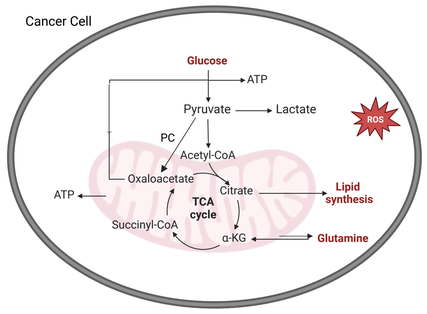
Screening for Inhibitors of Pyruvate Carboxylase

KLINGLER College of Arts & Sciences
MARQUETTE UNIVERSITY
Biological Sciences

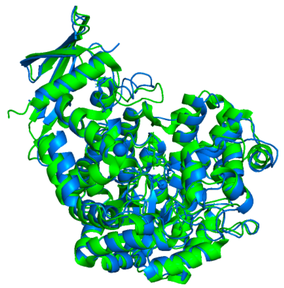
Helene N. Archer & Martin St. Maurice
Department of Biological Sciences, Marquette University, Milwaukee, Wisconsin

INTRODUCTION

- Pyruvate carboxylase (PC) plays a crucial role in the TCA cycle, a metabolic process in all of our cells that generates energy and pre-cursors for larger macromolecules.

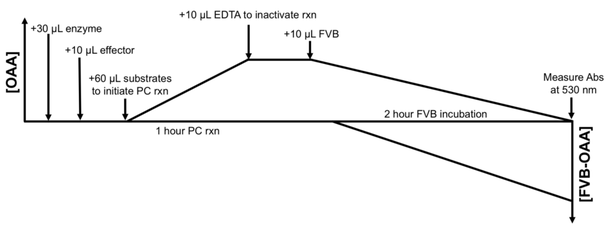


- Inhibiting pyruvate carboxylase with small molecule inhibitors can potentially be used to study, treat, and prevent diseases such as cancer and type 2 diabetes.
- Previous efforts to screen for inhibitors were against *Staphylococcus aureus* PC (*Sa*PC), where the small molecule inhibitors found were ineffective when tested against human cells.
- Why the inhibitors were not effective against the human cell PC resulted in two questions of species specific factors or allosteric modification factors influencing inhibition.
- *A. nidulans* PC (*An*PC) and a chimeric PC (ChiPC) made from part human PC and part *Sa*PC were used as they allow us to test PC activity & inhibition between different species and allosteric activation characteristics.



The carboxyltransferase (CT) domain of both human PC (green) and bacterial *Rhizobium etli* PC (blue) are completely superimposable, indicating high structural conservation in the CT domain active site.

METHODS



ASSAY DEVELOPMENT

- A FVB (Fast Violet Blue)-based assay was performed in 96-well plates as previously described (1) for *Sa*PC, *An*PC and chimeric PC to analyze the amount of oxaloacetate produced as a function of reaction time. All reactions were performed with substrates pyruvate, sodium bicarbonate, MgATP, and 33.3 ug/mL enzyme, and initiated by the addition of enzyme.
- Reactions were terminated at 15-minute intervals to a maximum time of 60 minutes by adding EDTA. Solutions were then incubated with FVB for 2 hrs to maximize formation of the colored adduct.
- The absorbance of each well was recorded using a Mini Plate reader, by measuring the maximum absorbance for the FVB-oxaloacetate adduct (530nm) and subtracted by the baseline absorbance recorded at 650nm.

SMALL LIBRARY INHIBITOR SCREENING

- A small library of inhibitors labeled A-Q were screened for inhibition of PC from different species. *Sa*PC, *An*PC, and a chimeric construct of S. aureus and human PC (chimeric PC) were screened either in the presence or absence of the allosteric activator, acetyl-CoA.
- All wells in the 96-well plate included the same substrates and concentrations as the assay development reaction.
- EDTA was used to stop the reaction after 1 hr, and absorbance values were recorded following a 2 hr incubation with FVB.

RESULTS

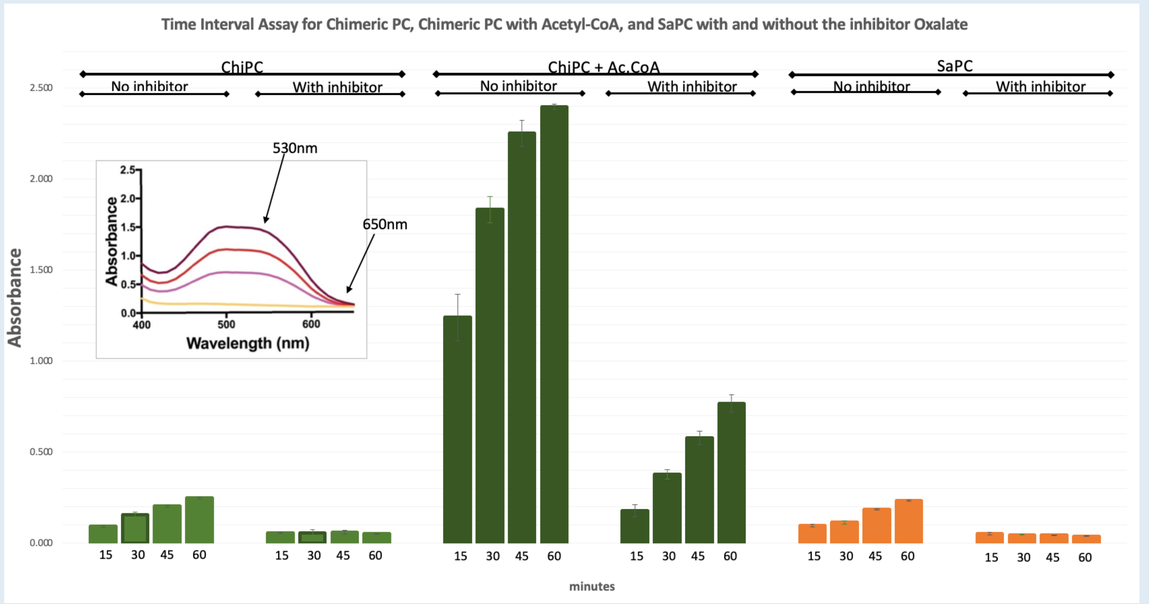


Figure 1: Normalized Absorbance values (A_{530} - A_{650}) over four different reaction times for Chimeric PC, Chimeric PC with acetyl-CoA, and SaPC, both with and without the inhibitor oxalate. The values were obtained by taking the absorbance at 530nm and subtracting the baseline absorbance values at 650nm. The inset on the left, illustrates the absorbance spectrum for FVB over a range of oxaloacetate concentrations (reproduced from Wyatt et al, 2018). These results show that, for all conditions, the largest difference in oxaloacetate production between the inhibited and uninhibited reaction occurs after 60 minutes.

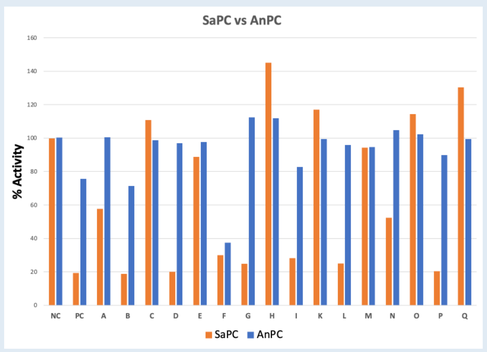


Figure 2. *Sa*PC vs. *An*PC in the absence of acetyl-CoA

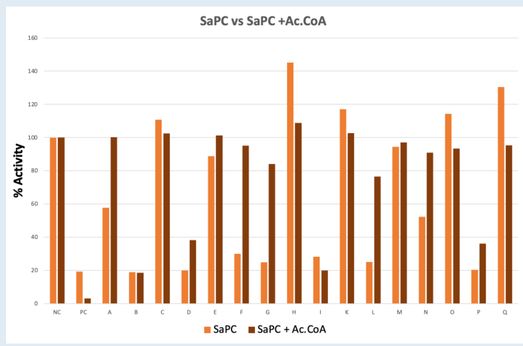


Figure 3. *Sa*PC in the absence of acetyl-CoA vs. *Sa*PC in the presence of acetyl-CoA

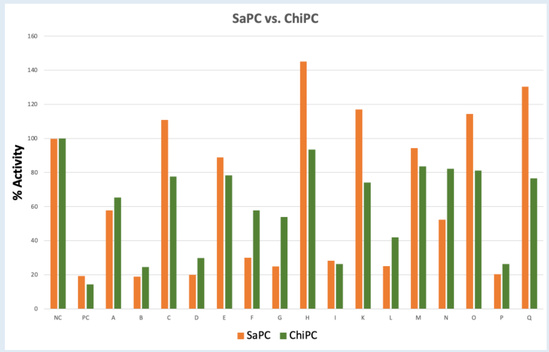


Figure 4. *Sa*PC vs. ChiPC in the absence of acetyl-CoA

Figures 2, 3 & 4: Inhibition against *Sa*PC was tested against *Sa*PC with acetyl-CoA, *An*PC, and ChiPC. The graphs represent the percent activation of the enzyme with each of the inhibitors. Figures 2 and 3 show a pattern of increased percent activation for the activated enzymes (*SaPC* + Ac.CoA and *An*PC), displaying less inhibition than the unactivated *Sa*PC. Figure 3 displays consistent percent activation for the *Sa*PC and the ChiPC with no activation.

CONCLUSION

- The activity of *Sa*PC without the activation of acetyl-CoA showed more inhibition with the library of inhibitors compared to less inhibition with the activated *Sa*PC + acetyl-CoA and the permanently activated *An*PC.
- The inhibition of *Sa*PC and the unactivated ChiPC demonstrated similar and consistent patterns.
- The ineffectiveness of the library of inhibitors on the *Sa*PC + acetyl-CoA and *An*PC is not due to the PC being from different species, but rather is dependent on the level of activation of the enzyme. The higher the level of activity of the enzyme, the more resistance it has to inhibition from small molecules.
- Further development of this research would require a screen of inhibitors on a PC that displays low levels of activity.

ACKNOWLEDGEMENTS

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2.Sellers, K., Fox, M.P., Bousamra, M., Slone, S.P., Higashi, R.M., Miller, D.M., et al. (2015). Pyruvate carboxylase is critical for non-small-cell lung cancer proliferation. J Clin Invest *125*, 687–98.