Light and metabolite regulation of the synthesis of ribulose-1,5-bisphosphate carboxylase/oxygenase and the corresponding mRNAs in the unicellular alga Chlorogonium

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Abstract. In the unicellular green alga Chlorogonium elongatum, the synthesis of the plastid enzyme ribulose bisphosphate carboxylase/oxygenase (RuBPCase) and its mRNAs is under the control of light and acetate. Acetate is the sole metabolizable organic carbon source for this organism. Light greatly promotes the synthesis of RuBPCase and the increase in the concentration of the mRNAs of both subunits of the enzyme while acetate has a strong inhibitory effect on this process. There is a good agreement between RuBPCase synthesis and the amount of translateable RuBPCase mRNA present in cells which are cultured under different conditions (autotrophic, heterotrophic, mixotrophic). During the transition period after transfer of the cells from heterotrophic to autotrophic growth conditions the amounts of the large and small subunits of the enzyme increase well coordinated. In contrast to the protein subunits the two subunit-mRNAs accumulate with different kinetics.

Key words: Chlorogonium – Light regulation – Metabolite regulation – Ribulose-1,5-bisphosphate carboxylase/oxygenase.

Introduction

In several algae the synthesis of the chloroplastic enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCase) is under the control of light and a metabolizable carbon source in the culture medium (Merrett 1976; Boege et al. 1981; Pineau 1982; Freyssinet et al. 1984a; Freyssinet et al. 1984b; Steinmüller and Zetsche 1984). We have shown in previous papers, that the amount of RuBPCase in the unicellular green alga Chlorogonium elongatum increases 10-fold when heterotrophically cultured cells (in the dark with acetate as carbon source) are shifted to autotrophic growth conditions (light+CO₂). In mixotrophic conditions (light+acetate) the concentration of RuBPCase increases to a smaller extent. By labeling experiments we have established that light promotes the de-novo synthesis of the enzyme while acetate represses this synthesis. Both factors exert their control by modulation of the concentration of the corresponding mRNAs (Boege et al. 1981; Westhoff and Zetsche 1981).

In the present paper we report in greater detail on the changes in concentration of the enzyme, its subunits and the subunit mRNAs after transfer of cells from heterotrophic to autotrophic and mixotrophic culture conditions. Especially, we studied the changes in enzyme and mRNA concentrations during the transition stage before the cells have reached new steady-state concentrations of these molecules. Since the enzyme is composed of eight large and eight small subunits which are coded for by plastid DNA and nuclear genes, respectively, it is of interest to know whether in the transition period the concentration of both subunits and their mRNAs change with the same kinetics. We have established conditions for cell-free translation of total cellular RNA that enable us to measure relative mRNA concentrations.

Material and methods

Culture of alga. Chlorogonium elongatum Dangeard, strain 2e (Algae culture collection, Institute of Plant Physiology, Gött...
tingen, FRG) was cultured as described elsewhere (Boege et al.
1981). In in-vivo labelling experiments with [35S]sulfate, sulfate-
anions were replaced by the same amounts of chlorides.

**Extraction of proteins.** Cells harvested by gentle centrifugation
were washed once with homogenisation buffer (50 mM 2-ami-
no-2-(hydroxymethyl)-1,3-propanediol-HCl-buffer; pH 7.4;
200 mM NaCl; 1 mM ethylenediaminetetraacetate (EDTA); 2 mM MgCl2; 5 mM dithioerythritol) and then sonicated with
a Branson sonifier (microtip; Branson, Danbury, Conn., USA)
5·10 s (55 W) under intensive cooling. The homogenate was
centrifuged for 15 min with 10000 g. The supernatant was col-
clected and centrifuged once more as above.

**In-vivo radioactive labelling of RuBPCase.** Cells were incubated
in a sulfate-free culture medium for 40 min. Then [35S]sulfate
(377 kBq ml–1 of culture, carrier free) was added. Samples were
taken and immediately cooled on ice. The cells were harvested
by gentle centrifugation and washed once with culture medium
supplemented with 2.225 mM sulfate.

**Quantitative determination of soluble protein and RuBPCase.**
Soluble protein was determined according to Esen (1978). The
RuBPCase concentration was measured by the single radial
immunodiffusion method of Mancini et al. (1965). In-vivo-lab-
elled RuBPCase was immunoprecipitated with antibodies
bound to p-aminobenzyl-cellulose (Westhoff et al. 1981). The
RuBPCase subunits synthesized in the cell-free translation sys-
tem were immunoprecipitated with subunit-specific antibodies
and the help of Staphylococcus aureus Cowan strain (Westhoff and

**Gel electrophoresis.** The labelled and immunoprecipitated sub-
units of RuBPCase were separated on 12.5% sodium dodecyl-
sulfate polyacrylamide gels using Laemli’s buffer system (1970).
The gels were fluorographed according to Bonner and Laskey
(1974).

**Radioactivity measurements.** Gel pieces containing the labelled
proteins were incubated with 200 µl of water and 1 ml of the
tissue solubilizer TS-1 (Zinsser, Frankfurt, FRG) for 3 h at
60°C, 8 ml of a toluene-based scintillation fluid were added
and the radioactivity determined in a liquid scintillation coun-
ter.

**Isolation and fractionation of RNA.** The RNA was isolated ac-
cording to Westhoff and Zetsche (1981). The precipitated RNA
was washed twice with 3 M Na-acetate, pH 6.0; 10 mM EDTA;
twice with 70% (v/v) ethanol; once with absolute ethanol; twice
with diethylether, dried and dissolved in bidistilled water. Two
volumes of ethanol were added to selectively precipitate impuri-
ties overnight. Then the solution was centrifuged at 10000 g
for 15 min. The supernatant was collected and the RNA precip-
itated by addition of 3 M Na-acetate, 10 mM EDTA (50 µl
per 1 ml supernatant) for 2 h at –20°C. The precipitated RNA
was washed again as described above. RNA was separated into
polyadenylated [poly(A)+] and non-polyadenylated [poly(A)––] RNA by chromatography on oligo(dT)-cellulose (Covey and
Grierson 1976).

**Translation of RNA in vitro.** The total cellular RNA or the
poly(A)+ and poly(A)––RNAs were translated in a nuclease-
treated rabbit reticulocyte-lysate system (Westhoff and Zetsche
1981). The amount of radioactivity incorporated in the subunits
of RuBPCase was taken as a measure for the concentration of
the corresponding mRNAs.

**Antibodies.** Antibodies specific for the small and large subunits
of RuBPCase were prepared in rabbits using standard proce-
dures.

**Chemicals.** p-Aminobenzyl-cellulose was obtained from Serva
(Heidelberg, FRG), oligo(dT)-cellulose from Collaborative Re-
search Inc. Waltham, Mass., USA. Nuclease and transfer
RNAs came from Boehringer (Mannheim, FRG). Staphylococ-
cus aureus Cowan strain was a gift from Dr. Schaeg, Institute
of Bacteriology and Immunology, Giessen, FRG. [35S]sulfate,
carrier-free (spec. activity >180 GBq mg–1) and [35S]methy-
onine (spec. activity >22 TBq mmol–1) were purchased from
Amersham Buchler, Braunschweig, FRG.

**Results**

**Quantitative translation of total cellular RNA in the rabbit reticulocyte-lysate system.** Because a separation
of total cellular RNA into poly(A)+ RNA and poly(A)––RNA by oligo(dT)-cellulose chromatography results in different losses of both mRNAs,
we used a second precipita-
tion step and wash cycle was included in the purifi-
cation procedure of the RNA as described in the
methods (Fig. 1). For our purposes the translation
system should fulfill the following requirements:
a) the amount of RuBPCase synthesized in the sys-
tem should relate to the concentration of RNA
applied to the system; b) mRNAs of both subunits
should be translated with the same efficiency; c) an
excess of other mRNAs should not compete with
the translation of RuBPCase mRNAs. All these
requirements can be fulfilled within a definite
range of RNA concentration. An acceptable linear
relationship between RNA and RuBPCase protein
synthesized was observed up to a concentration of
500 µg RNA per ml translation mix (Fig. 2).

In the range of 100–600 µg RNA, both subunits
of RuBPCase were synthesized nearly in the same
ratio. Lower RNA concentrations resulted in pre-
ferred synthesis of the small subunit (Fig. 2b).
When the RNA from autotrophic cells with a high propor-
tion of RuBPCase mRNA was mixed with RNA from heterotrophic cells with a low concen-
tration of RuBPCase mRNA and then translated
there was a linear relationship between the portion
of RNA of autotrophic cells in the mixture and
the amount of RuBPCase synthesized. Therefore,
no competition between the bulk of RNA and
RuBPCase-specific mRNAs was detectable in the
RNA concentrations which were applied to the
reticulocyte-lysate system (Fig. 3).