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PEGylation the key for increased thermostability of biopharmaceuticals: crisantaspase case-study

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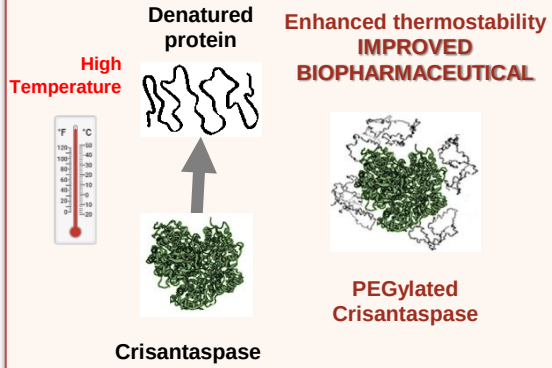
INTRODUTION

Thermostability represents an important parameter in the manufacturing process and is critical to the success or failure in the development of a viable drug. In this sense, PEGylation – covalent attachment of poly(ethylene oxide) to a protein surface - is an important strategy to improve protein drugs. It not only reduces the immune system activation but also increases the thermal and long-term stability of proteins.



RESULTS AND DISCUSSION

Circular dichroism

PEGylation preserved the enzyme secondary structure. Unfolding and refolding processes and suggested aggregation of crisantaspase and partial unfolding for PEG-crisantaspase.

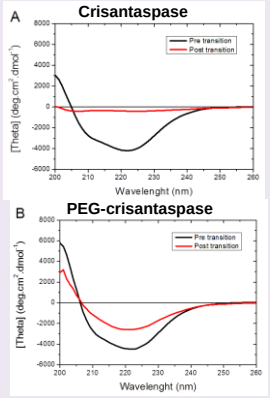


Figure 1. CD spectra of (A) native and (B) PEGylated crisantaspase at 20 °C before (black line) and after (red line) heating and cooling

Thermodynamic parameters

Table 1. Thermodynamic parameters of crisantaspase catalysed reaction and reversible unfolding of native crisantaspase and PEGylated crisantaspase (with 20 kDa PEG) estimated according to Arrhenius.

Protein	E^* (kJ.mol^{-1})	ΔH^*_U (kJ.mol^{-1})
Cris	22.3	32.5
Cris-PEG-20	11.0	25.8

The thermodynamic study is well described by first-order kinetics. The activation energy of denaturation of PEG-crisantaspase (307.1 kJ mol^{-1}) was higher than for crisantaspase (218.1 kJ mol^{-1}), which means that more energy is required to overcome the energy barrier of the unfolding process. Finally, higher and positive values of $\Delta H^{\ddagger}$ and $\Delta G^{\ddagger}$ demonstrated higher structural stability of PEG-crisantaspase

Thermostability and half-life

Half-life decreases progressively at high temperatures and higher half-life at 50 °C was observed for PEG-crisantaspase (87.74 min) in comparison to the native form (9.79 min).

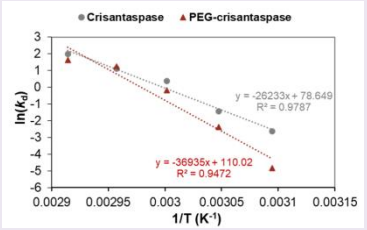


Figure 2. Semi-log plots of the first-order denaturation constant (k_d) vs. the reciprocal temperature (1/T). The slopes of the resulting straight lines were used to estimate the activation energies (E_d*) of irreversible inactivation (denaturation) of either native or PEGylated crisantaspase.

CONCLUSION

Our results demonstrated that site-specific PEGylation of crisantaspase provides protection it from thermal inactivation and improves its thermostability

FUNDING

FAPESP CNPq CAPES

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