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An Oxidation Stable and Chelator-resistant, Calcium-free α-Amylase from the Alkaliphilic *Bacillus* Isolate KSM-K38

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We found a novel α-amylase (AmyK38) in a culture of a novel, alkaliphilic *Bacillus* sp. strain KSM-K38. The enzyme was an alkaline, liquefying α-amylase, having a pH optimum of 8.0-9.5, and exhibiting strong resistance to chemical oxidants and chelating reagents. Therefore, the enzymatic properties of AmyK 38 fulfill the essential requirements for enzymes that can be used as effective additives in detergents. To further characterize and understand the unique features of AmyK38, we cloned and sequenced the gene for the enzyme. The amino acid sequence of the mature enzyme showed moderate homology with those of liquefying α-amylases from the genus *Bacillus*. By building a molecular model, we concluded that the high oxidative stability of AmyK38 was because the amino acid residue corresponding to Met197 in BLA is replaced by non-oxidizable Leu. We also suggested that the loss of coordination geometries of the Ca in AmyK 38 reflects its high resistance to chelating reagents. The previously reported α-amylases all contain one or more calcium per protein molecule. Surprisingly, AmyK38 was found to contain no Ca. Thus, this is the first report of calcium-free α-amylase. Additionally, AmyK38 required monovalent cations for manifestation of activity. Furthermore, we have determined the crystal structure of AmyK38, which revealed that sodium ions, instead of calcium ions, are used to retain the structure and function of this α-amylase. To make AmyK38 industrially useful, we improved the thermostability of this enzyme by protein engineering without any changes in the enzymatic properties.

Key words: calcium-free, α-amylase, *Bacillus*, oxidative stability, chelator resistance

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