UNDERSTANDING PROMISCUITY IN CLASS II PYRUVATE ALDOLASES

  u.hanefeld@tudelft.nl
Stefan Marsden, Biokatalyse, Afdeling Biotechnologie, Technische Universiteit Delft
Isabel Bento, EMBL Hamburg, Notkestrasse 85, 22607 Hamburg, Germany

Key Words:  aldolase; promiscuity; pyruvate; hydroxypyruvate; phosphate activation.

Aldolases are grouped based on their strict donor specificity. To expand the use of these versatile enzymes it is essential to identify aldolases that utilize different donors. Recently a number of pyruvate aldolases that accepted hydroxypyruvate was identified [1]. The class II hydroxy ketoacid aldolase A5VH82 from Sphingomonas wittichii RW1 (SwHKA) accepts hydroxypyruvate and was expressed for detailed studies. A thorough investigation including X-ray crystallography allowed new insights into the mechanistic details. This revealed a phosphate dependence of the enzyme and a preference for Mn (II) [2]. More importantly the substrate scope was studied. SwHKA is promiscuous for the donor, it accepted pyruvate, hydroxypyruvate and the halopyruvates as donor molecules. This expands the range of products that can be synthesized with aldolases. In particular since the halogen containing products can easily be further modified by S\textsubscript{N}2 reactions. Additionally, the substituted pyruvates yield products with not just one but two new stereocentres. Based on mutational studies, supported by structural elucidation by X-ray crystallography the stereochemical results will be discussed. Moreover, the acceptor molecules range far beyond the common sugar-type acceptors found in much pyruvate dependent aldolase chemistry. Overall SwHKA enables with its promiscuous properties a significant extension of the aldolase toolbox (Figure 1).

![Figure 1](image.png)

*Figure 1 – SwHKA is a promiscuous aldolase that accepts a range of donors and acceptors.*