The Crystal Structure of a Novel Subtilisin-like Alkaline Serine Protease, KP-43

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The crystal structure of an oxidatively stable subtilisin-like alkaline serine protease, KP-43 from *Bacillus* sp. KSM-KP43, with a C-terminal extension domain, was determined at 1.30-Å resolution. KP-43 consists of two domains, a subtilisin-like á/â domain and a C-terminal jelly roll â-barrel domain. The topological architecture of the molecule is similar to that of kexin and furin, which belong to the subtilisin-like proprotein convertases (SPCs), whereas the amino acid sequence and the binding orientation of the C-terminal â-barrel domain both differ in each case. Since the C-terminal domains of SPCs are essential for folding themselves, the domain of KP-43 is also thought to play such a role.

KP-43 is known to be an oxidation-resistant protease among the general subtilisin-like proteases. The structure analysis of oxidized form and the biochemical experiments have indicated that the oxidation of the methionine adjacent to the catalytic serine is not a dominant modification, but might alter the substrate specificities.

[1] Nonaka T., Fujihashi M., Kita A., Saeki K., Ito S., Horikoshi K., Miki K., *J. Biol. Chem.*, 2004, **279**, 47344. [2] Nonaka T., Fujihashi M., Kita A., Saeki K., Ito S., Miki K., *Acta Cryst.*, 2001, **D57**. 717.

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