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Title: ALDH Activity Assay Control Tests: K_m for ALDH and IC50 Determination for Disulfiram

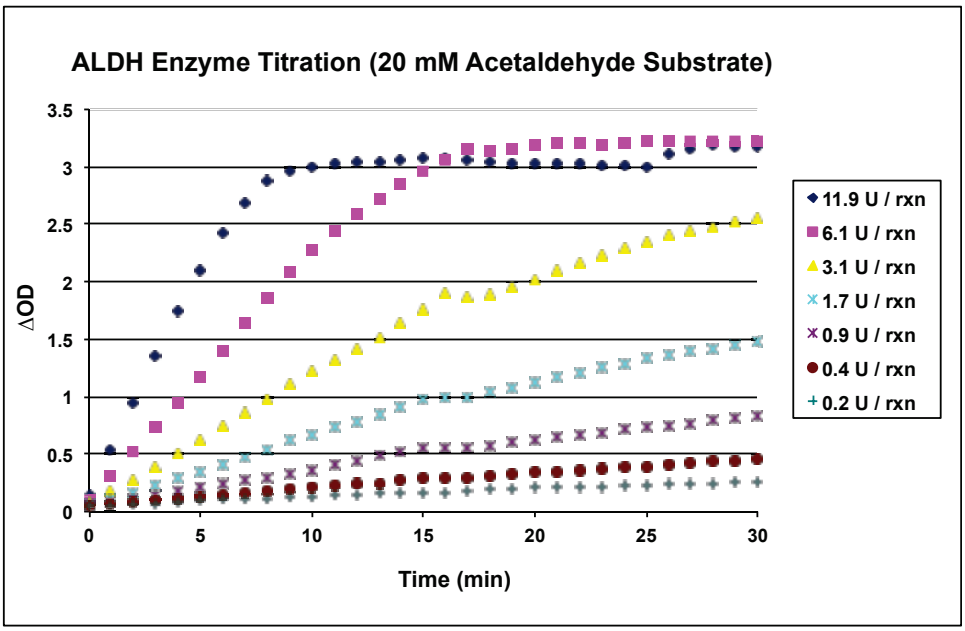
Summary

Before determining the IC50's for the Aldehyde Dehydrogenase (ALDH) inhibitor, we performed some initial experiments to establish appropriate assay conditions. We assessed conditions for baker's yeast potassium activated ALDH. First we titrated the enzyme using 20 mM acetaldehyde as the substrate. From these titrations we decided to use 1.3 U/reaction of ALDH. We next titrated the solvent DMSO to determine any inhibitory effects it may have on ALDH. The K_m of acetaldehyde for ALDH was then measured in the presence of the highest tolerable DMSO concentration. Finally, the IC50 for a known ALDH inhibitor, disulfiram, was measured. We determined the maximum DMSO concentration for the test compounds to be 100 v% (final reaction concentration of 5 v%). We determined the acetaldehyde K_m to be 900 ± 92 µM. Using 900 µM acetaldehyde, we observed an IC50 = 2.65 µM for disulfiram.

Results

ALDH Titration

In order to determine an appropriate amount of ALDH to use for the IC50 determinations, we titrated the ALDH enzyme. ALDH was titrated from 12 U/rxn. The titration was performed in the presence of 5 v% DMSO using 20 mM final [acetaldehyde] as the substrate. The ALDH titration was performed in a 96 clear well plate. From the results we opted to use 1.3 U/rxn ALDH since at this concentration the reaction would remain linear for at least 30 min.



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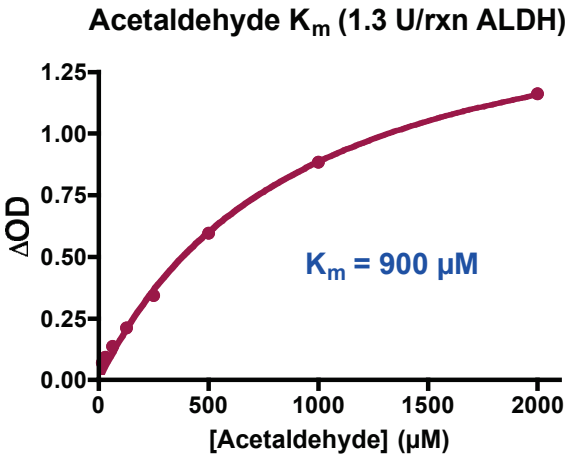
K_m of Acetaldehyde

In order to determine the K_m of acetaldehyde for ALDH, we titrated acetaldehyde from 0–2 mM with 1.3 U/rxn ALDH and computed the ΔOD ($OD_{30min} - OD_{0min}$), which is proportional to the rate of reaction, for each acetaldehyde concentration. The ΔOD's were then plotted versus [acetaldehyde] and the K_m was computed using Prism 4 (GraphPad Software Inc.). The ALDH K_m determination was performed in the presence of 5 v% DMSO (final reaction concentration). We found the K_m = 900 µM acetaldehyde.



DMSO Titration

In order to determine tolerance for DMSO of ALDH, we ran DMSO titrations from 0–5 v% in the reaction. For these titrations we used 1.3U ALDH per reaction. The reaction was initiated by adding 900 µM final [acetaldehyde] and was allowed to proceed for 30 min. We then computed the ΔOD ($OD_{30min} - OD_{0min}$), which is proportional to the rate of reaction, for each DMSO concentration. The ΔOD was then plotted versus DMSO concentration. From the result we discovered that at substrate concentration of 900 µM acetaldehyde, DMSO did not affect the activity of ALDH. For the ALDH inhibitor, we decided to move forward with 100 v% DMSO as the inhibitor solvent, which would result in a final 5 v% DMSO per reaction.

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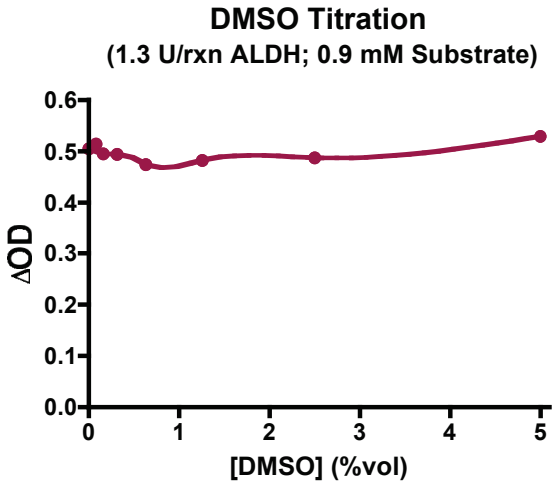

Disulfiram IC50 Determination

The IC50 determination required two steps: 1) 20 min pre-incubation of 45 µL ALDH (1.3 U/rxn) with 5 µL disulfiram in 100% DMSO, and 2) aldehyde dehydrogenase reaction with 900 µM final [acetaldehyde] for 30 min at 25˚C. We titrated disulfiram from 0-50 µM and computed the ΔOD for each inhibitor concentration. The ΔOD's were then plotted versus the inhibitor concentration and the IC50 was computed using Prism 4 (GraphPad Software Inc.). We observed an IC50 = 2.65 µM.