer solution of *Ct*AO (8 mg/ml) contained 25 mM NaCl and 100 mM Tris-HCl, pH 8.5. It was further dissolved in ratio 1:11 in a variety of buffer solutions with pH in range 3.0–9.0, all containing 50 mM NaCl. The composition of individual solutions is summarized in Table 1.

Results and Discussion

The results of the nanoDSF experiments for *Ct*AO in nine types of buffers are summarized in Table 1. *Ct*AO shows two main transition temperatures (T_{m1} and T_{m2}) in buffers with pH 5.5 and higher. It is likely that the two transition temperatures correspond to the transition points of the two domains of *Ct*AO, similarly as described previously for a related enzyme *Agaricus meleagris* pyranose dehydrogenase [4]. Our experiment showed that *Ct*AO is most stable in 50 mM Tris-HCl, pH 7.0 with T_m values 65.0 °C and 77.3 °C. On the contrary, the buffers with low pH (3.0 or 4.0) reach T_m values 41.8 °C and 55.7 °C, respectively, and are not suitable for *Ct*AO for its low thermal stability.

Figure 1 compares the four tested *Ct*AO buffers with the greatest positive effect on the *Ct*AO stability. These buffers have pH values in range 7.0–8.5, including the initial buffer (25 mM NaCl, 100 mM Tris-HCl, pH 8.5). The melting curves for the initial buffer and the buffer containing 50 mM NaCl and 50 mM Tris-HCl, pH 8.5 are similar and also the T_m values are comparable, so it is apparent that the change in molar concentration of both components does not significantly affect the *Ct*AO thermal stability.

Table 1: Melting temperatures (T_{m1}, T_{m2}) of *Ct*AO in buffers with pH between 3.0 and 9.0. The T_m values were determined by nanoDSF.

Buffer	Salt	pH	T_{m1} [°C]	T_{m2} [°C]
100 mM Tris-HCl (initial)	25 mM NaCl	8.5	61.2	72.1
50 mM Citric acid/ Na citrate	50 mM NaCl	3.0	41.8	-[a]
50 mM Na acetate	50 mM NaCl	4.0	55.7	-[a]
50 mM Bis-Tris	50 mM NaCl	5.5	60.4	72.2
50 mM MES	50 mM NaCl	6.0	63.0	76.8
50 mM Tris-HCl	50 mM NaCl	7.0	65.0	77.3
50 mM HEPES	50 mM NaCl	7.5	64.0	75.6
50 mM Tris-HCl	50 mM NaCl	8.5	60.3	72.2
50 mM Glycine	50 mM NaCl	9.0	61.9	71.7

[a] low thermal stability of *Ct*AO, the second transition point was not observed

Conclusion

The thermal stability of enzyme *Ct*AO was studied using nano differential scanning fluorimetry in a range of buffers with varied pH. Buffer containing 50 mM NaCl, 50 mM Tris-HCl, pH 7.0 had the greatest positive effect on the *Ct*AO stability; *Ct*AO was stable up to 65 °C. On the contrary, acidic buffers were not suitable for *Ct*AO because they significantly decreased its thermal stability.

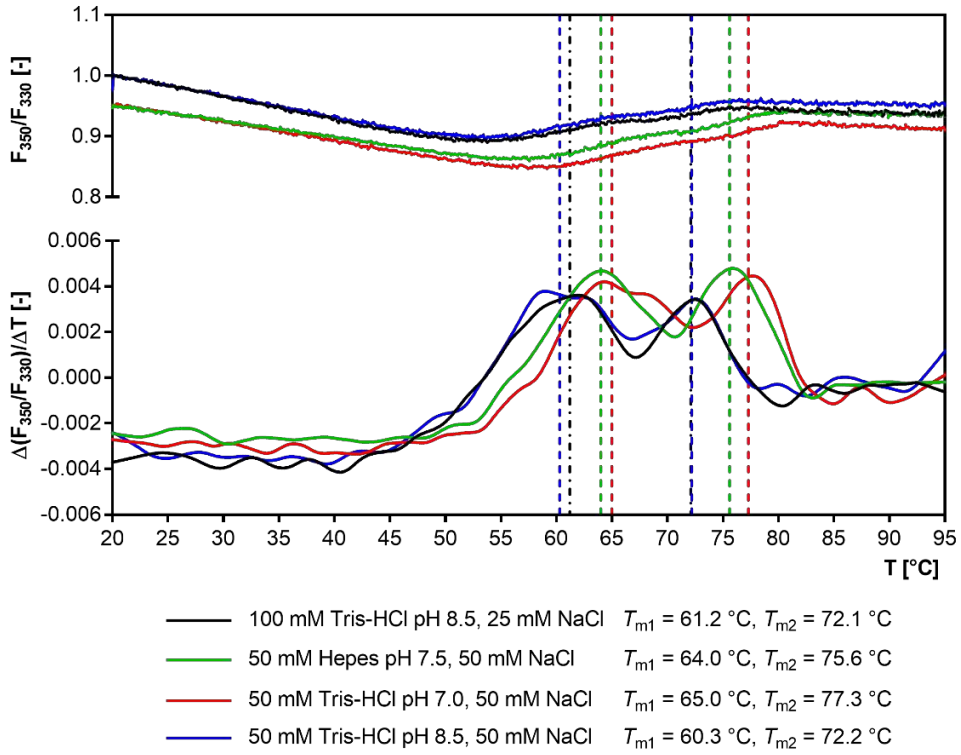

Figure 1: Melting curves of *Ct*AO in four buffers providing the highest stability (high T_m values) obtained by nanoDSF. Upper panel: ratio of integrated fluorescence at 350 nm and 330 nm against temperature. Lower panel: T_m calculation by first derivative analysis. The melting temperatures T_{m1} and T_{m2} are marked by dashed line (dot-dashed line for the initial buffer solution).

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