I’m a PC (Pyruvate Carboxylase)…
…and diabetes was not my idea!


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ABSTRACT

NIH estimates that 23 million Americans have diabetes, and 6.2 million are undiagnosed. If untreated, diabetes can cause complications, including heart disease and neuropathy. Type II diabetes patients cannot regulate glucose due to insulin resistance or deficiency. Pyruvate carboxylase (PC) plays an important role in insulin release from pancreatic β cells. Abnormal PC activity has been correlated with type II diabetes. PC is a dimer of dimers, each monomer a single chain with four domains: N-terminal biotin carboxylase (BC), central carboxyltransferase (CT), C-terminal biotin carboxyl carrier protein (BCCP), and allosteric domains. PC catalyzes the conversion of pyruvate to oxaloacetate (OAA). The process begins when biotin is carboxylated at the BC active site. The BCCP domain transfers the carboxybiotin to an active site in the CT domain. OAA is formed at the CT reaction site for Pyruvate & Carboxyl group. Researchers concluded that the BCCP domain swings between active sites on opposite chains, instead of sites on the same chain. The Brown Deer SMART Team (Students Modeling A Research Topic), in collaboration with MSOE, built a model of PC using 3D printing technology illustrating this movement of the BCCP domain. Current research is focused on increasing PC activity through controlling a binding site in the allosteric domain, which may increase insulin production. Supported by a grant from NIH-NCRR-SEPA.

I. What’s PC?

A possible contributing factor of type II diabetes is a decrease in activity in the enzyme, pyruvate carboxylase (PC), found in pancreatic β cells. PC catalyzes the ATP-dependent carboxylation of pyruvate to oxaloacetate. Normally, PC is involved in a cycle that leads to the release of insulin resulting in glucose absorption from the bloodstream, lowering blood sugar. Reduced PC activity correlates with reduced insulin release. Without proper release of insulin, cells do not absorb glucose and blood sugar increases. When the blood sugar level is too high, it can profoundly harm body tissues leading to complications including heart and kidney disease, stroke, hypertension, and blindness.

PC is a Tetramer

PC is a tetramer composed of two identical dimers, perpendicular to each other, with two chains each, as shown below. Before activation by acetyl coenzyme A (acetyl CoA), the chains on both dimers are equidistant from their partner on the same face. After activation by acetyl CoA, the top chains move together and become active, forcing the bottom chains to move apart.

II. Why Is PC Important In Diabetes?

1. Glucose is transported into pancreatic β cells by a specific glucose transporter.
2. Glucose is metabolized through glycolysis, resulting in pyruvate.
3. Pyruvate enters the mitochondria. About half of it combines with CoA and enters the TCA cycle (illustrated with green arrows). The other half is carboxylated by PC to generate oxaloacetate (illustrated with black arrows).
4. Oxaloacetate is converted to malate and malate exits the mitochondria where it is converted to pyruvate by malic enzyme. Pyruvate can then re-enter the mitochondria. Steps 3 and 4 are part of the pyruvate-malate shuttle. High throughput through this shuttle raises cytosolic NADPH levels.
5. Elevated cytosolic NADPH results in the release of insulin from the cell by influencing the activity of a number of other cytosolic enzymes.

II. How Does PC Work?

<table>
<thead>
<tr>
<th>IMAGE (Black is Deactivated)</th>
<th>CONSTRUCT</th>
<th>S.A. (MMOL MIN-1 MG-1)</th>
<th>ACTIVITY RELATIVE TO CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.5</td>
<td>0.2</td>
<td>100%</td>
</tr>
<tr>
<td>CT Deactivated</td>
<td>0.12</td>
<td>0.02</td>
<td>5%</td>
</tr>
<tr>
<td>BCCP Deactivated</td>
<td>0.00025</td>
<td>0.0008</td>
<td>0.1%</td>
</tr>
<tr>
<td>Chain A CT, Chain B BCCP Deactivated</td>
<td>0.49</td>
<td>0.06</td>
<td>20%</td>
</tr>
</tbody>
</table>

The data above is the result of experiments done by deactivation of specific domains on PC through the process of site specific mutagenesis. Enzymatic activity measured by spectrophotometry, demonstrates that the BCCP domain swings from the BC on the same chain to the CT on the adjacent chain. Model areas colored black in the table represent the deactivated domains.

The simplified model on the right illustrates the swinging action of the BCCP domain from the BC domains to the CT domains on adjacent chains. In the BC domain, a carboxyl group is attached to a tethered biotin on the BCCP domain of the same chain via energy from the hydrolysis of ATP. The BCCP domain then swings to the CT domain on the adjacent chain, where its tethered biotin is decarboxylated. The released carboxyl group then reacts with pyruvate to form oxaloacetate.

III. Where Does PC Go From Here?

When acetyl CoA (indicated in purple to the lower left) binds to PC, it makes PC more efficient by pushing the two chains on the top face of the tetramer closer together, allowing for faster carboxylation transfer. As of now, scientists are looking for ways to create a molecule more specific to PC that makes PC even more efficient, thereby overcoming deficiencies in the pancreatic β cell insulin release, lowering blood sugar and possibly allowing for a treatment for type II diabetes.

IV. Conclusion

Pyruvate carboxylase is distributed throughout various body tissues. In pancreatic β cells, reduced PC levels correlate with decreased insulin release. Since PC takes part in a cycle that releases insulin into the bloodstream, it may play an important role in the onset of type II diabetes. PC is allosterically activated by acetyl CoA. As of now, scientists are looking for a molecule that will activate PC more efficiently than acetyl CoA, thereby raising PC activity levels in the pancreas and decreasing the chance of contracting type II diabetes.