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Bienzymatic ATP-dependent Bacterial System Fueled by Metaphosphate as Efficient Tool to Break the Chemical Stability of δ -Lactams

AUTHORS

Mélanie HALL / UNIVERSITY OF GRAZ, HEINRICHSTRASSE 28, GRAZ

Sebastian ROTH / UNIVERSITY OF GRAZ, HEINRICHSTRASSE 28, GRAZ

Somayyeh GANDOMKAR / UNIVERSITY OF GRAZ, HEINRICHSTRASSE 28, GRAZ

Federico ROSSI / UNIVERSITY OF GRAZ, HEINRICHSTRASSE 28, GRAZ

PURPOSE OF THE ABSTRACT

Medium-sized 5- and 6-membered ring lactams are – in contrast to smaller 4-ring β -lactams – molecules with remarkable stability, a feature which appears connected to the increased resonance stabilization of the amide bond and the higher partial C-N double bond character in these lactams [1,2]. Chemical hydrolysis to the corresponding amino acids requires harsh reaction conditions (reflux and strong acid) and up to now, no enzyme active on monocyclic γ - and δ -lactams has been reported.

Our goal is to establish a biocatalytic platform for the ring opening of γ - and δ -lactams under mild reaction conditions. In this context, we explored the biocatalytic potential of bacterial ATP-dependent oxoprolinases, which function in pair (OplA and OplB) [3]. Strong activity in the presence of excess of ATP could be detected on δ -valerolactam and a range of derivatives. An ATP recycling system (Figure 1) based on cheap Graham's salt (sodium hexametaphosphate) and a polyphosphate kinase [4] allowed the use of catalytic amounts of ATP (1 mol%). Further improvements were obtained by co-expressing OplA and OplB using the pETDUET1 vector, a strategy which enhanced the soluble expression yield and the protein stability. Finally, a range of alternate phosphodonors were investigated in place of ATP. In some cases, activity was detected, providing hints on a possible mechanism, which was further studied by ^{31}P -NMR.

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FIGURES

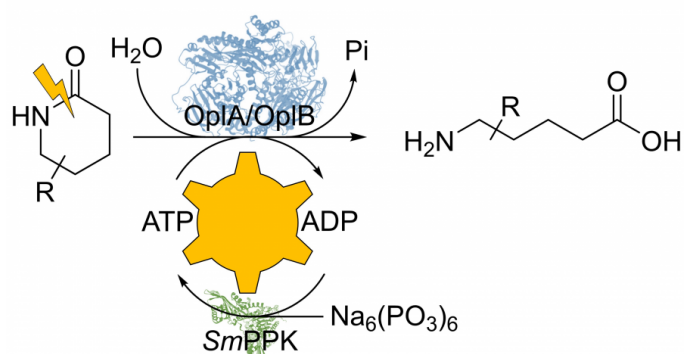


FIGURE 1

Biocatalytic metaphosphate-fueled ring-opening of δ -valerolactam derivatives by ATP-dependent-enzymes

FIGURE 2

KEYWORDS

valerolactam | ATP recycling | cascade | oxoprolinase

BIBLIOGRAPHY

- [1] M. Szostak, J. Aube, Chem. Rev. 2013, 113, 5701.
- [2] Z. Assaf, K. Faber, M. Hall, J. Biotechnol. 2016, 235, 11; Z. Assaf, E. Eger, Z. Vitnik, W. M. F. Fabian, D. Ribitsch, G. M. Guebitz, K. Faber, M. Hall, ChemCatChem 2014, 6, 2517.
- [3] A. Marjanovic, H. J. Rozeboom, M. S. de Vries, C. Mayer, M. Otzen, H. J. Wijma, D. B. Janssen, Proteins 2021, 89, 1079.
- [4] B. Nocek, S. Kochinyan, M. Proudfoot, G. Brown, E. Evdokimova, J. Osipiuk, A. M. Edwards, A. Savchenko, A. Joachimiak, A. F. Yakunin, Proc. Natl. Acad. Sci. USA 2008, 105, 17730.