REVERSIBLE COLD INACTIVATION AND HEAT REACTIVATION OF RuDP CARBOXYLASE ACTIVITY OF CRYSTALLIZED TOBACCO FRACTION I PROTEIN

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Summary: The specific RuDP carboxylase activity of crystallized tobacco Fraction I protein did not change during 12 days storage at room temperature. Whenever placed in an ice bath, 70% of the activity disappeared within 24 hours and remained lost during continued storage at 0°. However, the lost activity could be completely regained at any time during cold storage by a 20 min. treatment at 50°C. Loss or restoration of activity was not accompanied by a detectable change in the sedimentation velocity properties of Fraction I protein.

Using cold conditions normally employed for preserving labile materials, we were surprised to find a gradual loss in RuDP carboxylase activity of Fraction I protein which had been purified to the extent of crystallization. As the decay in activity seemed to follow first order kinetics, it appeared that inactivation could have resulted from a one-step process which might be reversed. As the following experiments will demonstrate, the lost enzymatic activity was completely regained by heating the protein.

Fraction I protein from tobacco leaves was highly purified as previously described (1) and then crystallized at room temperature according to a method recently reported (2). The crystalline protein was dissolved in 0.025 M Tris-HCl (pH 7.4) containing 0.2 M NaCl, adjusted to 5 mg/ml with the same buffer, and one sample was kept at room temperature (25°C ± 3°) and the other in an ice bath (0°C). The RuDP carboxylase activity of the protein was measured as previously described (1) except for time of incubation and temperature. Instead of 30°C where the activity of enzyme

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stored at 0°C was gradually reactivated during the assay, 20°C was used because the enzyme was neither activated nor inactivated during a 30 min. period.

As shown by the data plotted in Fig. 1, the specific activity did not change when the protein was stored at 25°C and assayed at intervals over a period of 12 days. However, when the same protein was stored at 0°C for 3 days, only 31% of the specific activity remained although no further reduction occurred during the next nine days of storage at 0°C. After

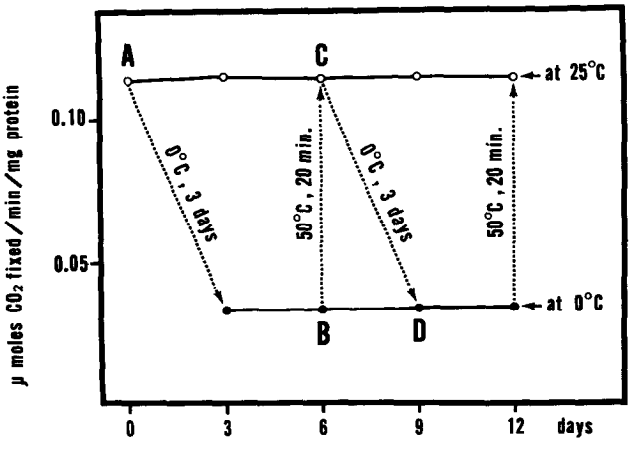

Fig. 1. Effect of various temperature conditions on the RuDP carboxylase activity of crystalline Fraction I protein. A-D designate condition of protein subjected to analytical centrifugation with results shown in Fig. 2. Enzyme activity measured at 20°C for 20 min. in all cases.

keeping in the ice bath for 6 days, the inactivated enzyme was heated at 50°C for 20 min., causing the difference in activity shown by points B and C in Fig. 1. The heat treatment completely restored the lost activity which now remained unchanged when the reactivated protein was stored at 25°C for an additional 6 days. However, when a sample of the reactivated enzyme was placed in an ice bath for another 3 days, the enzyme activity again dropped to the same level (D, Fig. 1) as found in the first cycle of the inactivation-reactivation experiments. The same phenomena were observed using samples kept in an ice bath for 12 days before enzyme assay. A further experiment showed the inactivation reaction was actually completed

by 24 hours, and the shape of the decay curve seemed to be semi-logarithmic.

The proteins in the various conditions indicated by A,B,C and D in Fig. 1 were subjected to analytical centrifugation with the results shown in Fig. 2. None of the samples--native protein (A), inactivated protein (B), reactivated protein after 6 days storage in the cold (C), or inactivated reactivated-inactivated protein (D)--showed any difference in sedimentation velocity or in the shape of the Schlieren pattern. Changing the temperature during centrifugation from 20°C to 10°C also produced no change in the Schlieren patterns between reactivated (E) and inactivated proteins

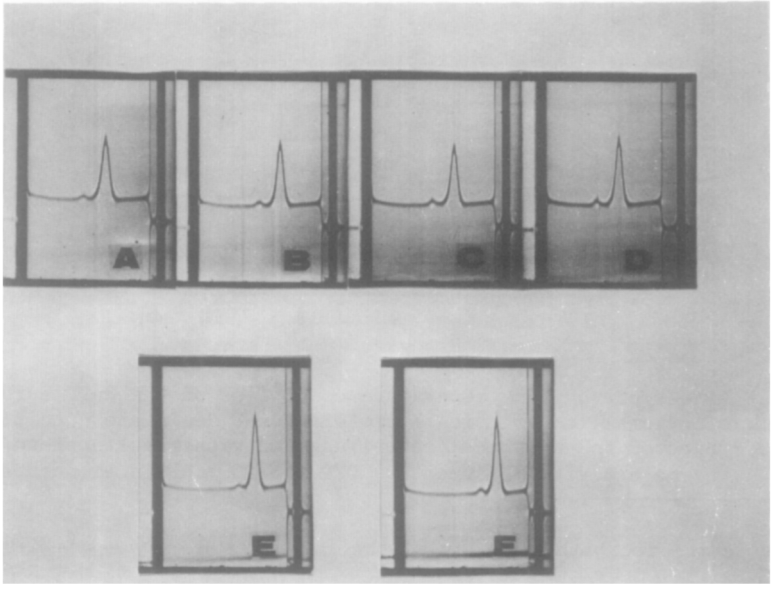

Fig. 2. Effect of temperature conditions which induce large changes in RuDP carboxylase activity on the sedimentation behavior of Fraction I protein. A-D, conditions as in Fig. 1; temperature of centrifugation, 20.0°C. E,F, same as B,C, except temperature of centrifugation, 10.0°C. All photographs taken 29 min. after AnD rotor had reached 44,770 rpm; protein conc., 5 mg/ml in 0.025 M Tris-HCl, pH 7.4, containing 0.2 M NaCl; bar angle 50°.

(F). Moreover, both of the samples could be induced to form the same dodecahedron-shaped protein crystals either at 5°C or at 25°C. Apparently the conformational change concerned with the cold inactivation is very small in physical dimensions.

Several reports have been made on other enzymes subject to cold inactivation and heat reactivation such as hydroxybutyrate dehydrogenase (3), nitrogenase (4) and pyruvate carboxylase (5). The cold inactivation of pyruvate carboxylase has been shown to be accompanied by dissociation of the protein into subunits whereas heat treatment induced the reassociation of subunits into tetrameric structures and also restored the enzymatic activity (5,6), leading to the interpretation that the catalytic site is located within the tetramer and is shared by each of the four subunits. Since no evidence could be found for dissociation-reassociation of subunits, or monomer $\rightleftharpoons$polymers accompanying loss and restoration of the RuDP carboxylase activity of Fraction I protein, it is probable that cold induces only a slight expansion in the volume of the protein. It was recently proposed that tobacco Fraction I protein is composed of 8 large subunits arranged as a cube and a sextet of small subunits, one of which occupies the center of each face of the cube (7). Because the large subunit contains 4 times more histidine than the small subunit (8), we suspect that the catalytic site is located with the large subunits. In addition, the enrichment of neutral amino acids together with other suggestions of the hydrophobic nature of the large subunits make it plausible that low temperatures induce a change in a more compact macromolecule to a slightly less compact form somewhat in the manner depicted by the models shown in Fig. 3. The change is accompanied by a distortion in the conformation of the catalytic site that is sufficient to be detected as a loss in specific enzymatic activity. By analogy to pyruvate carboxylase,

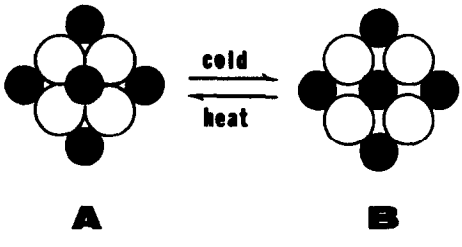

Fig. 3. Scheme to depict partial dissociation of octameric structure of Fraction I protein leading to loss in specific RuDP carboxylase activity.

perhaps cold induces dissociation of the octameric structure of the large subunits, but complete dissociation is arrested in some unknown manner by the sextet of hydrophylic, small subunits, the result being a change in conformation that still permits the enzyme to crystallize as well as to operate at 1/3 of its maximum catalytic capacity.

Fraction I protein constitutes about 40% of the total soluble proteins located in the mobile phase of higher plant chloroplasts (9). It is an interesting question therefore as to whether a readily reversible change in conformation of the protein is one way for regulating photosynthesis at the carbon dioxide fixation level in response to temperature changes in the plant's environment.

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