# SYNTHESIS OF THE SMALL SUBUNIT OF RIBULOSE 1, 5-DIPHOSPHATE CARBOXYLASE ON CYTOPLASMIC RIBOSOMES FROM GREENING BEAN LEAVES

J.C. GRAY* and R.G.O. KEKWICK

*Department of Biochemistry, University of Birmingham, Birmingham B15 2TT, UK*

## 1. Introduction

Ribulose 1, 5-diphosphate carboxylase (EC 4.1.1.39) is the most abundant protein in the leaves of higher plants, where it is confined to the chloroplasts. The enzyme is composed of two types of subunits which differ in size [1, 2]. The differential labelling of amino acids in the two subunits on incorporation of $^{14}CO_2$ by tobacco leaves suggested the possibility that the two subunits were synthesised at separate subcellular sites [3]. Further support for this suggestion came from Criddle et al. [4] who found that the incorporation of [$^{14}C$]leucine into the large subunit in greening maize leaves was inhibited by chloramphenicol whereas the incorporation into the small subunit was sensitive to cycloheximide. The synthesis of the large subunit by isolated chloroplasts of pea leaves was reported by Blair and Ellis [5]. Intact chloroplasts incorporated [$^{14}C$]leucine into the large subunit but not the small subunit, and this incorporation was inhibited by chloramphenicol. We now report that in greening bean leaves the small subunit of ribulose diphosphate carboxylase is synthesised on cytoplasmic ribosomes.

## 2. Materials and methods

Seedlings of the dwarf French bean, *Phaseolus vulgaris*, cv. Canadian Wonder were grown in darkness

for 7 days at $20°C$, and then illuminated at 11 000 lux for 24 hr.

Cytoplasmic polysomes were prepared from greening leaves by a modification of the method of Stutz and Noll [6]. Leaves were homogenised for 20 sec at top speed in an MSE Ato-Mix homogeniser with 4 vol of ice-cold 0.5 M sucrose in Buffer A (0.1 M Tris—HCl ph 7.5, 10 mM $MgCl_2$, 50 mM KCl, 5 mM 2-marcapto-ethanol) containing a complex of 2 mM guanosine and 0.2 mM vanadyl sulphate as a ribonuclease inhibitor [7]. The homogenate was strained through gauze and centrifuged at 26 000 g for 30 min. The supernatant was adjusted to 0.6% Triton-X-100 and recentrifuged at 26 000 g for 30 min. The supernatant was layered over 1.0 M Sucrose in Buffer A and centrifuged at 105 000 g for 3 hr. The pellets were suspended in Buffer A to give the polysome preparation.

For the completion and release of nascent poly-peptides, polysomes (0.14 mg RNA) were incubated for 30 min at $37°C$ in a volume of 1.0 ml with 1 $\mu$Ci of [$^{14}C$]algal protein hydrolysate (57 Ci/atom), 1 $\mu$mole ATP, 5 $\mu$mole PEP, 20 $\mu$g pyruvate kinase (8 units), 0.2 $\mu$mole GTP, rat liver 105 000 g supernatant (4.7 mg protein), rat liver pH 5 enzyme ng pro-(1.65 mg protein), 50 $\mu$mole KCl, 10 $\mu$mole $MgCl_2$, 5 $\mu$mole 2-mercaptoethanol and 100 $\mu$mole Tris—HCl pH 7.5. Labelled released protein was recovered in the supernatant after centrifugation at 165 000 g for 2 hr to pellet the ribosomes.

The small subunit of bean ribulose diphosphate carboxylase and antisera to the small subunit were prepared as described previously [8]. The supernatant (0.6 ml) containing the released protein was incub-ated with carrier small subunit (200 $\mu$g) and 0.2 ml

* Present address: Department of Biology, University of California, Los Angeles, California, 90024, USA.

anti-small subunit serum for 30 min at $37°C$ and then for 16 hr at $4°C$. Control precipitations of rabbit $\gamma$-globulin and horse anti-rabbit $\gamma$-globulin were carried out to give an estimate of the amount of non-specific absorption. All incubations were carried out in duplicate. The immunoprecipitates were collected by centrifugation at $1500\,g$ for 10 min and washed three times with 1.0 ml of 0.5% Triton-X-100 in Buffer A. The precipitates were suspended in 1.0 ml Buffer A and collected on Whatman GF/C discs. The discs were washed with 5% trichloroacetic acid, ethanol and diethyl ether, dried at $80°C$ for 10 min and counted in a toluene-based scintillation fluid.

Gel filtration in the presence of sodium dodecyl sulphate (SDS) was carried out on a 33.5 × 1.6 cm column of Sephadex G-100. Immunoprecipitates were dissociated in 1.0 ml of 1% SDS in 0.05 M sodium phosphate buffer pH 7.0, applied to the column and eluted with the SDS buffer at 5 ml/hr. Fractions (1 ml) were transferred to scintillation vials, 9 ml of Triton-X-100—toluene phosphor (1 : 2, v/v) was added and the samples were counted at 75% efficiency in a Phillips liquid scintillation counter, using an external standard.

## 3. Results

The cytoplasmic polysomes of greening bean leaves were shown to be free of contamination by chloroplast ribosomes by two criteria. Analysis in the Beckman Spinco model E ultracentrifuge showed that bean ribosomes co-sedimented with rat liver ribosomes, but sedimented faster than *E. coli* ribosomes, when centrifuged at 20 110 rev/min in the An-D rotor at $4°C$, indicating that the bean ribosomes were of the cytoplasmic 80 S type. Analysis by polyacrylamide gel electrophoresis [9] of RNA extracted from bean ribosomes showed the presence of only two RNA species of molecular weights $1.30 × 10^6$ and $0.7 × 10^6$ corresponding to the ribosomal RNA's of cytoplasmic ribosomes.

The synthesis of the small subunit of ribulose diphosphate carboxylase on cytoplasmic ribosomes was demonstrated with a cell-free system for the completion and release of nascent polypeptides. Incorporation of ^{14}C-labelled amino acids by polysomes into material precipitated by hot trichloroacetic acid was stimulated four-fold when pH 5 enzyme and 10 500 g

supernatant from rat liver was used in place of the corresponding fractions from bean leaves. The completion of polypeptide chains and their release in vitro was indicated by 25—30% of the total acid precipitable radioactivity remaining in the supernatant when the ribosomes were sedimented at the end of the incubation. The results of immunoprecipitation of the released protein are shown in table 1. The anti-small subunit serum precipitated 30% of the ^{14}C-labelled protein, whereas only 1% of the radioactivity was precipitated in control incubations with the $\gamma$-globulin—anti-$\gamma$-globulin system.

The ^{14}C-labelled protein precipitated by the anti-small subunit serum was eluted from a column of Sephadex G-100, equilibrated with 1% SDS in 0.05 M

Table 1
Immunoprecipitation of ^{14}C-labelled released protein.

| | dpm | % Released protein |
|---|---|---|
| Released protein | 22 310 | — |
| Control ppt | 249 | 1.1 |
| Small subunit ppt | 6 663 | 30.0 |

Precipitation was carried out as described in Methods and materials. The results are expressed as the means of duplicate determinations, duplicates agreed to within 5%.
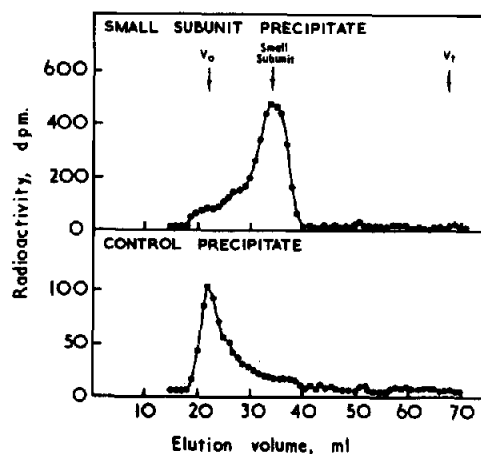


Fig. 1. Gel filtration of immunoprecipitates on Sephadex G-100 in the presence of sodium dodecyl sulphate. Gel filtration was carried out as described in Materials and methods.

sodium phosphate buffer pH 7.0, at the same position as authentic unlabelled small subunit (fig. 1). The radioactivity in the control immunoprecipitate was eluted at the void volume of the column.

## 4. Discussion

The present communication describes the synthesis of the small subunit of ribulose diphosphate carboxylase on cytoplasmic ribosomes from greening bean leaves. The synthesis of the small subunit is therefore spatially separated from the synthesis of the large subunit by the chloroplast membrane. The genes for the two subunits are also separated in the cell; nuclear DNA coding for the structure of the small subunit [10] and chloroplast DNA coding for the large subunit [11]. The control mechanisms necessary to ensure stoichiometric production of the subunits are therefore of great interest and it is hoped that future experiments with the in vitro system for the synthesis of the small subunit will give some insight into possible control mechanisms.

## References

[1] Rutner, A.C., and Lane, M.D. (1967) Biochem. Biophys. Res. Commun. 28, 531–537.
[2] Kawashima, N. (1969) Plant Cell Physiol. 10, 31–40.
[3] Kawashima, N. (1970) Biochem. Biophys. Res. Commun. 38, 119–124.
[4] Criddle, R.S., Dau, B., Kleinkopf, G.E., and Huffaker, R.C. (1970) Biochem. Biophys. Res. Commun. 41, 621–627.
[5] Blair, G.E. and Ellis, R.J. (1972) Biochem. J. 127, 4p.
[6] Stutz, E., and Noll, H. (1967) Proc. Natl. Acad. Sci. U.S. 57, 774–781.
[7] Lienhard, G.E., Secemski, I.I., Koehler, K.A. and Lindquist, R.N. (1971) Cold Spring Harbor Symp. Quant. Biol. 36, 45–51.
[8] Gray, J.C., and Kekwick, R.G.O. (1973) Biochem. Soc. Trans. 1, 455–458.
[9] Loening, U.E. (1967) Biochem. J. 102, 251–257.
[10] Kawashima, N., and Wildman, S.G. (1972) Biochim. Biophys. Acta 262, 42–49.
[11] Chan, P.-H., and Wildman, S.G. (1972) Biochim. Acta. 277, 677–680.