A large hinge bending domain rotation is necessary for the catalytic function of Escherichia coli 5 ‘-nucleotidase

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Two variants of Escherichia coli 5‘-nucleotidase with disulfide bridges that were engineered to link the two domains of the protein were used to demonstrate that a large domain rotation is required for the catalytic mechanism of the enzyme. Kinetic analysis demonstrates that the variant trapped in the open form is almost inactive but can be activated up to 250-fold by reduction of the disulfide bridge. The second variant can adopt a closed but also a half-open conformation despite the presence of the cystine linkage. As a result of this flexibility, the mutant is still active in its oxidized state, although it shows a more pronounced substrate inhibition than the wild-type protein. A theoretical model is proposed that allows estimation of the flexibility of the proteins in the presence of the disulfide domain cross-link. Despite the unexpected residual flexibility of the trapped mutants, the enzymes could be used as conformational reporters in CD spectroscopy, revealing that the wild-type protein exists predominantly in an open conformation in solution. The kinetic, spectroscopic, and theoretical data are brought together to discuss the domain rotation in terms of the kinetic functioning of E. coli 5‘-nucleotidase.
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