Rescuing the Function of Mutated Isocitrate Dehydrogenase

Authors: Joshua Strychalski, Rizwan Ahmed, and Ryan Lubelski

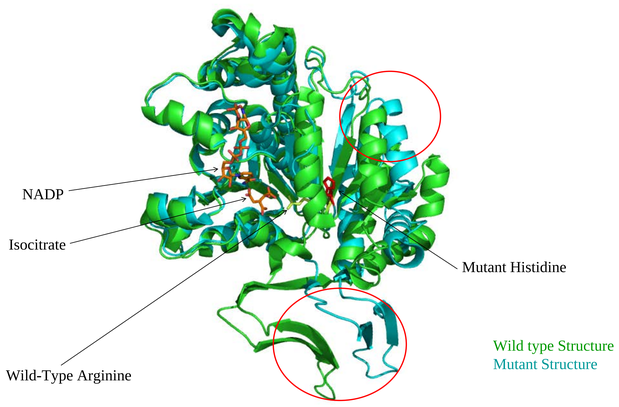
Indiana University South Bend, Department of Chemistry and Biochemistry, South Bend Indiana, USA 46634

Abstract

Isocitrate dehydrogenase (IDH1), an enzyme involved in the conversion of isocitrate into α-ketoglutarate, is an important enzyme in the citric acid cycle—a primary source of energy for the human body. A mutation in IDH1 disrupts its structure and overall functionality, leading to the high probability of developing aggressive brain tumors. In order to assess and repair this impairment, two synthetic binders, or FABs, were used to determine the effect on the activity of mutant IDH1. Comparison of the activities of these two different FABs revealed to what degree the activity of this enzyme was restored. The wild-type activity for IDH1 is greater than the activity of the mutant enzyme without the presence of any FABs. With the presence of FAB4, the activity was increased a significant degree. In comparison, FAB5 exhibits an opposite effect on the activity of the enzyme, lowering the enzyme's activity considerably. FAB4 helped to bring the activity of mutated IDH1 closer to that of wild-type IDH1; from this result, it's possible that FAB4 has the potential to lead to the development of a potential therapeutic agent.

In the citric acid cycle, isocitrate dehydrogenase (IDH1) catalyzes the conversion of isocitrate into α-ketoglutarate, a key metabolic step. In certain aggressive brain tumors, the IDH1 obtains a single point mutation at arginine 132, causing the enzyme to change its conformation so that it now converts α-ketoglutarate into 2-hydroxyglutarate. In order to repair the activity of this enzyme, randomly generated antibody fragments (FABs) could be selected to bind to the mutated IDH1. One or more of these fabricated FABs may restore the natural function of the mutated IDH1 by forcing the enzyme back into its native conformation. Is so, these FABs could potentially lead to the development of a drug that could be used to treat grade II-III gliomas.

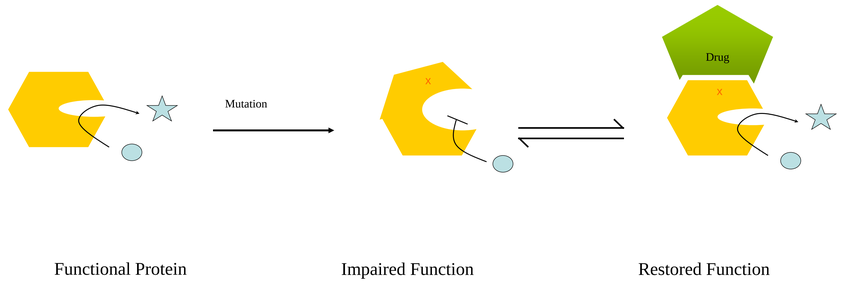
Structural Differences Between Wild-Type IDH1 and Mutant IDH1



Method

Effect of FABs on mutant IDH1 was measured using enzyme assay carried out in a micro plate reader. While holding [NADP] constant, concentrations of ICA (substrate) were increased. NADP is converted to NADPH, resulting in an increase in absorbance at 340 nm.

Method



Using Antibodies As Drugs to Restore Protein Function

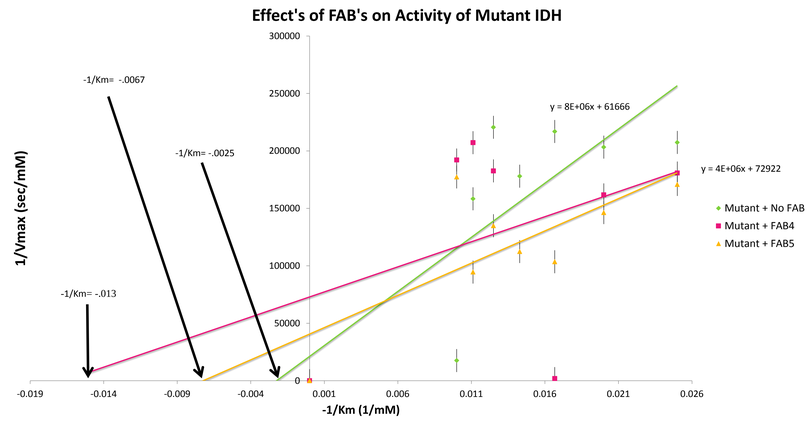


Figure 1: Double-reciprocal plot of $1/V_{max}$ vs. $1/K_m$ for mutant IDH1 and mutant IDH1 + FABs

Data

In the Lineweaver-Burke plot above, the x- and y-intercepts correspond to $-1/K_m$ and $1/V_{max}$ respectively. V_{max} indicates how fast an enzyme can produce product when the concentration of substrate is saturated. K_m is the concentration of substrate that is required to reach ½ V_{max}. For this experiment, changes in K_m are noteworthy. If the K_m is lower, then less substrate is required to reach half the maximal activity. If the K_m gets larger, then more substrate is required. Measuring the effect FABs have on K_m, will show whether the FABs activate (lower K_m) or inhibit (higher K_m) the mutated enzyme. K_m and V_{max} were calculated as follows:

Enzyme	K_m (mM)	V_{max} (mM/sec)
Wild-Type IDH1 (literature value)	0.0110	n/a
Mutant IDH1	130	1.6×10^{-5}
Mutant IDH1 + FAB4	76	1.3×10^{-5}
Mutant IDH1 + FAB5	150	2.5×10^{-5}

Figure 2: K_m values calculated for mutant IDH1 and mutant IDH1 + FABs.

Conclusions

Mutant IDH1 has been found in up to 80% of aggressive brain tumors. In order to repair the function of this mutant enzyme, the restorative potentials of two FABs were assessed. FAB5 appears to act as an inhibitor of IDH1, increasing the Km of mutant IDH1 from 130 mM to 150 mM. In contrast, FAB4 appears to have a positive effect on mutant IDH1 activity, decreasing the Km of mutant IDH1 from 130 mM to 76 mM. This suggests that FAB4 is acting as an activator, restoring the function of IDH1 activity to some degree. Although FAB4 does not fully restore the function of mutant IDH1 so that it better resembles the wild-type (Km = 0.001), it does have the potential to one day lead to the development of an effective therapeutic application.

Acknowledgments

Gretchen Anderson (Ph.D) – Indiana University South Bend
Shahir Rizk (Ph.D) – University of Chicago
Aaron Gill – Indiana University South Bend

References

Dean, A. M. and Koshland, D. E. (1993). Kinetic Mechanism of *Escherichia coli* Isocitrate Dehydrogenase. *Biochemistry*. 32, 9302-9309