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Specific effect of ribulose-1,5-diphosphate on the solubility of tobacco Fraction I protein

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SUMMARY

After incubation with either ribulose-1,5-diphosphate or Mg^{2+} and HCO_3^-, Fraction I protein was subjected to conditions optimal for high yield of crystalline protein. With Mg^{2+} and HCO_3^-, crystals formed by 1.5 h, and within 24 h nearly 70% of the total protein was recovered as crystals, whereas with ribulose-1,5-diphosphate, the protein remained in solution beyond 3 days. The Fraction I protein crystals were almost instantaneously solubilized by addition of ribulose-1,5-diphosphate, but upon addition of Mg^{2+} and HCO_3^- the crystals would again rapidly appear. Speculations are advanced on how such profound changes in solubility might regulate photosynthetic carbon fixation and ameboid movement of the mobile phase of chloroplasts.

That binding of ribulose-1,5-diphosphate to ribulose diphosphate carboxylase (EC 4.1.1.39) induces a conformational change in the protein was first indicated by a difference spectrum obtained by Rabin and Trown[1]. Further support for this view has come from findings by the Akazawa group[2] that enzyme pretreated with ribulose-1,5-diphosphate was less susceptible to proteolytic degradation than untreated enzyme. We have now found that ribulose-1,5-diphosphate has a profound effect on the solubility of crystalline tobacco Fraction I protein, the latter being inseparable from ribulose diphosphate carboxylase activity even after repeated recrystallization of Fraction I protein[3].

Fraction I protein was isolated from *Nicotiana tabacum* (Turkish Samsun) leaves and crystallized by a high yield method[3] utilizing room temperature conditions and resulting in maximum activity and stability of the specific ribulose diphosphate carboxylase activity[4]. The protein crystals were dissolved in 0.025 M Tris–HCl, pH 7.4, containing 0.2 M NaCl and kept at room temperature until use. Aliquots of the solution containing

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10 mg of protein were incubated with 0.55 μmole of ribulose-1,5-diphosphate at 33° for 10 min in a final volume of 1 ml which contained 0.1 M NaCl. Other aliquots were treated in an identical manner except for omission of ribulose-1,5-diphosphate. The aliquots were then placed in Collodion bags (Schleicher and Schull) and the conical ends of the bags submerged in 200 ml of the Tris buffer which contained no NaCl. Where ribulose-1,5-diphosphate was absent, crystals appeared within 1.5 h. After 3 days of dialysis, nearly 70% of the protein was recovered as crystalline protein. No crystals or precipitates appeared in the aliquots treated with ribulose-1,5-diphosphate. To see whether inhibiton of crystallization was a specific property of ribulose-1,5-diphosphate, various phosphorus-containing compounds were tested with the results shown in Table I. Of the six compounds tested, only ribulose-1,5-diphosphate prevented crystallization. Inorganic phosphate has been reported to compete with ribulose-1,5-diphosphate for a binding site on ribulose diphosphate carboxylase while 3-phosphoglyceric acid appeared to compete for the HCO_3^--binding site[5]. Thus, the effect of solubilization of Fraction I protein by ribulose-1,5-diphosphate has the attributes of a highly specific process.

TABLE I

EFFECT OF PHOSPHORUS COMPOUNDS ON CRYSTALLIZATION OF TOBACCO FRACTION I PROTEIN

Aliquots containing 5 mg of crystalline Fraction I protein dissolved in 0.5 ml of 0.025 M Tris–HCl buffer, pH 7.4, containing 0.1 M NaCl were incubated for 10 min at 33° in the presence of 0.11 μmole of ATP, ribose-5-phosphate, 3-phosphoglyceric acid, NaH_2PO_4, carbamyl phosphate, or 0.055 μmole ribulose-1,5-diphosphate. All dialyzed 16 h against Tris buffer without NaCl whereupon crystalline protein was recovered by centrifugation at 5000 × *g* for 10 min. Protein determined by 280 mμ absorption, 1 *A* unit corresponding to 0.7 mg Fraction I protein.

Additions	*% of Fraction I protein recovered as crystals*
Ribose-5-phosphate	58.6
ATP	60.2
3-Phosphoglyceric acid	61.8
Inorganic phosphate	64.8
Carbamyl phosphate	59.4
None	67.2
Ribulose-1,5-diphosphate	0

When aliquots of the protein solution were pretreated with HCO_3^- and Mg^{2+} before being placed under optimal crystallization conditions as before, crystallization of Fraction I protein was greatly accelerated as shown by the data in Fig. 1. Since it was not known whether enzyme–CO_2 and/or enzyme–ribulose-1,5-diphosphate complexes were already present in the Fraction I protein preparation, 50 mg of the protein in 1 ml of Tris buffer was first incubated with 5.5 μmoles of ribulose-1,5-diphosphate at 33° for 10 min to convert all of the CO_2 bound to the enzyme to 3-phosphoglyceric acid. Moreover, the great excess of ribulose-1,5-diphosphate remaining would provide for a completely soluble protein to begin with and which would not crystallize even after prolonged dialysis

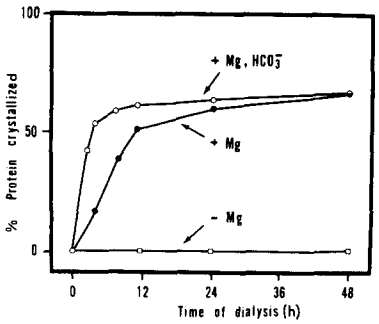

Fig. 1. Effect of Mg^{2+} and HCO_3^- on solubility of Fraction I protein. Protein in crystals determined as in legend for Table I. The bottom line (−Mg) was obtained for protein that was only treated with ribulose-1,5-diphosphate, or the same protein further treated with 6.4 μmoles $NaHCO_3$.

to remove unbound ribulose-1,5-diphosphate as shown by the bottom line in Fig. 1. The same condition was obtained when 6.4 μmoles of $NaHCO_3$ was added to this protein in a final volume of 0.5 ml Tris buffer containing 0.1 M NaCl and incubated at 33° for 10 min prior to dialysis against NaCl-free Tris buffer. However, when 1.4 μmoles of $MgCl_2$ or the latter plus 6.4 μmoles of $NaHCO_3$ were added under the same conditions, the results shown by the upper two lines in Fig. 1 were obtained. Addition of Mg^{2+} alone appeared to be sufficient to reverse the ribulose-1,5-diphosphate solubilization effect since crystals of Fraction I protein appeared within 4 h and increased to the maximum yield within 24 h. It should be noted that no attempt was made to prevent diffusion of atmospheric CO_2 into the open dialysis system in these experiments. However, addition of HCO_3^- together with Mg^{2+} greatly accelerated the formation of crystals, maximum recovery being achieved after 12 h dialysis suggesting that diffusion of atmospheric CO_2 into the dialysis system may have been a limiting factor resulting in the slower rate of crystallization when only Mg^{2+} was present. If so, it would appear that CO_2 is required together with Mg^{2+} to transform the bound ribulose-1,5-diphosphate into 3-phosphoglyceric acid before crystallization of the protein can occur.

The solubilization of Fraction I protein crystals by ribulose-1,5-diphosphate and reversal by Mg^{2+} and HCO_3^- could be repeated in a cyclic manner. When 0.055 μmole of ribulose-1,5-diphosphate was added to the suspension of Fraction I protein obtained from the Mg^{2+} and HCO_3^- treatment in Fig. 1, the crystals immediately dissolved although the ionic strength of the added material was not in itself sufficient to dissolve the crystals. The protein continued to remain in solution even after prolonged dialysis under the optimal conditions for crystallization. Crystals of Fraction I protein appeared from this solution very shortly after dialysis began following the addition of Mg^{2+} and HCO_3^-. Thus, there is a cyclic change in the solubility of Fraction I protein depending on which of the two substrates is present. We estimate the enzyme–ribulose-1,5-diphosphate complex to be around 100 times more soluble than the enzyme in the presence of excess HCO_3^- and Mg^{2+}.

As the solubility of a protein molecule is generally a reflection of the nature of the surface exposed to external environment, we suspect that a significant conformational change accompanies the great change in solubility of Fraction I protein. The latter is a huge molecule ($5.25 \cdot 10^5$ daltons) composed perhaps of eight large (50 000 daltons) and six small

(25 000 daltons) subunits, the latter being much more hydrophilic than the former[6]. Thus, a change in conformation which would result in a greater exposure of the smaller subunits to the external environment might serve as a plausible mechanism to account for the much greater solubility of the enzyme–ribulose-1,5-diphosphate complex.

Because of the location and abundance of Fraction I protein in higher plant chloroplasts, the speculation has been advanced that this protein not only functions as the enzyme catalyzing the first step in photosynthetic carbon dioxide fixation, but also serves as the principal structural element of the mobile phase of chloroplasts[7]. Thus, the cyclic change in the solubility of Fraction I protein induced by ribulose-1,5-diphosphate on the one hand, and Mg^{2+} and HCO_3^- on the other, suggests that these substrates might be antagonists which control the degree of fluidity or solidity of the mobile phase and thereby regulate the degree of its ameboid movements and interaction with other organelles in the leaf cells. The further understanding of the physical dimensions of the conformational change in Fraction I protein induced by the two different substrates might therefore provide a very useful hint to explain the basis for the motion of the mobile phase.

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