Rubisco (by Pat Delwiche and JoAnn Miller)

Ribulose-1,5-biphosphate carboxylase/oxygenase (rubisco) fixes carbon dioxide gas (carbon fixation) from the atmosphere at the beginning of the Calvin-Benson-Bassham cycle. This catalytic reaction is one of the first reactions in the multistep process of photosynthesis, and is responsible for the most significant production of biomass, in the form of complex carbohydrates, in all ecosystems. The carbon backbones of those carbohydrates become the backbones of the carbohydrates, lipids, proteins, and nucleic acids of organisms in every trophic level. To say that the function carried out by rubisco is fundamental to life itself is modest: carbon fixation by rubisco is the only means by which living things transform the energy captured by the light reactions into chemical energy used by almost all life on Earth (Taylor and Andersson, 1997).

The carboxylation reaction is the rate-limiting step in carbon fixation. RuBP and carbon dioxide, the substrates of carbon fixation (photosynthesis) compete for the active sites of the enzyme with RuBP and oxygen, the substrates of RuBP oxidation (photorespiration). Carbon fixation efficiency is compromised by the competing oxidation reaction of the same substrate (RuBP), at the same active site as that used for carbon fixation (Gutteridge & Gatenby, 1995). The relative rates of carbon fixation and oxidation thus determine how efficiently carbon is fixed. A reduction in carbon fixation between 30%-50% has been estimated due to these competing functions of rubisco (Andersson & Backlund, 2008).

The ratio of carboxylation rate to oxygenation rate is a measure of plant carbon fixation efficiency. Efficiencies are dependent on environmental conditions such as temperature and/or CO_2 and O_2 concentrations as well as the intrinsic characteristics of the rubisco enzyme itself. Values that describe efficiencies vary from 5 to 240 across all photoautotrophs. Higher plants have values in the range of 60 – 100 (Andersson & Backlund, 2008).

There is substantial interest in engineering the enzyme to be more efficient because rubisco has such a pivotal role in the carbon cycle, and because there is considerable variation in carbon-fixation efficiencies. One approach that has been considered is to reduce or eliminate rubisco's oxygenase activity (Raines, 2006), thereby increasing crop productivity.

Rubisco Structure

Across all organisms, rubisco enzymes consist of more than one subunit. There are two structural forms of rubisco based on number of subunits. The simplest rubisco (form II) is found in some photosynthetic bacteria, and is a dimer of two large subunits (L), each of which is composed of approximately 475 residues. Form I is the more common type of rubisco. It is composed of two different sized subunits, small (S) and large (L). Large subunits of form I are, like those of form II, approximately 475 residues, and small subunits are approximately 120 residues in length. Form I rubisco is a hexadecamer, made of eight L and eight S subunits (8L8S). All eight L subunits are identical to each other and are arranged in

the hexadecamer complex as four dimers around a central axis. This octameric core is roughly spherical; a cluster of four S subunits forms a cap on both ends of the sphere.

The L subunit is roughly pear-shaped in all forms of rubisco, The narrower, amino-terminal end of the L subunit is composed of the first 150 residues. The larger, carboxy-terminal end is built of residues 151 to 475, and consists of an α / β barrel structure made of eight α helices and eight β-strands.

The Active Site

Each large subunit of all forms of rubisco has one substrate-binding site which becomes an intact active site with the head-to-toe, antiparallel association of the coordinating large subunit in the dimer (Anderson and Backlund, 2008). The formation of a dimer is necessary to complete two active sites, with each large subunit providing opposite halves of the active site to the other. Thus, there are two active sites per dimer, and eight per rubisco complex. The active site of the enzyme is found at the interface of the L subunits in each dimer; the active site is constructed of elements from both subunits. Most of the active site residues are contributed by the loops connecting α -helices with β -strands at the carboxy-terminal end of the α/β barrel structure in the carboxy-terminal domain (wide end) of one of the L subunits of the dimer. However, two loops in the amino-terminal domain (narrow end) of the second L-subunit contribute additional residues to the active site. The entrance to each active site faces the outer surface of the hexadecameric molecule. The S subunits do not contribute directly to the active site, but interactions between L and S subunits affect enzyme function and CO_2/O_2 specificity (Andersson & Backlund, 2008).

Activation of the Active Site

Pre-catalytic modifications activate the substrate-binding sites of the dimers. The activation begins with CO_2 addition to Lysine 201 (carbamylation) within the pocket that is destined to become the active site. Activation is completed with the addition of an Mg^{2+} ion to the active site (Andersson & Backlund, 2008). The carbamylated lysine is located on the C-terminal end of one of the β-strands of the α/β barrel structure. Carbamylation stabilizes a Mg^{2+} ion that is crucial to the activation of the site. Now the active site is ready to accept the substrates RuBP and CO_2 or O_2. It is noteworthy to mention the carbamylating carbon dioxide molecule that modified lysine 201 is not the substrate carbon dioxide. The substrate CO_2 molecule is incorporated into the 3-phosphoglycerate products from the rubisco-catalyzed reaction.

The Catalytic Reaction

Sidechains within the active site are nitrogen-rich, establishing a positive potential for electrostatic interactions with the partial negative charges on the oxygen atoms of the substrate. Once the substrate

interacts with those sidechains, Loop 6 of the same subunit rotates so as to cover the substrate within the active site. The carboxy terminus of the same subunit slides into a position over loop 6, stabilizing closure of the active site. Loop 6 acts as a lid over the active site, and the carboxy terminus acts as a latch to hold the lid closed during catalysis.

The role of the magnesium ion in the active site is to create an environment conducive to catalysis. Specifically, it acts as a proton shuttle among the substrate, polar sidechains, and the Mg^{2+} ion itself during coupled oxidation-reduction reactions as CO_2 and water are being added to RuBP (Andersson & Backlund, 2008).

References:

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