BULG. J. PLANT PHYSIOL., 1999, 25(3–4), 31–44

BARLEY LEAF RUBISCO, RUBISCO BINDING PROTEIN AND RUBISCO ACTIVASE AND THEIR PROTEIN/PROTEIN INTERACTIONS*

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Received 25 January 1999

Summary. The existence of protein/protein interactions between Rubisco (R), Rubisco binding protein (RBP) and Rubisco activase (RA) was proven independently in the presence or in the absence of the ATP-regenerating system. Sandwich and inhibitory ELISA, immunoprecipitation and immunoblotting methods were used with monoclonal antibodies (Mabs) against barley Rubisco or polyclonal antibodies against barley RBP or RA. The results show that the protein/protein interactions are localized on the Rubisco large subunits at a distance from the active site and from the sites connected with Mabs.

Key words: *Hordeum vulgare* L., immunoblotting, immunoprecipitation, monoclonal antibodies, Rubisco, Rubisco activase, Rubisco binding protein

Abbreviations: ATP – adenosine-5-triphosphate; BSA – bovine serum albumin; CABP – 2-carboxyarabinitol-1,5-bisphosphate; EDTA – ethylene diamine tetra-acetic acid; ELISA – enzyme-linked immunosorbent assay; LS – large subunits; Mab – monoclonal antibody; PAGE – polyacrylamide gel electrophoresis; PBS – 20 mM phosphate buffered saline (pH 7.2); PMSF – phenyl-methyl sulphonyl fluoride; R – Rubisco; RA – Rubisco activase; RBP – Rubisco binding protein; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase; RT – room temperature; RuBP - ribulose-1,5-bisphosphate; SDS – sodium dodecyl sulphate; SS – small subunits; Tris – tris (hydroxymethyl)-aminomethane; TBS – tris buffered saline (pH 7.4)

* This paper is dedicated to the 70-th anniversary of Prof. Dr. Sci. Ivan Yordanov.

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Introduction

Rubisco is a very abundant bifunctional oligomer chloroplast enzyme (560 kDa) which catalyses photosynthetic carboxylation or oxygenation in plant leaves. In higher plants the holoenzyme is composed of eight large (LS) and eight small (SS) subunits with a molecular mass of about 53–55 kDa and 12–14 kDa, respectively. The catalytic sites are placed on the large subunits (Andrews et al., 1987; Knight et al., 1990). The LS are synthesized in chloroplasts whereas the SS are synthesized in the cytoplasm and transported into the chloroplasts for assembly. Thus, the assembly of the holoenzyme is under the dual chloroplast and nuclear genetic control. It is a complex process that may require helper proteins called "chaperonins" (Roy and Cannon, 1988; Roy at al., 1988). One nuclear-encoded chloroplast protein named Rubisco binding protein (720 kDa) is able to take part in Rubisco assembly (Roy et al., 1988). This RBP consists of 14 subunits of equal amounts of two isoforms called α-subunits (61 kDa) and β-subunits (60 kDa) (Hemmingsen and Ellis, 1986). The hypothesis was advanced that the function of the RBP is to keep the newly synthesized LS in a soluble form suitable for assembly with SS entering the chloroplasts from the cytoplasm (Barraclough and Ellis, 1980). Musgrove and Ellis (1986) proposed that RBP is a "molecular chaperone" that ensures the correct assembly of Rubisco but the mechanism by which the RBP functions in the assembly is still largely unknown. The RBP occurs in at least two states: monomers, and the high molecular mass complex (Roy et al., 1988). It is clear that assembly of Rubisco can take place when the high molecular weight complex containing the RBP has been dissociated in the presence of ATP and Mg^{2+} ions (Cannon et al., 1986), so it is an ATP dependent process. It is also known that RBP forms intermediate complexes with LS of the enzyme (Cannon et al., 1986). Immunological data of Barraclough and Ellis (1980) indicate that LS in the complex are not available for reaction with LS specific antibodies, so it seems likely that the LS are probably surrounded by RBP subunits when it complexes with the RBP. In our earlier investigations a cross-reaction was established between Rubisco and RBP (Mladjova et al., 1992). We have suggested that common determinants in the molecules of Rubisco and RBP exist, probably related to the function of RBP in the Rubisco holoenzyme assembly. Such cross-reaction was observed by Musgrove and Ellis (1986), Hemmingsen and Ellis (1986), Demirevska-Kepova et al. (1993) with polyclonal antibodies against Rubisco and RBP. Further investigations could explain such results.

To be catalytically competent the holoenzyme must be activated through carbamylation (Andrews and Lorimer, 1987). Full activation of Rubisco *in vivo* could be achieved not only with CO_2 and Mg^{2+} but with a specific nuclear-encoded chloroplast protein named Rubisco activase (Somerville et al., 1982; Salvucci et al., 1985), which possesses ATP-ase activity (Streusand and Portis, 1987; Robinson and Portis, 1989). The complete mechanism by which RA acts remains also unclear (Salvucci

and Ogren, 1996). It is considered that RA specifically removes sugar phosphates from Rubisco catalytic sites taking off their inhibitory effect on the enzyme activity and allowing CO_2 and Mg^{2+} a more rapid access to carbamilation and activation (Robinson and Portis, 1988; Portis, 1990). It is suggested that the activation of Rubisco by RA is a multistep process involving conformational changes in Rubisco and RA, formation and dissociation of putative Rubisco-RA complex (Wang et al., 1992). Presumably, the energy from ATP hydrolysis is used to induce specific conformational changes in Rubisco that alter its binding affinity for sugar phosphates (Salvucci, 1993). Possibilities of interactions between Rubisco and RA within some binding domain placed on Rubisco molecule exist.

Süss et al. (1993, 1995) prove the connection of Rubisco with other enzymes from Calvin's cycle. That is why, it could be supposed that the molecules of Rubisco have sites for association with other proteins necessary for its function – RBP or RA. In the present study this hypothesis was tested by immunochemical methods using purified Rubisco, RBP and RA.

The aim of the study was to obtain more immunochemical evidences about protein/protein interactions between Rubisco, RBP and RA using monoclonal antibodies against barley Rubisco and polyclonal antibodies against barley RBP and RA.

Materials and Methods

Plants. Barley **(***Hordeum vulgare* L.**)** plants were grown under photothermostate conditions with 12 h photoperiod (180 $\mu E.m^{-2}.s^{-1}$) at 20–25 °C. After 10 days, when the primary leaves were fully expanded, they were harvested and rapidly frozen in liquid nitrogen.

Antigen preparation. Rubisco, RBP and RA were extracted and purified as described by Demirevska-Kepova and Simova (1989), Demirevska-Kepova and Juperlieva-Mateeva (1990) and Demirevska-Kepova et al. (1995) with some modifications. Barley leaves were homogenized (1:4 m/v) in ice-cold 100 mM Tris-HCl (pH 8) buffer containing 10 mM $MgCl_2.6H_2O$, 20 mM $NaHCO_3$, 1 mM EDTA, 20 mM β-mercaptoethanol, 0.1 M PMSF, 10 μM leupeptin, 3% Polyclar AT, 1% ovalbumin (m/v) and 12.5% glycerol (v/v) (including 0.4 mM ATP for RA isolation and storage) and precipitated by 35–55–70% saturation with ammonium sulphate. The precipitate was dissolved and separated on a Sephacryl S-200 column (1.48×50 cm). The Rubisco, RBP and RA enriched fractions were purified on a DEAE-Sephacel column (3.45×10.5 cm). RA was eluted with 200 mM KCl, Rubisco – with 300 mM KCl, RBP – with 400–500 mM KCl and further purified on a Sephacryl S-300 column (2.5×90 cm). All procedures were carried out at 4 °C. Purified antigens were stored under liquid nitrogen and were characterized electrophoretically on 15% SDS-PAGE.

Antibodies. Hybridoma lines 2G2, 2F4, 2H5 and 2D11 (Mladjova et al., 1992) secreting specific Mabs against Rubisco from barley were restored from frozen state and after cloning by limiting dilutions were cultured in mass cultures. Supernatants containing Mabs were collected and stored at – 20 °C. Polyclonal antibodies against Rubisco binding protein and Rubisco activase were prepared and checked according to Demirevska-Kepova et al. (1990, 1995).

In some experiments the exhausted polyclonal antibodies against RBP were obtained. After 3 h polymerisation of Rubisco with 2.5% glutaraldehyde in the presence of bovine serum albumin and PBS, pH 7.2 at RT, polyclonal antibodies against RBP were exhausted to Rubisco holoenzyme molecules at 4 °C. The exhausted antibodies were precipitated with 50% ammonium sulphate.

Sandwich ELISA. Wells of microtiter plates were coated with purified antigens: RBP or RA (2.10^{-8} M or 2.10^{-9} M), or R (2.10^{-9} M in different forms). The controls are described under the figures. R or RBP, or RA (1.10^{-8} M) were added. The sandwich was completed with Mab 2G2, 2F4, 2D11 added in constant concentration (1:8) and Mab 2H5 (not deluted). In some experiments polyclonal antibodies against RBP or RA (1:1000) were used. R or R forms connected with RBP, or RA were revealed with peroxidase-rabbit antimouse Ig G conjugate, or goat anti-rabbit Ig G peroxidase conjugate.

Inhibition ELISA. Wells of microtiter plates were coated with purified Rubisco (1.10^{-9} M antigen in Tris-HCl buffer, pH 8.0). Mab in constant concentration (1:16 for 2G2, 2F4 2D11 and 1:1 for 2H5) was incubated separately with R, RBP or RA in concentrations (from 5.10^{-10} M to 5.10^{-8} M in 1% ovalbumin) for 1 h at 30 °C and then transferred to the plates with immobilized Rubisco. The Mabs bound to immobilized R were developed with peroxidase-rabbit antimouse Ig G conjugate.

Immunoprecipitation. The procedures were performed according to Sanches de Jimenes et al. (1995). Purified barley Rubisco samples were mixed (1:2) with purified barley RBP or RA samples and incubated in the reaction mixture containing 20 mM Tris HCl pH 8, 20 mM $NaHCO_3$, 20 mM $MgCl_2$, 5 mM ATP, 5 mM phosphoenol pyruvate, 20 units pyruvate kinase and 4 mg/ml BSA at 25 °C or without ATP regenerating system for RA, or on the presence or on the absence of ATP and Mg^{2+} for RBP. After 15 min incubation the reaction was stopped with 200 µl of buffer with 10 mM TBS pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.5% Tritone ×100, 1 mM PMSF, 15 µM leupeptin and addition of 30 µl of polyclonal antibodies against barley Rubisco. The mixture was allowed to stand overnight at 4 °C. Then the samples were supplemented with 20 µl of protein A-Sepharose and incubated for an additional 2 h at 25 °C. The immunoprecipitate was collected by centrifugation, dissolved in 20 µl of sample buffer, and analysed by SDS-PAGE (Laemmli, 1970). Some of the gels were stained with Coomassie blue and others were immunoblotted onto nitrocellulose sheets for Western blot analyses. Differences in variants are explained under the figures.

Immunoblotting. After 12.5% SDS-PAGE proteins were electrophoretically transferred onto nitrocellulose sheets and the sheets were cut in vertical strips. The strips were blocked by 5% (w/v) BSA in PBS (pH 7.2) for 30 min at RT. After a thorough wash, hybridoma supernatants (1:1) were added to each well and incubated for 20 min at RT. The nitrocellulose strip was washed again and peroxidase-conjugated anti-mouse Ig serum diluted 1:100 in Tris-HCl buffer was added for 1 h at RT. The strips were washed and the bound enzyme activity was revealed by incubating them with 3,3-diaminobenzidine tetrahydrochloride in Tris-HCl buffer, pH 7.4 containing H_2O_2. To stop the reaction the strips were washed with 7% acetic acid.

Protein quantification. Soluble proteins were measured by the method of Bradford (1976).

Statistics. All statistical analyses were carried out using ORIGIN version 3.0 software. Some of the means were compared for significance of the observed differences by the Student's t-test.

Results

Mab 2G2, 2F4, 2D11 and 2H5 were checked about the existence of cross-reactivity between Rubisco, RBP and RA in inhibition ELISA (Fig. 1). For RA we obtained a small cross-reactivity only with Mab 2H5. RBP revealed cross-reactivity with all investigated Mabs, but a different one with rather low intensity with Mab 2F4.

In sandwich ELISA we tested protein/protein interactions between Rubisco and RBP or RA using Mabs 2G2 and 2F4 (Fig. 2). The presence of pure RBP did not influence the binding of Mab 2G2, but influenced the binding of Mab 2F4. Under the same experimental conditions the presence of purified RA influenced the binding of both Mabs especially Mab 2G2. All the data are in accordance with the existence of the protein complexes with RBP or RA and Rubisco.

In the next type of experiments immobilizing RBP or RA and albumin we tried to examine the specificity of such protein/protein complexes. When ELISA was used with immobilized RA we obtained big differences in optical density which shows the specific character of the interactions between Rubisco and RA (Fig. 3). The ELISA data with immobilized RBP showed that cross-reactivity between RBP and Mabs influenced the specific revealing of theirs interactions especially in concentrations of RBP 10^{-8} M (data not presented).

If all the investigated Mabs could detect such kind of protein/protein complexes, the site of protein binding on the Rubisco molecule is in a position different to that responsible for Mabs binding, because there were no stereo hindrances for sandwich formation in ELISA.

In another experimental series of sandwich ELISA the different forms of Rubisco (activated – with CO_2 and Mg^{2+}, non activated – without CO_2 and Mg^{2+} and the com-

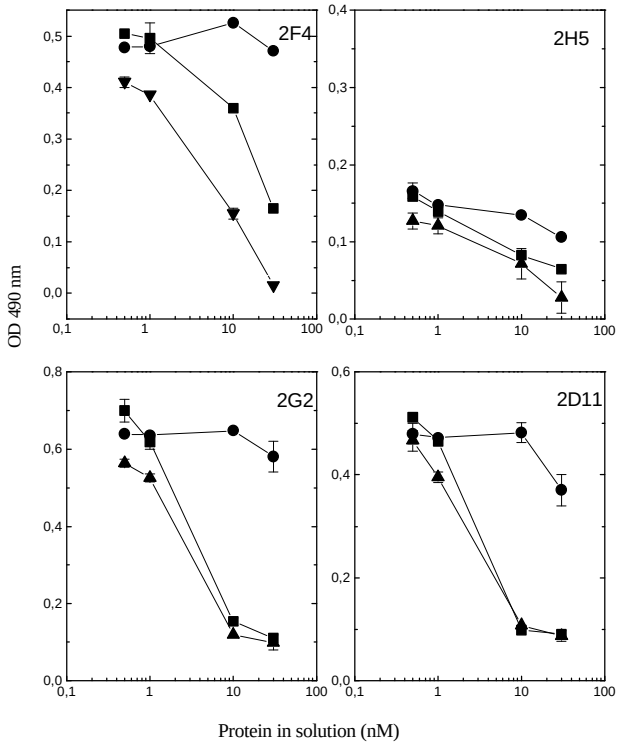

Fig. 1. Cross-reactivity between Rubisco activase (RA), Rubisco binding protein (RBP) and Rubisco (R) in inhibition ELISA. Wells of microtiter plates were coated with purified R (1.10^{-9}M antigen in Tris-HCl buffer, pH 8.0). Mab in constant dilution (1:16 for 2G2, 2F4 and 2D11, and 1:1 for 2H5) was incubated in another plate with R (triangle), RBP (square) or RA (circle) in concentrations (from 5.10^{-10} to 5.10^{-8}M in 1% ovalbumin) for 1 h at 30 °C and then transferred to the plates with immobilized Rubisco. The Mabs bound to immobilized R were developed with peroxidase-rabbit antimouse Ig G conjugate. On the ordinate, OD 490 nm absorption proportional to the Mab bound by the immobilized antigens. On the abscissa, R, RBP or RA concentration in solution (nM).

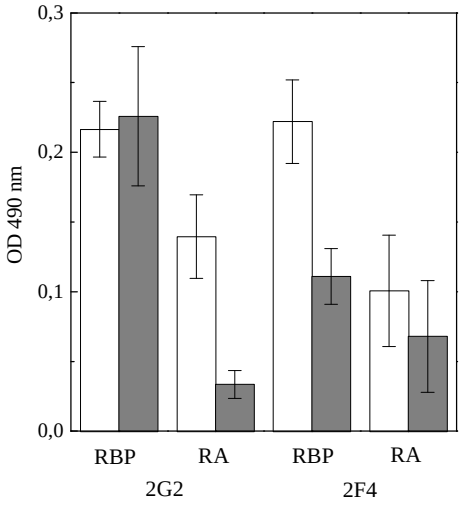

Fig. 2. Interactions between Rubisco (R) and Rubisco binding protein (RBP) or Rubisco activase (RA) in sandwich ELISA. Wells of microtiter plates were coated with purified RBP or RA (2.10^{-9}M). After blocking with ovalbumin (1%) for 1h the sandwich was completed with Rubisco (1.10^{-8}M). The controls were with 1% ovalbumin only. Mab 2G2 or 2F4 were added in constant dilution (1:16). R connected with RBP or RA was revealed with peroxidase-rabbit antimouse Ig G conjugate. On the ordinate, OD 490 nm – absorption proportional to the Mab bound by the immobilized antigens. On the abscissa, immobilized proteins with R (white) or without R (black).

plex of Rubisco with ribulose-1,5-bisphosphate (RuBP)) were investigated for their binding with RA in the presence or absence of ATP-regenerating system including ATP, PEP and Pyruvate kinase (Fig. 4). The results show the formation of complexes

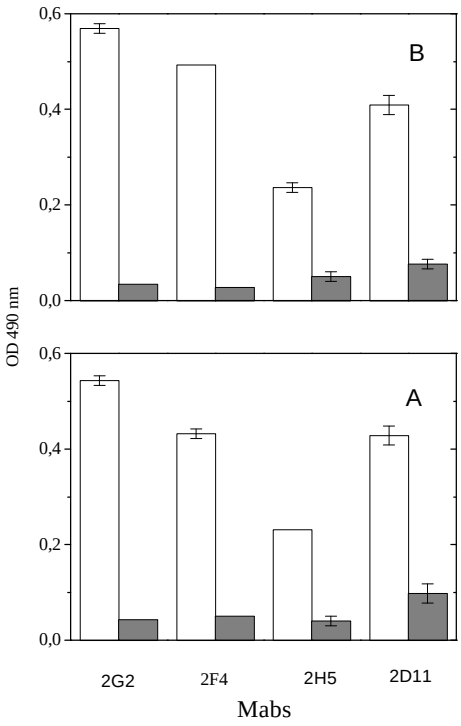

Fig. 3. Interactions between Rubisco (R) and Rubisco activase (RA) in sandwich ELISA. Wells of microtiter plates were coated with purified RA (white column) in two different concentrations (1.10^{-8} M – A or 1.10^{-9} M – B in Tris–HCl, pH 8) or with 1% ovalbumin (black column). To the immobilized proteins purified R (5.10^{-9}M) in 1% ovalbumin was added. Mab 2G2, 2F4, 2D11 were added in constant dilution (1:8), Mab 2H5 was not diluted. R connected with RA or with ovalbumin was revealed with peroxidase-rabbit antimouse Ig G conjugate. On the ordinate, OD 490 nm – absorption proportional to the Mab bound by the immobilized antigens. On the abscissa, Mabs.

between different forms of Rubisco with RA. The major interactions were revealed between R-RuBP and RA for all the Mabs (more than 50% interactions). No differences were observed between activated and non-activated Rubisco. RA reacted with all investigated forms of Rubisco with Mabs 2F4 and 2D11. Only Mab 2D11 detected differences between activated and non-activated Rubisco connected with RA.

When we used polyclonal antibodies against RA instead of Mabs in similar conditions of sandwich ELISA we could not detect differences in interactions between activated or non-activated Rubisco with RA also (Fig. 5). Only higher absorptions were observed in case of interactions of activated Rubisco with RA. It is possible that activated Rubisco could be immobilized better on the microtitration plate in comparison with non-activated Rubisco or Rubisco-RuBP complex.

In another sandwich ELISA we wanted to determine the interactions between RBP and Rubisco in solution with or without ATP and Mg^{2+} (Fig. 6). The microtiter plates were coated with RBP and then incubated with Rubisco. The possible complexes were revealed with Mabs 2G2, 2F4 and 2D11. We did not observe differences in interactions of RBP with Rubisco in the presence or in the absence of ATP and Mg^{2+}. A big cross-reactivity was also revealed, but may be it was due to a lower Rubisco concentration used in the experiments (1.10^{-9} M in comparison to RBP – 2.10^{-8} M).

All data obtained from sandwich ELISA prove the existence of protein/protein interactions between Rubisco and RBP or RA independently in the presence or in the

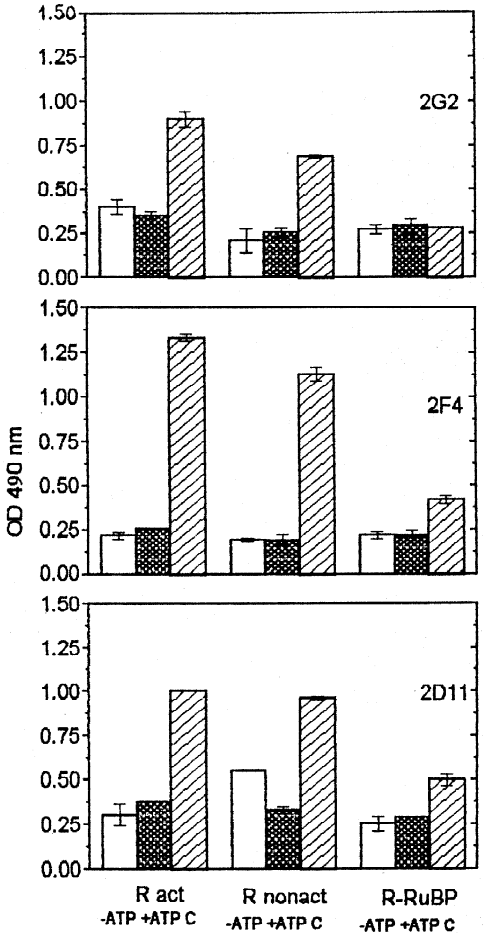

Fig. 4. Interactions between different forms of Rubisco (R) – activated (R act), non activated (R nonact) or complex with RuBP (R-RuBP), with Rubisco activase (RA) in the presence or in the absence of ATP-regenerating system in sandwich ELISA revealed with Mabs against R. Wells of microtiter plates were coated with purified RA (2.10^{-8}M). The controls (C) were with different forms of R instead of RA. Rubisco (1.10^{-8} M) in different forms with or without ATP regenerating system was added. The sandwich was completed with Mab 2G2, 2F4 or 2D11 in constant dilution (1:8). R forms connected with RA were revealed with peroxidase-rabbit antimouse Ig G conjugate. On the ordinate, OD 490 nm – absorption proportional to the Mab bound by the immobilized antigens. On the abscissa, the forms of Rubisco.

absence of ATP-regenerating system for RA and in the presence or in the absence of ATP and Mg^{2+} for RBP as well as independently from the different forms of Rubisco.

In an attempt to choose another type of specific interactions between investigated proteins the methods of immunoprecipitation and immunoblotting were used. These methods have an advantage to visualize the obtained immunoprecipitates of the complexes. To minimize the cross-reactivity of RBP to Rubisco, we exhausted polyclonal antibodies against RBP from cross-reactivity to Rubisco (see Materials and Methods).

The results show that two closely situated bands corresponding to RA subunits (41–43 kDa), that are visible as one band in barley varieties coprecipitate with LS and SS of different tested forms of Rubisco – R-RuBP, R-CO_2-Mg^{2+}, R-CO_2-Mg^{2+}-CABP (Fig. 7). Hence, the sites of binding are in a distance from the active site of the enzyme. Mab 2F4 reveal LS (56 kDa) and complexes of LS-RA subunits with molecular weight above 90 kDa. Small differences between variants with ATP-regenerating sys-

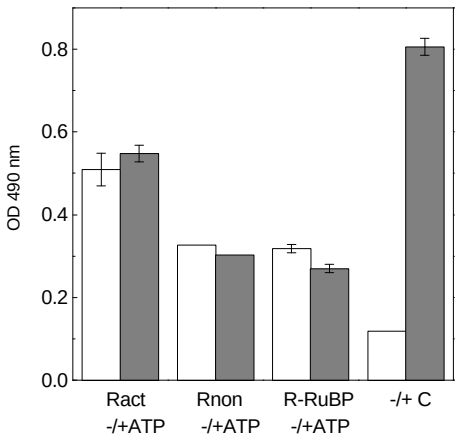

Fig. 5. Interactions between different forms of Rubisco (R) – activated (R act), non activated (R nonact) or complex with RuBP (R-RuBP), with Rubisco activase (RA) in the presence or in the absence of ATP-regenerating system in sandwich ELISA, revealed with polyclonal antibodies against RA. Wells of microtiter plates were coated with purified R (2.10^{-9} M). Other controles (C) were coated with R(-) or RA(+) for checking the specificity of antibodies. RA (1.10^{-8} M) with (black column) or without ATP (white column) regenerating system was added except to variant C. The sandwich was completed with polyclonal antibodies against RA (1:1000). R forms connected with RA were revealed with goat anti-rabbit Ig G peroxidase conjugate. On the ordinate, OD 490 nm – absorption proportional to the polyclonal antibodies bound by the immobilized antigens. On the abscissa, immobilized proteins.

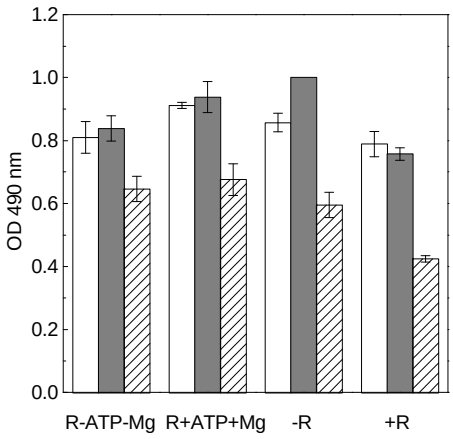

Fig. 6. Interactions between Rubisco binding protein (RBP) and Rubisco (R) in the presence or in the absence of ATP and Mg^{2+} in sandwich ELISA revealed with Mabs against R. Wells of microtiter plates were coated with purified RBP (2.10^{-8} M) in Tris-HCl, pH 8. R (1.10^{-9} M) with or without ATP (2 mM) and Mg^{2+} (20 mM) was added. The controls were with 1% ovalbumin only instead or R (-R) or immobilized R only (+R). The sandwich was completed with Mabs – 2G2 (white), 2D11 (black) and 2F4 (grey) (1:8). R connected with RBP was revealed with peroxidase-rabbit antimouse Ig G conjugate. On the ordinate, OD 490 nm – absorption proportional to the Mabs bound by the immobilized antigens. On the abscissa, immobilized proteins.

tem or without ATP-regenerating system were observed. The variants with the presence of ATP-regenerating system were revealed more slightly.

The subunits of RBP (60–61 kDa) also coprecipitated with LS and SS of different forms of Rubisco – activated at room temperature, denatured at 55°C and oxidized by 1% H_2O_2 using polyclonal antibodies against RBP and revealed with Mab 2F4 (Fig. 8). The complexes LS-RBP subunits with molecular weights about 110 kDa were revealed clearly. Differences between variants with ATP regenerating system or without ATP regenerating system were not observed.

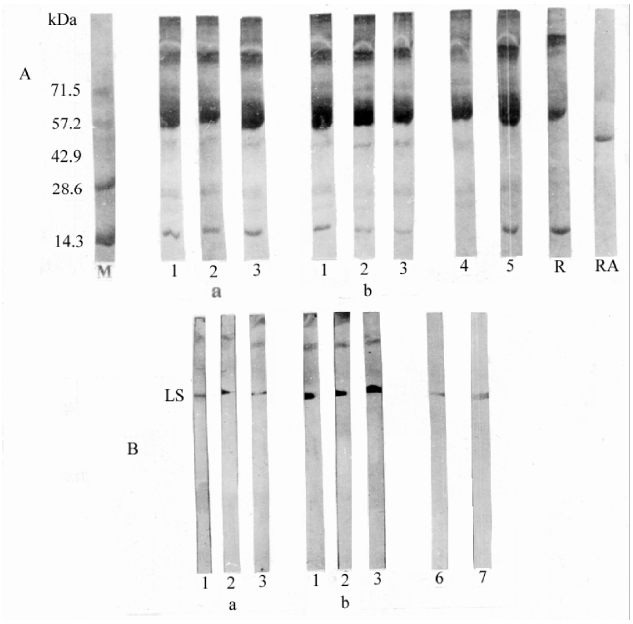

Fig. 7. Immunoprecipitation of Rubisco activase (RA) associated with different forms of Rubisco (R) – non activated complex with RuBP (1), with CABP (3), or activated with CO_2 and Mg^{2+} (2) in the presence (a) or in the absence (b) of ATP regenerating system. Immunoprecipitates were analysed after SDS-electrophoresis and revealing with Coomassie blue (A) or after immunoblotting on nitrocellulose sheets and revealing with Mabs (B). M – molecular weight markers (Sigma, 14–70 kDa); 1 – RA/R-RuBP/ARBL; 2 – RA/R-CO_2-Mg^{2+}/ARBL; 3 – RA/R-CO_2-Mg^{2+}/CABP/ARBL; 4 – RA/ARBL; 5 – R/ARBL; 6 – RA/ARBL/Mab; 7 – RA/R/ARBL/Mab; ARBL – Polyclonal antibodies against barley R.

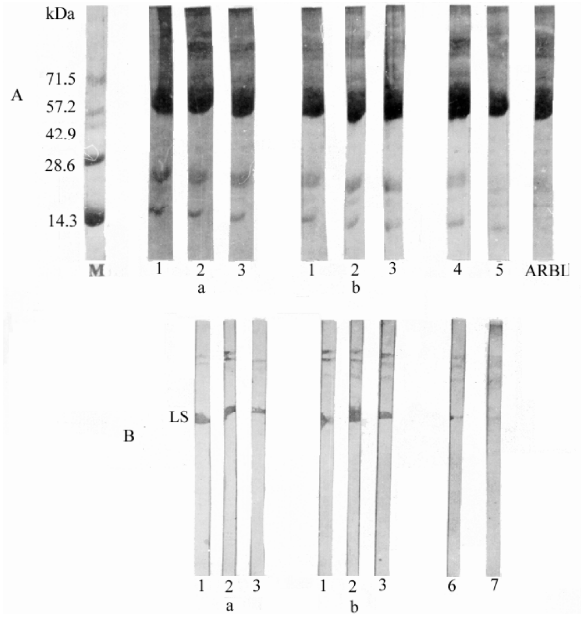

Fig. 8. Immunoprecipitation of Rubisco binding protein (RBP) associated with different forms of Rubisco (R) – activated (1), denatured (2), or oxidized (3) in the presence (a) or in the absence (b) of ATP and Mg^{2+}. Immunoprecipitates were analysed after SDS-electrophoresis and revealing with Coomassie blue (A) or after immunoblotting on nitrocellulose sheets and revealing with Mabs (B). M – molecular weight markers (Sigma, 14–70 kDa); 1 – RBP/R-act./ARBL; 2 – RBP/R-den./ARBL; 3 – RBP/R-ox./ARBL; 4 – RBP/ARBL; 5 – R/ARBP; 6 – RBP/ARBP; 7 – R/ARBP; ARBL – Polyclonal antibodies against barley R; ARBP – Polyclonal antibodies against RBP.

Discussion

Rubisco binding protein has two isoforms (61 and 60 kDa) and their cross-linked isoforms with Rubisco LS were recognized by Mabs to Rubisco or antibodies against

RBP after immunoprecipitation. The obtained results prove the formation of Rubisco-RBP complexes. In references RBP is known as a chaperonin, so it is possible to interact with some binding domain placed on Rubisco LS. The nature of the function of RBP requires protein/protein interactions with many proteins including Rubisco. Probably, Rubisco LS were preferred from SS. The results confirm specific binding of Rubisco LS with RBP subunits.

Rubisco activase has two isoforms (41 and 44 kDa) but their molecular masses are in a species dependence variation. It was established that Rubisco/Rubisco activase interaction varies between species (Wang et al., 1992). Rubisco activase from barley plants has isoforms in closer position which are seen as one band after SDS electrophoresis. In all our experiments the cross-linked isoforms of Rubisco activase were recognized by individual antisera (Mabs to Rubisco or antibodies against RA) and confirm the formation of the Rubisco-Rubisco activase complexes after their mixing.

Assuming that Rubisco is one of the most abundant soluble leaf proteins and that Rubisco activase comprises only 2%, it is very difficult to explain such unequal interactions. Sanchez de Jimenes et al. (1995) consider that Rubisco activase protein is not a conventional enzyme and belongs to the molecular chaperone group. Their postulate was supported by the existence of conformational changes in the Rubisco molecule through its activation-inactivation process according to Bowien and Gottschalk (1992). It is possible that the nature of the function of Rubisco activase requires protein/protein interactions with Rubisco. It has been supposed that binding of Rubisco activase to one of the eight large subunits of Rubisco may cause the remaining subunits to develop another protein conformation, which increases the affinity of the activation sites of Rubisco for CO_2 (Yokota and Tsujimoto, 1992). The exact nature of the interaction between Rubisco and Rubisco activase is not yet established. Our results also prove that Rubisco activase may bind predominantly to Rubisco LS.

Chaperonins including RBP or RA are supposed to interact transiently with other proteins, promoting different processes (assembly of the proteins into functional complexes or other). Our results show that these proteins might associate to each other and form protein supercomplexes thus to facilitate directly Rubisco assembly and Rubisco activation. The protein/protein interactions between the investigated proteins are at places different from the active site of Rubisco LS, because they could be obtained not only with activated but also with non-activated enzyme molecules with active sites denatured or blocked by RuBP or CABP. Following this interpretation we could suppose that the sites for Rubisco-RBP or Rubisco-RA interaction are at a distance from the sites for Mabs binding. It seems that these interactions don't depend considerably on the presence or absence of ATP-regenerating system for RA, or on the presence or on the absence of ATP and Mg^{2+} for RBP but the presence of ATP-regenerating system inhibits slightly the formation of coprecipitates with RA. All the investigated Mabs reveal different degree of interactions, so they have different specificity.

Conclusion

The results indicate the existence of protein/protein interactions between Rubisco, RBP and RA and that in the interaction between them groups determining the association concern the primary structure of Rubisco. Thus, the recognition between Rubisco, RA or RBP requires the precise amino-acid sequence and it is based on protein/protein interactions between the investigated proteins at places different from the active site of Rubisco. The sites for Rubisco-RA or Rubisco-RBP interaction are at a distance from the active site and the sites for Mabs binding and they don't depend to a great extent on the presence or on the absence of ATP in ELISA. By means of immunoprecipitation and immunoblotting methods used, qualitative differences between variants with or without ATP-regenerating system were obtained that is why Rubisco activase binds preferably to Rubisco in the absence of ATP.

Acknowledgements: This work was supported by Grant No K-443 from the National Science Fund, Bulgaria.

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