Mn^{2+} binding enhances mesophilic properties of an Antarctic esterase

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Abstract:

The production of cold-active enzymes is one of the most endorsed strategies by psychrophilic and psychrotolerant organisms to thrive in cold environments. Usually, cold-active enzymes are distinguished by high activities at low temperatures, high structural flexibility and enhanced thermolability [1].
A poorly considered aspect in cold adaptation mechanisms is the role of metal ions. In general, in psychrophilic enzymes, metal ion - protein interactions are minimized to promote their structural flexibility, while in thermophilic enzymes are widespread and enhance their structural rigidity [2].
In this work we report the characterization of a GDSx esterase (*M*-Est) and the role of metal ion binding manganese (Mn^{2+}) in its cold adaptation [3]. *M*-Est has been identified in the genome of *Marinomonas* sp. ef1, an Antarctic marine bacterium able to grow in the temperature range from 4 to 22°C. M-Est is specific for short chain esters and can be considered a true cold-active enzyme, as it displays a T_{opt} of 5 °C and marked thermolability, with a melting temperature of 31,7 °C.
The binding between Mn^{2+} ion and *M*-Est has been extensively investigated through a complementary set of biochemical, biophysical, and computational techniques, and our results suggest that *M*-Est binds Mn^{2+} ions with a single binding site located on the enzyme surface, close to the active site. Our analyses reveal that Mn^{2+} ion enhances catalytic efficiency and thermostability of *M*-Est, besides promoting conformational changes. Overall, our results suggest that manganese binding is a peculiar solution adopted by *M*-Est to mitigate the detrimental effect of mild temperature.

1 Mangiagalli M, Brocca S, Orlando M & Lotti M (2020) The “cold revolution”. Present and future applications of cold-active enzymes and ice-binding proteins. *New Biotechnology* **55**, 5–11.
2 Feller G, Payan F, Theys F, Qian M, Haser R & Gerday C (1994) Stability and structural analysis of alpha-amylase from the antarctic psychrophile Alteromonas haloplanctis A23. *Eur J Biochem* **222**, 441–447.
3 Marchetti A, Orlando M, Mangiagalli M & Lotti M (2022) A cold-active esterase enhances mesophilic properties through Mn $^{2+}$ binding. *The FEBS Journal*, febs.16661.