Chapter 1

Rubisco: attempts to reform a promiscuous enzyme

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Abstract: Despite its unique role in incorporating carbon from atmospheric CO\textsubscript{2} into the organic substances of the biosphere, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco; EC 4.1.1.39) is an inefficient enzyme; it has a low turnover number and catalyses several competing reactions, including oxygenation of ribulose-1,5-bisphosphate (ribulose-P\textsubscript{2}), in addition to the carboxylation of ribulose-P\textsubscript{2}. Information on the relative specificity for CO\textsubscript{2} and O\textsubscript{2} and the turnover number for mutant and native Rubisco from diverse species complements the increasing knowledge of the 3-dimensional structure of Rubisco at atomic resolution. We report progress towards improving the catalytic function by protein engineering and consider future experimental objectives. In particular, we have focused on loop 6 of the large subunit $\alpha/\beta$ barrel domain and its interaction with the C-terminus of the large subunit. Rubisco is a target of great agronomic importance and genetic engineering offers the prospect of increased net carbon assimilation by increasing the specificity factor. Whilst the technologies are available to achieve this, additional mutants and 3-dimensional structures are needed to distinguish the structural and ionic components that determine specific catalytic properties of Rubisco.

1. INTRODUCTION

Incorporation of carbon from atmospheric CO\textsubscript{2} to organic carbon depends on the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Rubisco catalyses the carboxylation of...
ribose-1,5-bisphosphate (ribulose-P₂) to generate two molecules of 3 phosphoglycerate (3-P-glycerate). However, in spite of its fundamental importance, Rubisco is a grossly inefficient catalyst: it is slow and it also catalyses several wasteful alternative reactions, including the oxygenation of ribulose-P₂. This oxygenation initiates photorespiratory metabolism in which, typically, more than 20% of fixed carbon is lost as CO₂. The relative partitioning between the carboxylase and oxygenase reactions is not constant but differs considerably between Rubiscos isolated from diverse species (Parry et al., 1987; Read and Tabita, 1994; Uemura et al., 1994). The highest reported value for the Rubisco specificity factor (i.e. the ratio of $V_{c.K_o}/V_o.K_c$) is 238, found in the red alga Galderia partita (Uemura et al., 1997). This is almost 3-fold greater than the specificity factors reported for Rubisco from most crop plants and about 6-fold greater than those reported from the photosynthetic bacteria. Plants also require large amounts of Rubisco to photosynthesize rapidly because Rubisco has a low turnover number (e.g. 3 per sec for each wheat Rubisco catalytic site). Although an apparent negative correlation between specificity factor and turnover has been reported (Bainbridge et al., 1995), (Figure 1), the linkage between these kinetic properties is not invariant since some point mutations have decreased $k_{cat}$ without increasing the specificity factor (Wildner et al., 1996).

Genetic engineering offers the prospect of increased net carbon assimilation by increasing the specificity factor and/or the rate of turnover. The natural variation in kinetic properties of Rubisco from various species offers a key to understanding how differences in catalytic properties are determined by primary and tertiary structure. The genes encoding the large and small subunits of Rubisco, $rbcL$ and $rbcS$, from a number of species have been cloned and expressed together in E. coli. The expression of both higher plant $rbcL$ and $rbcS$ in E. coli has not yet resulted in the production of functional enzyme. In contrast, expression of bacterial or cyanobacterial $rbcL$ and $rbcS$ in E. coli yield functional proteins which have been used extensively to investigate structure and function.

2. ENZYME STRUCTURE

In most species Rubisco is a hexadecamer composed of 8 large (M, approximately 50–55,000) and 8 small (M, 12–14,000) subunits. High resolution 3-dimensional structures have been reported for Rubisco from two higher plants, tobacco (Chapman et al., 1987, 1988; Curmi et al., 1992; Schreuder et al., 1993) and spinach (Andersson, 1996; Knight et