

**EVOLUTIONARY ADAPTATION OF RUBISCO SPECIFICITY
TOWARD CARBOXYLATION OF RuBP****GÜNTER F. WILDNER¹ and JÜRGEN SCHLITTER²**¹Lehrstuhl für Biochemie der Pflanzen, ²Lehrstuhl für Biophysik der Ruhr-Universität Bochum D-44780 Bochum, Germany

Rubisco as the most abundant protein of the biosphere is the key enzyme for two opposing pathways, the Calvin cycle and the photorespiration. The flow of carbon through both processes is determined by its kinetic properties (specificity factor) and the ratio of both substrates [CO₂] vs [O₂]. The specificity factor (SF= $V_c K_o / K_c V_o$) is an important parameter for the survival of an organism. It has increased during evolution from 40 for cyanobacteria to 80-250 for higher plants and red algae. This SF-value of Rubisco is constant for an organism and varies considerably with changing temperatures.

Experimental results demonstrate that the C-terminal tail of the large subunit determines both functions (carboxylase and oxygenase) and provide a basis for understanding differential specificity. On the basis of those experimental results and structural analysis we propose a reaction mechanism that includes the opening/closing mechanism of the binding niche, a large conformational change involving the C-terminal tail, K-128 and loop 6.

In our hypothesis, enzyme specificity can be explained by differences in the velocities of the fast carboxylation and the slow oxygenase reaction and by a variation in the time t_{closed} . The hermetically closed binding niche can transiently open after a time t_{closed} if no binding has taken place. Once the substrate CO₂ or O₂ is in the niche, the time t_{closed} is the actual reaction time available for the ligand to react with the substrate RuBP. If the time is long enough, either ligand has the same chance to react and hence there is no specificity. However, when the time is rather short, the carboxylation reaction is favored and Rubisco has a high specificity. This "time window" changed during the evolution and caused the variation in enzyme specificity.

The lifetime of the closed state is determined by the primary and secondary structure of the C-terminal tail and the underlying protein surface and their resulting interactions. In particular the C-terminal tail has a strictly conserved residue, D-473, which forms a salt bridge to R-134. An additional salt bridge as seen in cyanobacteria (E-470 to R-131) stabilizes stronger the closed enzyme conformation in low specificity enzymes.

In general, a remarkable variability in length and composition of the C-terminal tail can be observed. The *rbcl* gene of marine organisms differs considerably from cyanobacteria, green algae and land plants. Lower plants have a similar C-terminus as green algae but some families in higher plants, like the Asteraceae, gained up to 10 additional residues with a special repeating DK motive. The

variation in length and charge of the C-terminus will be discussed in respect to altered enzyme properties.

The best way to study and possibly develop further our guiding idea, the “time window” hypothesis, is certainly a combination of biochemical and biophysical methods with theoretical approaches. The comparison of crystal structures with open vs activated and closed binding niche gives a first impression as to which residues are involved in the closing mechanism. Temperature factors indicate the existence of residual motions in the relevant elements although the niche seems to be firmly locked. More information is obtained by performing molecular dynamics (MD) simulations under realistic conditions. Note that simulations are the only way to study an enzyme with its natural substrate at natural temperature and pressure, instead of an inhibitor and/or unnatural conditions suppressing the proper catalysis.

First simulations were performed on a fragment covering a binding niche and all neighbouring parts of the two relevant large subunits. The system was prepared by inserting RuBP for which a reasonable charge distribution was determined before, together with 13 water molecules seen in the crystal structure and 4 further waters to fill the internal cavity determined by analysis tools. The Rubisco fragment was put into a box filled with water and subject to a thermostat and barostat.

As expected the appropriately modelled system stays in an equilibrium with closed niche. A variety of information is gained, however, from a correlation analysis of fluctuations. Movements of the substrate and catalytic residues are correlated with elements of the closing mechanism. Unexpectedly, correlations were also found with more distant residues, which may help to explain the impact of mutations that are not understood yet and to propose new mutagenesis experiments.

Another approach is the application of a constraint in order to push the C-terminus away from its “closed” position. Such simulations carried out with the Targeted MD technique for solvent and vacuum conditions confirm the stabilization of the closed state, and indicate a variety of motions that are possible without completely opening the niche. Complete opening turns out to require a large amount of energy.

The behaviour of Rubisco in simulations is compatible with our ideas concerning the influence of conformational motions on the proper enzyme catalysis. The simulation approach gives new insight into the realization of this influence in terms of motions and residues involved and is promising to stimulate new experiments.