proteinspotlight
> ONE MONTH, ONE PROTEIN <

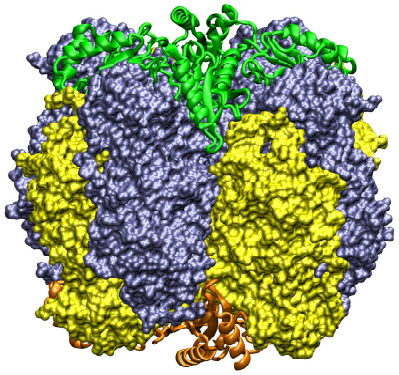
Issue 38, September 2003

www.proteinspotlight.org

The plant kingdom's sloth

Vivienne Baillie Gerritsen

Most of us take the oxygen we breathe for granted. Yet were it not for the plant kingdom, and a large and slothful enzyme, none of us would be here. Rubisco is the key enzyme which – in the process of photosynthesis – swallows up atmospheric carbon dioxide and deals with it in such a way that oxygen is released into the air. The release of oxygen is really just a side effect. Rubisco has no particular feelings for humans; it just uses the carbon from the carbon dioxide, which it recycles as sugars for its own selfish purposes. In the same way that we breathe in oxygen for life's sake and recycle the waste as CO_2.



RuBisCo

Courtesy of Fabrice David, SIB Geneva

Spinach is at the heart of the discovery of Rubisco. Shortly before the outbreak of World War II, a British biochemist by the name of A.C. Chibnall separated the proteins of spinach leaves into two components: a non soluble and a soluble component. S.G. Wildman, an American biochemist, was so intrigued by the finding that he bought some spinach at the local market, took his mixer to it, performed the same separation and went one step further. He extracted the soluble component and added a saturated solution of ammonium sulfate until a precipitate appeared. 'In those days', Wildman writes, 'the resolution of protein mixtures was more art than science.' The ongoing practice was to keep on adding ammonium sulfate in the hope of producing yet another precipitate. In this case, there was no further precipitate and he called the fraction spinach had yielded, 'Fraction I'.

A new electrophoresis apparatus had just been put into operation following the design of the Swedish investigator Arne Tiselius, who subsequently received the Nobel Prize for his invention in 1948. In 1947, Wildman subjected his Fraction I to electrophoresis and was astonished to see that the whole lot migrated as a homogeneous component, hinting that it consisted of one single protein. The finding was met with a little scepticism.

The construction of an analytical centrifuge – also invented by a Swede: Teodor Svedberg – was on its way but was interrupted by the war because some of its parts required metals which were not available. At the end of the war, the Svedberg centrifuge took its first spin with spinach soluble proteins as its clients. And doubt was dismissed. The entire fraction migrated as one compact blob confirming what electrophoresis had initially suggested. And 'Fraction I' became 'Fraction Protein I'.

No one knew what Fraction Protein I did though. And it took another few years before an inkling of what its function might be emerged. By 1954, the process of photosynthesis and its Calvin cycle had been defined in detail. What scientists needed were enzymes that were part of the cycle. There is a crucial step in the Calvin cycle where CO_2 is plucked from the atmosphere and combined with ribulose-1,5-diposphate (RuDP), thereby producing two molecules of 3-phospho-glyceric acid (3-PGA). Scientists already knew of an enzyme which performed this step. What is more, it presented the same sedimentation constant as Fraction Protein I. It was not long before researchers caught on that Fraction Protein I and this novel carboxylation enzyme were the same.

From 1956 to 1979, as research evolved, so did Fraction Protein I's name. From Fraction

Protein I, it changed to carboxydismutase, ribulose diphosphate, RuDP carboxylase, ribulose bisphosphate, RuBP carboxylase and finally the sexy 3-phospho-D-glycerate carboxylase (dimerizing) EC 4.1.1.39. It had become such a mouthful that – as a joke – it was referred to as Rubisco (*Ru* stems from *r*ib*u*lose, and *bisco* from *bis*-*c*arboxylase-*o*xygenase) by one of Wildman's friends. And it stuck.

Rubisco is certainly easier to say, yet it is the tongue-twister 3-phospho-D-glycerate carboxylase which gives us a better idea of what Rubisco actually does. Rubisco fixes atmospheric CO_2 and offers it to RuDP, a short sugar chain. It then clips the new chain into two identical phosphoglycerate molecules or 3-PGAs. 3-PGA is used in one of three ways: it is either fed back into the Calvin cycle or whipped off to make sucrose which is either used or stored.

Rubiscos are huge globular enzymes, usually involving eight large subunits – which club up into pairs – and eight small subunits. The large subunits bear the active sites and harbour the CO_2 molecules for subsequent 3-PGA formation. However, Rubisco is one of the laziest – if not the laziest – enzyme on earth. Most enzymes can process one thousand molecules per second; Rubisco plods along at a mere three molecules per second… To bypass such slothfulness, plants synthesize a gross amount of Rubisco, sometimes up to 50% of their total protein content! Which is a perfect illustration of quantity as opposed to quality. As a consequence, Rubisco is probably also the most abundant protein on earth. Scientists estimate that the biosphere boasts about 40 million tons of Rubisco, i.e. the equivalent of almost 8kg per person!

Not only is Rubisco sluggish but it is also unselective. Oxygen can easily settle in the active site thus preventing the fixation of carbon dioxide. It is then added to RuDP and a series of energy-consuming reactions occur to mend the wrong. Hence, Rubisco is slothful and inefficient. Yet quite popular. Just goes to show.

The small subunits have no active site but their role in the Rubisco enzyme is not just decorative. The eight large subunits form the core of the enzyme. The eight small ones are located both at the top and the bottom of the core, where they are bound to each other and establish multiple contacts with the large subunits – much in the fashion of tentacles feeling their way around. The small subunits probably have a structural role in the formation of the holoenzyme as well as some say in catalytic efficiency and CO_2/O_2 specificity – which in the case of Rubisco can only be to its advantage.

Rubisco's large subunits have long been the subject of research in the understanding of enzyme mechanisms. Now that it is becoming apparent that the small subunits are also important in the enzyme's function, more attention is being given to the enzyme as a whole. Consequently, any novel design of a better Rubisco also has to take into account the role of the smaller subunits. What is the point of designing new Rubiscos? Given the enzyme's natural propensity for laziness, the design of more effective Rubiscos could enhance the growth of crops in countries which need it, for example.

Rubisco may be idle and undemanding, yet you cannot help but feel a certain empathy for it…perhaps because its qualities are vaguely familiar …

Cross-references to Swiss-Prot

RuBisCO large subunit, *Spinacia oleracea* (Spinach): P00875
RuBisCO small subunit, *Spinacia oleracea* (Spinach): P00870

References

1. Wildman S.G.
   Along the trail from Fraction I protein to Rubisco (*r*ib*u*lose *bis*phosphate *c*arboxylase-*o*xygenase)
   Photosynth. Res. 73:243-250(2002)

2. Rubisco, an enzyme of global significance
   Marine Microbiology Group, University of South Florida
   http://www.marine.usf.edu/~bwawrik/RubisCO/rubisco.html

3. Spreitzer R.J.
   Role of the small subunit in ribulose-1,5-bisphosphate carboxylase/oxygenase
   Arch. Biochem. Biophys. 414:141-149(2003).
   PMID: 12781765