Studying the effects of divalent metals on the stability and activity of His-SUMO-hAMPD2-2$^{\Delta 128}$

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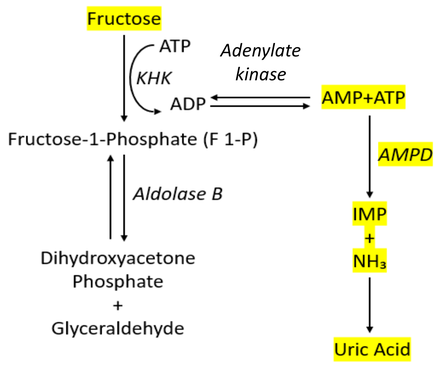
Introduction

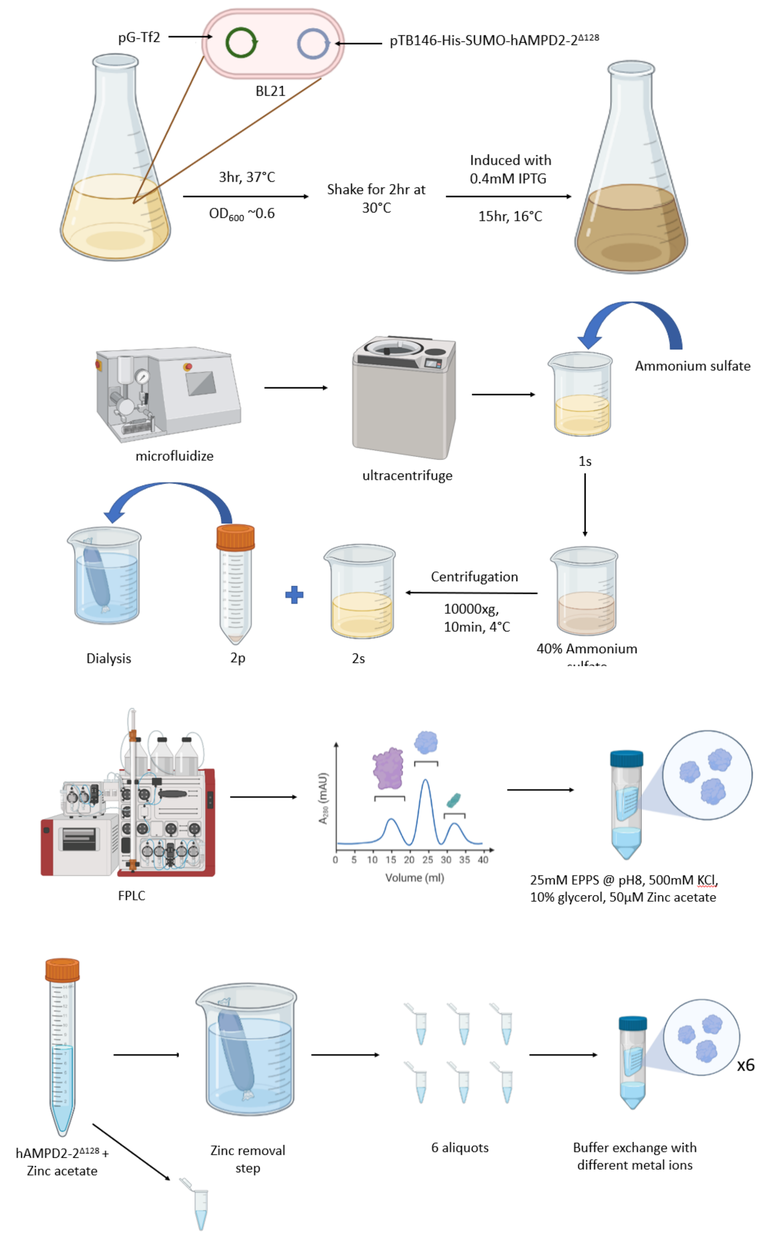
Hereditary fructose intolerance (HFI) is a rare inherited autosomal recessive disease in which a person cannot metabolize fructose. Approximately 1 in 10,000 people have HFI. It is caused by a mutation in the aldolase B gene, which codes for the aldolase B protein enzyme. This enzyme is essential in breaking down fructose in the body. There are two main pathways in the fructose metabolic pathway. In normal individuals, fructose is catalyzed with ATP into fructose 1-phosphate (F 1-P) and ADP. F 1-P is then converted into dihydroxyacetone phosphate (DHAP) and glyceraldehyde by the aldolase B enzyme. In the second pathway, ADP is catalyzed into adenosine 5'-monophosphate (AMP), which is irreversibly catalyzed by a zinc metalloenzyme called AMP deaminase (hAMPD) into inosine 5'-monophosphate (IMP) and ammonia. This ultimately leads to a series of reactions resulting in uric acid formation. When the aldolase B enzyme does not function properly, a variety of health problems can occur such as hypoglycemia, necrosis, metabolic acidosis, and liver and kidney failure. Furthermore, uric acid accumulates, causing hyperuricemia.[1]

Fructose Metabolic Pathway

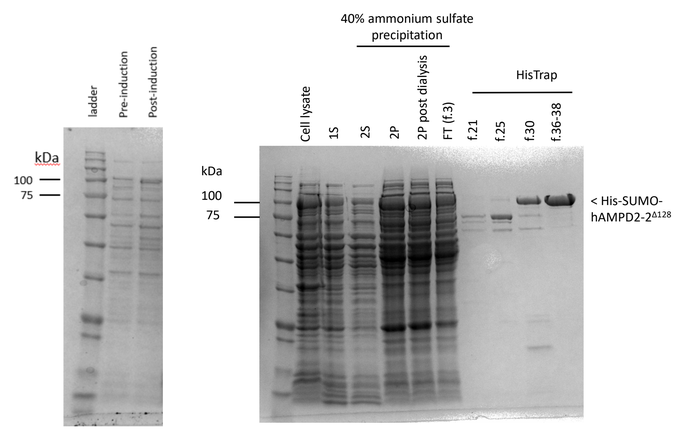


AMPD is the enzyme that catalyzes AMP to IMP in the fructose metabolic process.[2] It has both a regulatory domain and a catalytic domain. Additionally, there are different isoforms of AMPD, including AMPD1 (found in muscles), AMPD2 (found in the liver), and AMPD3 (found in erythrocytes).[3] This project focuses on the catalytic domain of AMPD2. To block the uric acid pathway, hAMPD (human AMPD) can be targeted for drug development through structure-activity relationship (SAR) studies. This project aims to further characterize hAMPD via binding affinity studies of divalent metal ions other than zinc (Ca^{+2}, Co^{+2}, Cu^{+2}, Mg^{+2}, Mn^{+2}), which is the metal cofactor proposed in the literature.[4] These studies determine which metals change the melting temperature (T_m) and activity of the enzyme. An increase in melting temperature suggests an increase in the enzyme's thermal stability, making it more amenable for future crystallography work.

Method: Expression and Protein Purification

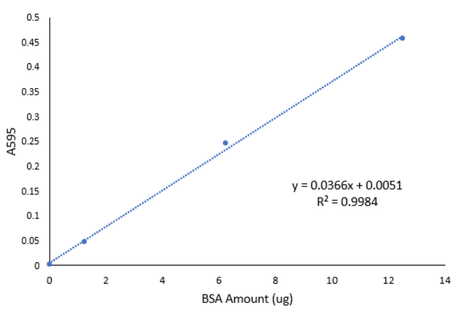


SDS-PAGE



His-SUMO-hAMPD2-2$^{\Delta 128}$ was successfully purified from *E. coli*. The band below 100 kDa was identified as His-SUMO-hAMPD2-2$^{\Delta 128}$. In the pre-induction (before IPTG) column, the band is lighter than in the post-induction column. After cell lysis, the soluble fraction (1S) containing the protein was precipitated using 40% ammonium sulfate. The resulting precipitated 2P fraction was dialysed to remove the ammonium sulfate, and further purified using nickel (HisTrap) affinity chromatography. The pure protein eluted in the fractions labeled f.36-38.

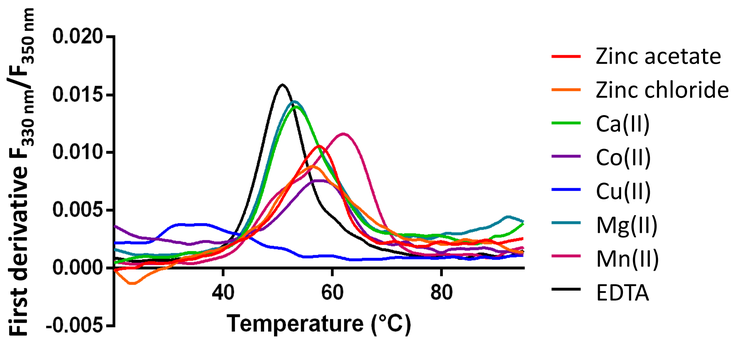
Bradford Assay

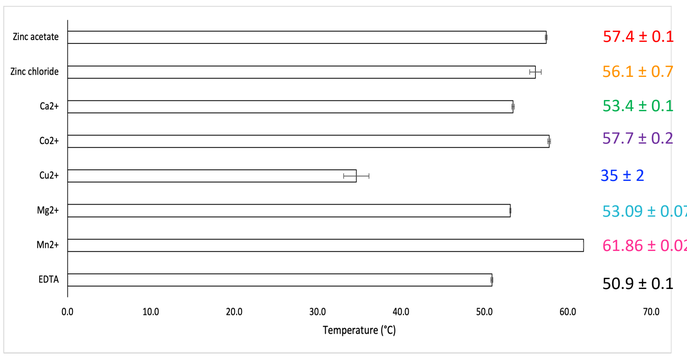


Metal (µM)	mg/mL
Zinc acetate (50 µM)	0.51
Zinc chloride (50 µM)	0.69
Ca^{2+} (500 µM)	0.70
Co^{2+} (500 µM)	0.19
Cu^{2+} (500 µM)	0.39
Mg^{2+} (500 µM)	0.81
Mn^{2+} (500 µM)	0.24
EDTA	0.34

The final concentrations for each metal were determined by using a Bradford assay. Using a bovine serum albumin (BSA) standard curve, the amount of protein with different metals was determined. These protein concentrations were determined for subsequent NanoDSF and activity assays.

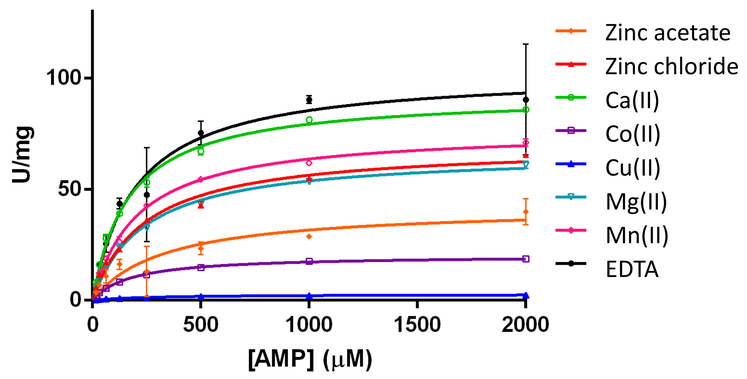
Nano Differential Scanning Fluorimetry (DSF)





His-SUMO-hAMPD2-2$^{\Delta 128}$ bound to Mn^{2+} displays the highest melting temperature. Proteins bound to different divalent metals were assayed in triplicate using NanoDSF to determine the effect of each metal on the melting temperature (T_m). Mn^{+2} increased the T_m of the enzyme by almost 4.5 °C compared to Zinc acetate, the positive control. In addition, Cu^{+2} severely decreased the T_m of the enzyme from 57.4 °C to 35 °C. Both zinc chloride and zinc acetate had similar melting temperatures. Co^{+2} yielded a similar melting temperature compared to Zn^{+2}. Ca^{+2} and Mg^{+2} had slightly lower melting temperatures compared to Zn^{+2}, with melting temperatures ~53 °C. Finally, EDTA lowered the T_m of the enzyme to 50.9 °C.

Kinetic Assay



Metal (µM)	V_{max} (U/mg)	K_M (µM)	k_{cat} (s^{-1})
Zinc acetate (50 µM)	70 ± 2	240 ± 20	106 ± 1
Zinc chloride (50 µM)	42 ± 4	330 ± 102	64 ± 3
Ca^{2+} (500 µM)	93 ± 1	168 ± 7	141.0 ± 0.8
Co^{2+} (500 µM)	20.3 ± 0.3	178 ± 10	30.9 ± 0.2
Cu^{2+} (500 µM)	2.70 ± 0.08	264 ± 25	4.12 ± 0.05
Mg^{2+} (500 µM)	66 ± 1	237 ± 12	101.1 ± 0.7
Mn^{2+} (500 µM)	77 ± 1	208 ± 10	116.9 ± 0.8
EDTA	103 ± 7	202 ± 44	156 ± 5

His-SUMO-hAMPD2-2$^{\Delta 128}$ bound to Mg^{+2} and Mn^{+2} suggests no difference in activity compared to that of zinc acetate bound protein. To determine the effect on the activity of different metals bound to the enzyme, a kinetic assay was performed using a coupled enzyme assay, under Michaelis-Menten conditions. The zinc chloride-bound enzyme decreased the activity of the enzyme, whereas Ca^{+2} increased the activity of the enzyme compared to zinc. Both Co^{+2} and Cu^{+2} decreased the enzyme's activity. The enzyme with no metal bound to the active site (EDTA sample) showed the highest activity, which was not expected.

Results / Discussion

- His-SUMO-hAMPD2-2$^{\Delta 128}$ increases in thermal stability and does not decrease in activity when bound to Mn^{+2}
- Cu^{+2} decreases both stability and activity of the enzyme
- There is no significant difference in stability between zinc acetate and zinc chloride. However, zinc acetate shows greater activity compared to zinc chloride
- Co^{+2} does not change stability but decreases activity
- Ca^{+2} decreases the stability of the enzyme but increases activity, which merits further study
- The apoenzyme (EDTA sample) decreases the enzyme's thermal stability but displays higher activity. This could be due to the presence of $MgCl_2$ in the assay.
- The presence of the His tag could affect the activity of the enzyme, as it can bind to divalent metals

Conclusion / Future Work

- Cu^{+2} severely compromised both the stability and activity of His-SUMO-hAMPD2-2$^{\Delta 128}$
- Since Mn^{+2} did not affect the activity of hAMPD and increased its stability, it can be used for crystallography studies
- The experiment will be repeated without the His-SUMO tag
- The activity assay will be repeated with hAMPD2-2$^{\Delta 128}$ to confirm these preliminary results
- $MgCl_2$ will be removed and replaced with the metal ion of interest in the assay condition to study enzyme activity
- Other divalent metals such as Fe^{+2} and Ni^{+2} will be tested using NanoDSF and Michaelis-Menten kinetic assays

References

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