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Determination of Amino Acid Residues Responsible for Salt-Tolerant Mechanisms of Glutaminase from *Micrococcus luteus* K-3

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Summary

Glutaminase from *Micrococcus luteus* K-3 (*Micrococcus* glutaminase) is a salt-tolerant enzyme that shows 40% residual activity even in 3 M NaCl. Though the crystal structure of the fragment of *Micrococcus* glutaminase consists of N-teminal and C-terminal domains has been determined, the overall structure of the intact glutaminase has been unknown. To investigate its salt-tolerant mechanisms in detail, its overall structure was determined. An addition of 0.3 M Tris(hydroxymethyl)aminoethane (Tris), which increases the enzyme activity and its salt-tolerance, to crystallization solution was a determinant to obtain crystals for the overall structure determination. Furthermore, a structure of the intact glutaminase which had been co-crystallised with its product glutamine reveals a movement of Tyr27 to bind its product glutamic acid, for which electron density was observed. By the addition of Tris, the C-terminal domain moved to affect the position of Tyr27 through 1-34 amino acid residues. These results suggest that Tris activate *Micrococcus* glutaminase by influencing the position of Tyr27 in the active site through the movement of C-terminal domain and 1-34 amino acid residues.