myo-Inositol phosphate isomers generated by the action of a phytate-degrading enzyme from _Raoultella terrigena_ on phytate

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The properties of *myo*-inositol phosphates strongly depend on the number and distribution of the phosphate residues on the *myo*-inositol ring. *myo*-Inositol phosphates containing the 1,2,3-trisphosphate cluster, for example, are iron binding anti-oxidants (Phillippy and Graf, 1997), and D-*myo*-inositol (1,2,3,6) tetra*kis*phosphate [D-Ins(1,2,3,6)P_4] is moderately effective in opening Ca channels (Burford *et al*., 1997). In addition, D-Ins(1,2,6)P_3 has anti-inflammatory and anti-secretory properties (Claxon *et al*., 1990). Until now, the diversity and unavailability of individual *myo*-inositol phosphate intermediates precluded investigation of their bioactivity. Phytate-degrading enzymes, especially the histidine acid phytate-degrading enzymes (Konietzny and Greiner, 2002), are good candidates for the production of lower *myo*-inositol phosphates from phytate, because they release only one major *myo*-inositol penta*kis*-, -tetra*kis*-, tris-, -bis-, and -monophosphate (Greiner and Konietzny, 1996). Only the alkaline phytate-degrading enzymes from *Bacillus* species have been demonstrated to degrade phytate using a dual pathway (Kerovuo, 2000). To exploit the full potential of naturally occurring phytate-degrading enzymes, identification of the *myo*-inositol phosphates generated during enzymatic phytate degradation is of great importance.

The phytate-degrading enzyme of *Raoultella terrigena* is a cytoplasmatic enzyme belonging to the group of histidine acid phosphatases (Greiner *et al*., 1997). The final product of enzymatic phytate degradation was identified by gas chromatography coupled with mass spectrometry as Ins(2)P, with phosphate released only slowly from this compound. The full sequence of phytate hydrolysis by the phytate-degrading enzyme from *R. terrigena* was elucidated using a combination of high-performance ion chromatography (HPIC) and kinetic studies. The enzyme generated a single *myo*-inositol penta*kis*phosphate identified as D/L-Ins(1,2,4,5,6)P_5. Kinetic studies revealed that D-Ins(1,2,4,5,6)P_5 was the first intermediate of enzymatic phytate degradation (Table 1). Therefore, the phytate-degrading enzyme from *R. terrigena* is a 3-phytase (E.C: 3.1.3.8).

Table 1. Kinetic constants for enzymatic *myo*-inositol phosphate dephosphorylation.

Substrate	K_m [µmol L^{-1}]	k_{cat} [sec^{-1}]
Ins(1,2,3,4,5,6)P_6	300 ± 23	180 ± 13
D-Ins(1,2,4,5,6)P_5	345 ± 21	165 ± 8
D-Ins(1,2,3,5,6)P_5	715 ± 19	45 ± 3
D-Ins(1,2,3,4,5)P_5	721 ± 35	48 ± 3
D-Ins(1,2,5,6)P_4	372 ± 15	143 ± 9
Ins(2,4,5,6)P_4	384 ± 12	149 ± 12
D-Ins(1,2,6)P_3	415 ± 18	128 ± 7
Ins(2,4,6)P_3	no degradation	observed
$InsP_5$*	352 ± 11	162 ± 14
$InsP_3$*	419 ± 14	131 ± 9

*Generated by the phytate-degrading enzyme from *Raoultella terrigena*

Hydrolysis of D-Ins(1,2,4,5,6)P_5 resulted in equimolar amounts of two *myo*-inositol tetra*kis*-, tris-, and -bisphosphate intermediates, suggesting two alternative pathways. The *myo*-inositol tetra*kis*phosphates were identified by HPIC as Ins(2,4,5,6)P_4 and D-Ins(1,2,5,6)P_4. The existence of two alternative pathways was established by using D-Ins(1,2,5,6)P_4 as a substrate. Its enzymatic dephosphorylation resulted in a single *myo*-inositol trisphosphate [D-Ins(1,2,6)P_3] and a single *myo*-inositol bisphophosphate [D-Ins(1,2)P_2]. Therefore, one pathway of hydrolysis of D-Ins(1,2,4,5,6)P_5 proceeds via D-Ins(1,2,5,6)P_4, D-Ins(1,2,6)P_3, and D-Ins(1,2)P_2 to finally Ins(2)P (Figure 1).

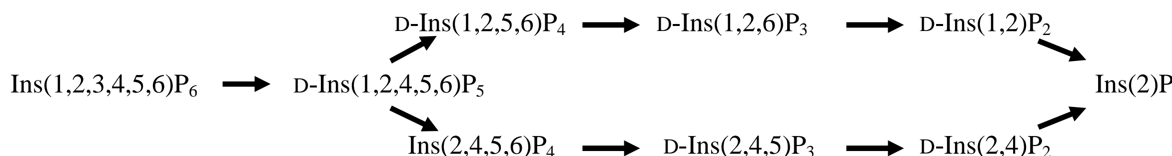

Figure 1. Degradation pathway of phytate by a phytate-degrading enzyme from *Raoultella terrigena.*

Dephosphorylation of Ins(2,4,5,6)P_4, the second *myo*-inositol tetra*kis*phosphate generated by the action of the phytate-degrading enzyme from *R. terrigena* on phytate, could be degraded via: (a) D-Ins(2,4,5)P_3 and D-Ins(1,2)P_2, (b) D-Ins(2,5,6)P_3 and D-Ins(2,6)P_2 and/or Ins(2,5)P_2,or (c) D-Ins(2,4,6)P_3 and D-Ins(2,4)P_2 and/or D-Ins(2,6)P_2. D-Ins(2,4,6)P_3 has to be excluded as an intermediate of enzymatic phytate degradation, since it was not accepted as a substrate by the phytate-degrading enzyme from *R. terrigena* (Table 1) and an accumulation of a *myo*-inositol trisphosphate during phytate degradation did not occur. Discrimination between D-Ins(2,4,5)P_3 and D-Ins(2,5,6)P_4 [= L-Ins(2,4,5)P_3] by HPIC was not possible, since these compounds are an enantiomeric pair and their kinetic evaluation was not possible due to their unavailability in pure form. However, the degradation pathway from Ins(2,4,5,6)P_4 can be rationalized by noting the similarities of the structures of "inverted" Ins(2,4,5,6)P_4 and D-Ins(1,2,5,6)P_4. Provided these similarities satisfy the requirements for substrate recognition and that the nature and orientation of the C-2 and C-3 substituents are not critical determinants of substrate recognition, the 1-hydroxy, 6-phosphate, 5-phosphate, and 4-phosphate of "inverted" Ins(2,4,5,6)P_4, respectively, mimic the configuration of the 4-hydroxy, 5-phosphate-, 6-phosphate, and 1-phosphate of D-Ins(1,2,5,6)P_4. Therefore, it was suggested, that enzymatic hydrolysis of Ins(2,4,5,6)P_4 proceeds via D-Ins(2,4,5)P_3, and D-Ins(2,4)P_2 to finally Ins(2)P (Figure 1).

This is the first report of a histidine acid phytate-degrading enzyme with two alternative pathways for the hydrolysis of phytate, resulting in one *myo*-inositol penta*kis*phosphate [D-Ins(1,2,4,5,6)P_5] and two *myo*-inositoltetra*kis*- [D-Ins(1,2,5,6)P_4, Ins(2,4,5,6)P_4], tris- [D-Ins(1,2,6)P_3, D-Ins(2,4,5)P_3], and bisphosphate [D-Ins(1,2)P_2, D-Ins(2,4)P_2] intermediates (Figure 1).

References

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