APPENDIX

DISCOVERY, CHARACTERIZATION AND KINETIC ANALYSIS OF AN ALDITOL OXIDASE FROM STREPTOMYCES COELICOLOR

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Running title: Alditol oxidase from S. coelicolor

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Steady-state kinetics
We used the determinant method for obtaining the steady-state rate equations of the proposed ping-pong and ternary complex mechanisms (1). From these rate equations the steady-state kinetic parameters were calculated. Next to this, we also show the measured and simulated Lineweaver-Burk plots of steady-state kinetics of AldO at varying oxygen and xylitol concentrations. The plots and the calculated steady-state kinetic parameters suggest that AldO follows a ternary complex mechanism.

Scheme 1. Proposed kinetic scheme (I = ping-pong mechanism, II = ternary complex mechanism).

Ping-pong mechanism
To solve the steady-state rate equation for the proposed ping-pong mechanism, the steady-state concentrations of all enzyme species need to be solved. This is accomplished by using the determinant method (1). Because of the relatively fast equilibrium between $E_{ox}$ and $E_{ox} \sim S$ these species are lumped yielding: $d([E_{ox}+E_{ox} \sim S]dt = -[E_{ox} \sim S] \cdot k_{red} + [E_{red}] \cdot k_{ox,1} \cdot O_2$ where $[E_{ox} \sim S] = ([E_{ox}] + [E_{ox} \sim S]) \cdot S/(K_d + S)$. Substitution of $[E_{ox} \sim S]$ by the latter equation then yields:

\[
d(E_{ox}+E_{ox} \sim S)/dt = -([E_{ox}] + [E_{ox} \sim S]) \cdot S/(K_d + S) \cdot k_{red} + [E_{red}] \cdot k_{ox,1} \cdot O_2
\]
From this matrix the concentration for \( (E_{\text{ox}} + E_{\text{ox}} - S) \) is obtained by deleting the first row and first column and then calculating the determinant of the remaining smaller matrix. In a similar way the concentrations of the remaining enzyme species can be obtained. These values can then be used to write down the steady-state rate equation in the form of a Michaelis-Menten equation:

\[
V = \frac{k_{\text{ox,4}} O_2 [E_{\text{red}}]}{[E_{\text{ox}} + E_{\text{ox}} - S] + [E_{\text{red}} - P'] + [E_{\text{red}} - P] + [E_{\text{red}}]}
\]

\[
V = \frac{k_3 k_4 k_{\text{ox,1}}} {(k_4 + k_{-3}) k_{\text{ox,1}} k_{\text{red}} O_2 S + k_3 (k_{\text{ox,1}} k_{\text{red}} O_2 S + k_4 (K_d k_{\text{ox,1}} O_2 + k_{\text{red}} S + k_{\text{ox,1}} O_2 S))}
\]

From this rate equation the kinetic parameters \( K_M \) and \( k_{\text{cat}} \) can be derived:

\[
K_M = \frac{k_3 k_4 K_d k_{\text{ox,1}} O_2} {(k_4 + k_{-3}) k_{\text{ox,1}} k_{\text{red}} O_2 S + k_3 (k_{\text{ox,1}} k_{\text{red}} O_2 S + k_4 (k_{\text{red}} O_2 + k_{\text{ox,1}} O_2 S))}
\]

\[
k_{\text{cat}} = \frac{k_3 k_4 k_{\text{ox,1}} k_{\text{red}} O_2} {(k_4 + k_{-3}) k_{\text{ox,1}} k_{\text{red}} O_2 S + k_3 (k_{\text{ox,1}} k_{\text{red}} O_2 S + k_4 (k_{\text{red}} O_2 + k_{\text{ox,1}} O_2 S))}
\]

\[
\frac{k_{\text{cat}}}{K_M} = \frac{k_{\text{red}}} {K_d}
\]

**Ternary complex mechanism**

The steady-state rate equation for the proposed ternary complex mechanism can be derived in a similar way. The result is:

\[
V = \frac{k_3 k_5 k_{\text{ox,2}} k_{\text{red}} O_2 S} {(k_7 k_{\text{red}} (k_{-3} + k_{\text{ox,2}} O_2) S + k_3 (k_{\text{ox,2}} k_{\text{red}} O_2 S + k_7 (K_d k_{\text{ox,2}} O_2 + k_{\text{red}} S + k_{\text{ox,2}} O_2 S)))}
\]

From this rate equation the following equations for \( K_M \) and \( k_{\text{cat}} \) were derived:
Steady-state kinetics of AldO at varying oxygen concentrations

Steady-state kinetic parameters of AldO were determined at 0.25 and 1.25 mM O$_2$ ($k_{cat}$ is respectively 13 s$^{-1}$ and 20 s$^{-1}$, $K_M$ is respectively 0.32 mM and 0.49 mM). The set of Lineweaver-Burke plots obtained in this way gives information on the kinetic mechanism that is operative. A set of parallel lines suggests that a ping-pong mechanism is employed by the enzyme, however it is known that in certain cases a ternary complex mechanism yields the same result (2,3). For AldO a set of parallel lines was obtained and correlated to a ternary complex mechanism by simulating the Lineweaver-Burk plot according to the abovementioned equations.

![Graph showing Lineweaver-Burk plots for AldO at varying oxygen concentrations](image)

**Tested compounds for substrate profiling**

D-ribose, L-arginine, diglycerol, L-arabinose, L-alanine, cholesterol, D-xylose, D-alanine, D-lyxose, L-asparagine, Dglucose, L-aspartate, ethyleneglycol, D-mannose, DL-aspartate, diethyleneglycol, D-galactose, L-cysteine, hexaethyleneglycol, L-histidine, PEG, L-proline, D-fructose, L-threonine, 3-buten-1-ol, 3-buten-2-ol, glycerol, sarcosine, cis-2-Butene-1,4-diol, meso-erythritol, DL-homoserine, L-threitol, L-ornithine, ribitol, DL-norvaline, 2-aminoethanol, D-arabitol, 1-amino-2-propanol, xylitol, 2-amino-1-propanol, D-sorbitol, methanol, 4-amino-1-butanol, D-mannitol, ethanol, galactitol, 1-propanol, 2-propanol, 1-butanol, 1,4-diaminobutane, L-rhamnose, 2-butanol, Lfucose, 1-pentanol, 2-pentanol, benzyl alcohol, 1-octanol, 2-phenylethanol, L-gulono-1,4-lactone, isoamylalcohol, α-methylbenzyl alcohol, α-methylbenzyl amine, 1-phenyl-1,2-ethanediol, maltose, 1,2-propanediol, 2-amino-1-phenylethanol, lactose, 1,3-propanediol, 2-amino-2-phenylethanol, sucrose, 1,2-butanediol, 2-amino-2-phenylethanol, 1,3-butanediol benzyl methylether, 1,4-butanediol, cinnamyl alcohol, D-melizitose, meso-2,3-butanediol, 2-methoxy-4-methylphenol, N-acetylglucosamine, butanediol, vanillyl alcohol, butanediol, 1,2-pentanediol, 1,2-hexanediol, cis-1,2-cyclohexanediol.
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