RIBULOSE-1,5-DIPHOSPHATE FROM AND CO_2 FIXATION BY *TETRAGONIA EXPANSA* LEAVES EXTRACT*, **

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Previous work[1] in this laboratory on algae has shown that the primary CO_2 fixation in photosynthesis operates by the carboxylation of ribulose-1,5-diphosphate (RuDP) producing two molecules of phosphoglyceric acid (PGA) in the following manner:

$$\text{RuDP} + CO_2 + H_2O = 2\ \text{PGA} \qquad (1)$$

The carboxylation of RuDP has been shown to take place primarily in the presence of a catalyzing enzyme from a cell-free extract of *Chlorella*[2]. The relation has not been proved in an unequivocal manner. Thus, if the enzymic extract was put in contact with unlabeled RuDP in the presence of $NaH^{14}CO_3$, phosphoglyceric acid appeared as the main product of the carboxylation. However, the formation of this last substance was accompanied by other products when the experiment was undertaken in the presence of radioactive RuDP and unlabeled CO_2.

Evidence of such carboxylation was obtained with a cell-free extract of spinach leaves, which permitted the fixation of CO_2 in the presence or ribose-5-phosphate[3], indicating that the leaf extract contains phosphopentoisomerase[4] and phosphopentokinase[5] permitting, in the presence of ATP, the transformation of ribose-5-phosphate to RuDP.

Later, from the same leaves, WEISSBACH AND HORECKER[6] obtained a partially purified carboxylation enzyme. RACKER[7], in order to synthesize *in vitro* the carbohydrates from CO_2 and H_2 in a cell-free system, added the RuDP-carboxylase. This enzyme preparation corresponds to the fraction obtained by the precipitation of the proteins of leaf extract, between concentrations from 20 to 50% $(NH_4)_2SO_4$ (corresponding to 0.3 to 0.7 saturated ammonium sulfate (SAS)), followed by alcohol precipitation and isoelectric fractionation. This preparation contained transketolase, transaldolase and phosphopentokinase in undetermined quantities.

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A carboxylation enzyme system has been prepared from *Tetragonia expansa* leaf extract (New Zealand spinach), which was free of all other enzyme systems capable of acting upon RuDP. As the carboxylation produced an internal dismutation of the substrate (RuDP), the name "carboxydismutase" has been suggested for the enzyme[8].

MATERIAL AND METHODS

A. *Preparation of the crude extract*

All operations were carried out at 0° C. Fifty g (wet weight) of fresh *Tetragonia* leaves were ground in a blender for 1 min with 50 ml of distilled water. To the supernatant from centrifugation (at 3000 r.p.m. for 5 min), which has been filtered through filter paper, is added washed charcoal (5 g/100 ml extract)*. The pH is maintained at 7.0 by addition of 2 *N* NH_4OH. The solution is centrifuged at 40,000 r.p.m. for 10 min in a Spinco centrifuge. The pale yellow supernatant is then adjusted to pH 7.0 with 2 *N* NH_4OH. The solution can be kept in an ice bath for more than 2 or 3 days.

B. *Dialysis of the crude extract*

Five ml of crude extract (A) were placed in a cellophane tube and dialyzed at 0° C against one liter of distilled water with moderate agitation, the water being changed every two hours. Dialysis was also carried out in the presence of the salts (EDTA K) Mg^{++}, Fe^{+++}, Al^{+++} and Cu^{++} in the concentration of $5 \cdot 10^{-5} M$ at pH 7.0.

C. *Fractional precipitation of the crude extract proteins by addition of* $(NH_4)_2SO_4$

All operations were carried out at 0° C. To the crude extract, to which no charcoal had been added, a sufficient quantity of $(NH_4)_2SO_4$ was added** to obtain 0.33 SAS, the pH being adjusted to 7.0 with 2 *N* NH_4OH. After centrifugation at 20,000 r.p.m. for 5 min, the clear supernatant was raised to 0.4 SAS with $(NH_4)_2SO_4$. The pH was adjusted to 7.0 and the proteins were centrifuged at 20,000 r.p.m. for 5 min, recovered and redissolved in 10 ml of 0.05 *M* phosphate buffer, pH 7.0. The same operations were repeated for the protein fractions precipitating between 0.4–0.5 SAS and 0.5–0.75 SAS. The proteins which were dissolved were dialyzed 6 h against 1 l of neutral Mg^{++} (EDTA K) $5 \cdot 10^{-5} M$, which was changed every 2 hours.

D. *Preparation of the carboxydismutase*

All operations were carried out at 0° C. Fifty g (wet weight) of fresh leaves were ground in a blender for 1 min with 50 ml 0.1 *M* phosphate buffer at pH 7.0. The supernatant from centrifugation at 3000 r.p.m. for 5 min was filtered through filter paper and then centrifuged twice at 40,000 r.p.m. for 10 min. To the clear yellow supernatant, $(NH_4)_2SO_4$ sufficient to achieve 0.35 SAS was added. The pH was adjusted to 7.0 with 2 *N* NH_4OH and centrifuged at 20,000 r.p.m. for 5 min. The pellet was discarded and the pale supernatant was brought to 0.39 SAS. The precipitate was collected by centrifugation, also for 5 min at 20,000 r.p.m. It was then dissolved in 5 ml of 0.05 *M* phosphate buffer, pH 7.0, and dialyzed 6 h against 1 liter of solution (changed every two hours) of $5 \cdot 10^{-5} M$ Mg^{++} (EDTA K) at pH 7.0 or $10^{-4} M$ sodium acetate, pH 7.0.

E. *Preparation of the substrate*

RuDP was prepared following a method previously described[2]. RuDP-^{14}C was prepared in a similar way by shaking for 3 min in the light a suspension of *Scenedesmus* (2.5 g of cells in 200 ml water), with 2.5 ml of $NaH^{14}CO_3$ (0.025 *M* $NaH^{14}CO_3$; 400 μc/ml). A rapid stream of nitrogen was bubbled through. The suspension was agitated for 30 sec and then emptied in 800 ml of boiling ethanol. The suspension was centrifuged at 3000 r.p.m. for 10 min and the cell products extracted with 50 ml of 20% boiling ethanol.

The extract was evaporated *in vacuo* to 1 ml and the proteins were eliminated by centrifugation at 40,000 r.p.m. for 10 min. The RuDP-^{14}C was isolated by "strip chromatography" in a phenol-water solvent on oxalic acid-washed Whatman paper No. 4. The strips containing the RuDP-^{14}C determined by autoradiography of the chromatograms were cut out and washed with absolute ethanol and dried. The RuDP-^{14}C was then eluted with water and these washings were collected and concentrated to 1 ml. Fifty μl of this solution contained approximately 0.1 μmole of RuDP-^{14}C. The solution was conserved by deep freezing***.

* Charcoal was prepared by suspending it in boiling HCl (10%) for 1 hour. Then it was filtered and washed with distilled water to neutrality and dried at 100° C.

** The saturation solubility used is 68.5 g $(NH_4)_2SO_4$ for 100 ml initial solution volume.

*** Note that the RuDP during chromatographic separation partially decomposed to pentose monophosphates (7%), see Table III, experiment 5.

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F. *CO_2 fixation experiments*

The carbon fixation experiments were done at ordinary temperature in closed tubes, each tube containing 10 μl of enzymic solution, orthophosphate (2.5 μM) at pH 6.8, RuDP ($\sim$0.1 μM), $NaH^{14}CO_3$ (1.2 μM) with a specific activity of $4.8 \cdot 10^6$ c.p.m./μM, pH 7.6. Distilled water was added to raise the total volume to the indicated value.

The reaction was stopped after 10 min by putting one drop of acetic solution (2 %) into the tube which was then plunged into boiling water for 1 min. If necessary the solution can be centrifuged to eliminate proteins which have been precipitated. The solution is then transferred quantitatively onto oxalic acid-washed Whatman No. 4 filter paper sheets and dried with a gentle stream of cool air. The $^{14}CO_2$ fixed by the extract was determined by measuring the radioactivity from the point of "origin" with the aid of a Scott Geiger tube with a large end window.

To determine the substances produced, the paper was chromatographed in two dimensions with phenol-water and butanol-propionic acid-water as solvents. The radioactive substances, the emplacement of which was determined by autoradiography, were identified by their R_F values and by cochromatography. The activity of these different radioactive substances was determined directly on the paper by means of a Scott Geiger tube.

Similar experiments were conducted in tubes with the radioactive substrate. Each tube contained, besides the enzymic extract, orthophosphate (2.5 μM), pH 6.8, RuDP-^{14}C ($\sim$0.1 μM) and $NaHCO_3$ (1.2 μM), pH 7.6. The incubation time was fixed at 15 to 30 min.

RESULTS AND DISCUSSION

A. *Crude extract and dialysis*

An experiment conducted on the crude extract prepared as described in Part A of the previous section, in the presence of RuDP and $NaH^{14}CO_3$, showed that the fixed carbon is distributed among five compounds: phosphoglyceric acid, phosphoenolpyruvic acid, phosphoglycolic acid, glyceric acid and alanine (Fig. 1). The quantitative

TABLE I

CARBOXYLATION REACTION OF THE CRUDE AND DIALYZED EXTRACT
(Results expressed in counts per minute)

A. RuDP + $NaH^{14}CO_3$*

Expt.	*Step*	*Total counts on origin before run*	*PGA*	*P-pyruvic*	*P-glycolic*
1	Crude extract	3,500	1,340	127	207
2	Extract dialyzed 3 h against H_2O	4,100	2,906	58	96
3	6 h against Mg^{++} (EDTA K) $5 \cdot 10^{-5} M$	4,300	3,553	—	—
4	6 h against Fe^{+++} (EDTA K) $5 \cdot 10^{-5} M$	3,820	3,190	—	—
5	6 h against Cu^{++} (EDTA K) $5 \cdot 10^{-5} M$	2,180	1,667	—	—

B. RuDP-^{14}C + $NaHCO_3$**

Expt.	*Step*	*Total counts on origin before run*	*Counts on origin after run*	*RuDP remaining*	*PGA*	*P-pyruvic*
6	Extract dialyzed 6 h against Mg^{++} (EDTA K) $5 \cdot 10^{-5} M$	4,700	250	—	2,824	312

* Initial amounts of reactants:RuDP ($\sim$ 0.1 μM), $NaH^{14}CO_3$ (1.2 μM); ** RuDP-^{14}C ($\sim$ 0.1 μM) ment: 400 μl leaves extract (50 ml per 50 g wet leaves), orthophosphate (2.5 μM) pH 6.8. Total volume time: Expt. 1–5, 10 min; Expt. 6, 30 min. Values expressed in counts per minute fixed on paper.

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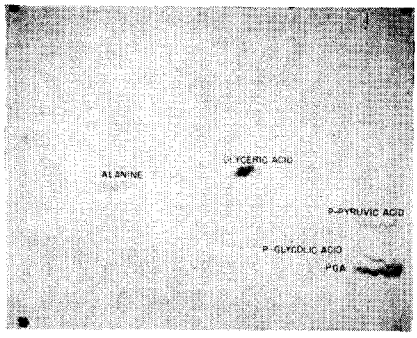

Fig. 1. RuDP + $H^{14}CO_3^-$ + crude extract.

distribution of the activity is given in Table I, Expt. 1. Although only 40% of the radioactive carbon is fixed in PGA, it is evident that other enzymes present can transform the PGA initially formed as well as other substances.

After dialysis for three hours against distilled water (see B under MATERIALS AND METHODS) it was seen that the enzymic activity of the extract was increased. The distribution of the radioactive products formed is found in Table I, Expt. 2. PGA-^{14}C passed from 40 to 70% and appeared as the principal product formed in addition to glyceric acid. Alanine has disappeared. Phosphoenolpyruvic acid and phosphoglycolic acid were reduced to traces. It is not possible, however, to continue the dialysis of the plant extracts against distilled water because the proteins begin to precipitate after a time and the carboxylation activity is lost.

The addition of small amounts of Versene Fe^{+++} or Versene Mg^{++} was sufficient to stabilize the proteins and maintain the enzymic activity.

After six hours of dialysis against Mg^{++} (EDTA K) $5 \cdot 10^{-5}$ *M*, the extract showed that 80% of ^{14}C was fixed in the PGA. The only product that appeared besides PGA was glyceric acid (Table I, Expt. 3; also Fig. 2).

The extract dialyzed six hours against Fe^{+++} (EDTA K) $5 \cdot 10^{-5}$ *M*, showed a smaller proportion of glyceric acid. However, the fixed ^{14}C was also less (see Table I, Expt. 4).*

Cu^{++} (EDTA K) at the same concentration induced an appreciable lowering of the carboxylation (Table I, Expt. 5).

These experiments on the crude extract seem to show that the activity of carboxylation is not sensitive to dialysis as is, for example, the malic enzyme or the greater part of the glycolytic enzyme system. That the carboxylation enzyme acts as an independent system indicates that, for the fixation of CO_2 with RuDP to produce PGA as the principal product, the participation of dialyzable cofactors such as ATP, DPN^+ and TPN^+ is not necessary. The metals in the Versene complex are not specific.

When the crude extract was dialyzed against Mg^{++} (EDTA K) and put into the presence of RuDP-^{14}C and unlabeled HCO_3^-, the activity was distributed, besides PGA, between the pentose monophosphates, phosphoenolpyruvic acid, glyceric acid and several other unidentified

Glyceric	*Alanine*
1,286	470
764	—
518	—
243	—
144	—

Glyceric	*Pentose mono-P*	*Other compounds*
237	271	602

$aHCO_3$ (1.2 μM). For each experi- o μl. Temperature: 20°. Incubation

* The increase of free glyceric acid seen after dialysis against Mg^{++} (EDTA K) $5 \cdot 10^{-5} M$, may be understood as a specific activation of phosphatase by Mg^{++}.

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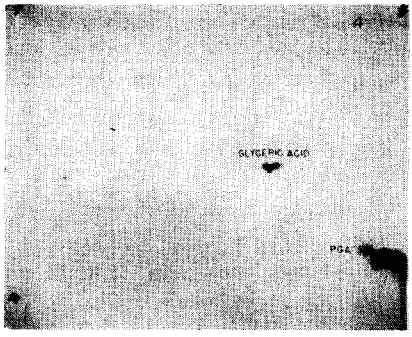

Fig. 2. RuDP + $H^{14}CO_3^-$ + extract dialyzed 6 h against Mg^{++} (EDTA K) $5 \cdot 10^{-5} M$.

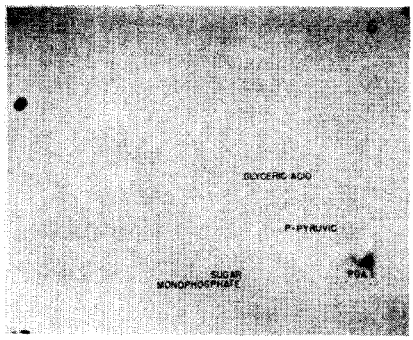

Fig. 3. RuDP-^{14}C + HCO_3^- + extract dialyzed 6 h against Mg^{++} (EDTA K) $5 \cdot 10^{-5} M$.

substances. PGA constitutes approximately 60% of the initial RuDP-^{14}C (Table I, Expt. 6; Fig. 3).

Thus, reaction (1) was not proved in an unequivocal way. The different compounds from RuDP-^{14}C indicate the presence of a variety of enzymes and suggest that further purification was necessary.

B. *Fractional precipitation of the crude extract*

In order to determine with which of the protein fractions the enzymic activity is associated, the crude extract was fractionated by addition of a progressively increasing amount of $(NH_4)_2SO_4$ (see C under MATERIALS AND METHODS). Table II shows the dry weight of each fraction after dialysis compared with its ability to fix the $^{14}CO_2$. Quantitatively, the strongest activity was found in the protein fraction precipitating between 0.33 – 0.40 SAS. This fraction represents more than half of the total soluble proteins precipitated by $(NH_4)_2SO_4$. It was also shown that only PGA appeared as the final product of the reaction when 50 μl of the protein fraction 0.33 – 0.40 SAS were incubated with RuDP and $NaH^{14}CO_2$ (Table III, Expt. 1; Fig. 4). The phosphatase which hydrolyzed the PGA to glyceric acid was thus eliminated. On the other hand, when the protein fraction 0.33 – 0.40 SAS was incubated with RuDP-^{14}C and HCO_3^-, it was found that only 40% of the transformed RuDP-^{14}C appeared in the PGA (Table III, Expt. 2; Fig. 5). This yield of PGA is lower than that obtained when the

TABLE II

FRACTIONAL PRECIPITATION OF THE CRUDE EXTRACT PROTEINS BY ADDITION OF $(NH_4)_2SO_4$

Saturation $(NH_4)_2SO_4$	*Dry weight in mg of each protein fraction*	*Fixed* $^{14}CO_2$*	*Enzymic specific activity counts/min/mg protein*
0–0.33	20	587	3,000
0.33–0.40	65	6,150	15,000
0.40–0.50	10	571	600
0.50–0.75	15	—	—

* Initial amounts of reactants: 100 μl for protein fraction 0–0.33 SAS; 0.40–0.50 SAS; 0.50–0.75 SAS; 50 μl for protein fraction 0.33–0.40 SAS (10 ml protein fraction per 50 g wet leaves).

RuDP ($\sim$0.1 μM); NaH $^4CO_3$ (1.2 μM); orthophosphate (2.5 μM) pH 6.8. Total volume: 250 μl. Temperature: 20°. Incubation time: 10 min. Values expressed in counts per minute fixed on paper.

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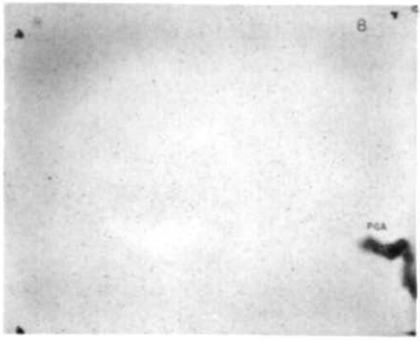

Fig. 4. RuDP + $H^{14}CO_3^-$ + protein fraction, 0.33–0.4 SAS.

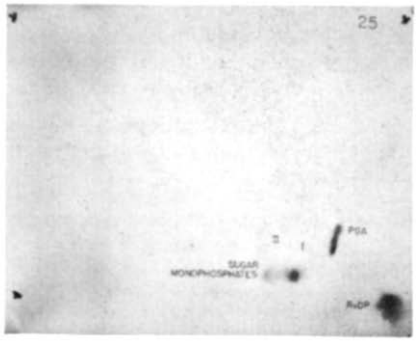

Fig. 5. RuDP-^{14}C + HCO_3^- + protein fraction, 0.33–0.4 SAS.

TABLE III

CARBOXYLATION REACTION OF PROTIEN FRACTION 0.33–0.40 SAS

Expt.	*System**	*Total counts put on origin before run*	*Counts on origin after run*	*RuDP remaining*	*PGA*	*Pentose mono-P*	*Other compounds*
	Protein fraction 0.33–0.40 SAS						
1	+ RuDP + $H^{14}CO_3$	6,150	—	—	6,024	—	—
2	+ RuDP-^{14}C + HCO_3^-	4,453	—	1,751	1,130	1,415	186
3	+ RuDP-^{14}C (no HCO_3^-)	4,650	112	2,588	—	1,880	96
	Boiled protein fraction 0.33–0.40 SAS						
4	+ RuDP-^{14}C + HCO_3^-	4,500	126	4,177	107	316	17
5	+ RuDP-^{14}C (no HCO_3^-)	4,550	203	3,894	—	300	61

* Initial amounts of reactants added as indicated: 50 μl protein fraction 0.33–0.40 SAS (10 ml per 50 g wet leaves); RuDP ($\sim$0.1 μM); RuDP-^{14}C ($\sim$0.1 μM); $NaHCO_3$ (1.2 μM); $NaH^{14}CO_3$ (1.2 μM). In each experiment: orthophosphate (2.5 μM) pH 6.8. Total volume: 250 μl. Temperature: 20°. Incubation time: Expt. 1, 10 min; Expt. 2–5, 30 min. Values expressed in counts per minute fixed on paper.

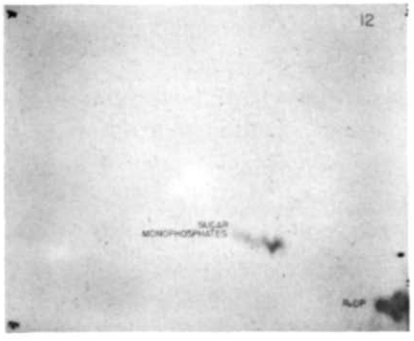

Fig. 6. RuDP-^{14}C + protein fraction, 0.33–0.4 SAS.

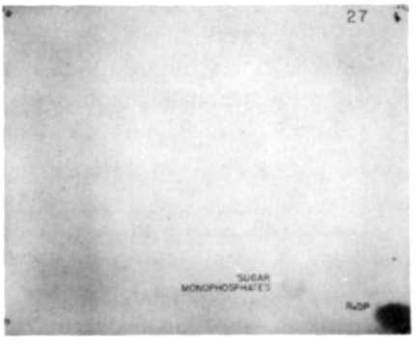

Fig. 7. RuDP-^{14}C + boiled protein fraction, 0.33–0.4 SAS.

crude extract was used. However, with the protein fraction 0.33 – 0.40 SAS, in addition to PGA, only sugar monophosphates appeared on the chromatograms. These sugar phosphates after elution from the paper and hydrolysis by addition of the phosphatase "Polidase S" (Schwarz Laboratories) showed after chromatography that the sugar fraction was respectively ribose and ribulose. Table III, Expt. 3 and Fig. 6

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show that the pentose monophosphate formation is independent of the carboxylation. The transformation of RuDP giving pentose monophosphates is due to the action of a phosphatase. Thus, when RuDP was incubated with or without HCO_3^- in the presence of boiled enzyme extract, pentose monophosphates appear at a lower concentration (Table III, Expt. 4 and 5; Fig. 7).

This RuDP phosphatase does not hydrolyze the PGA which was formed. No trace of glyceric acid was obtained during Expt. 1 and 2 (Table III).

C. *Carboxydismutase*

The separation of the carboxydismutase from the RuDP phosphatase by further fractional precipitation with $(NH_4)_2SO_4$ was not achieved. It was shown that the activity of the RuDP phosphatase in the protein extract seemed to depend on the method by which the crude extract and the protein fraction were obtained. The activity of the RuDP phosphatase became negligible when the grinding of the leaves and the protein fractionation were carried out in phosphate buffer, 0.1 *M*, pH 7.0. This difference in the activity of the RuDP phosphatase may be explained by the denaturation of the proteins in the process of preparation. The process by which the RuDP phosphatase activity became negligible in the case of *Tetragonia* leaves was not successful with spinach.

The total protein fraction 0.33–0.40 SAS was not taken to obtain the carboxylation enzyme. Actually, the carboxylation enzyme showed the greatest activity in the protein obtained between 0.35–0.39 SAS[9]. Between these limits the protein fraction was homogeneous to precipitation with $(NH_4)_2SO_4$, to electrophoresis and to sedimentation, and it was from this fraction that the carboxydismutase was obtained as described under MATERIALS AND METHODS, Part D.

Properties of carboxydismutase

1. Carboxydismutase is an independent enzyme system: carboxydismutase can be dialyzed indefinitely against Mg^{++} (EDTA K) $5 \cdot 10^{-5}$ *M* or sodium acetate, 10^{-4} *M*, without losing activity.

2. Reaction (1) is unequivocal: when carboxydismutase solution (50 g wet leaves for 5 ml enzymic solution) is incubated either with RuDP-^{14}C and HCO_3^- (Table IV, Expt. 2; Fig. 8), only PGA appears as the final product according to reaction (1). Pen-

TABLE IV

CARBOXYLATION REACTION OF THE CARBOXYDISMUTASE

Expt.	*System**	*Total counts put on origin before run*	*Counts on origin after run*	*RuDP remaining*	*PGA*	*Pentose mono-P*	*Other compounds*
	Carboxydismutase						
1	+ RuDP + $H^{14}CO_3^-$	7,100	—	—	7,000	—	—
2	+ RuDP-^{14}C + HCO_3^-	5,600	274	639	4,218	369	81
	Boiled carboxydismutase						
3	+ RuDP-^{14}C + HCO_3^-	5,600	100	5,142	58	379	—

* Initial amounts of reactants added as indicated: 50 μl carboxydismutase solution; RuDP ($\sim$0.1 μM); RuDP-^{14}C ($\sim$0.1 μM); $NaHCO_3$ (1.2 μM); $NaH^{14}CO_3$ (1.2 μM). In each experiment: orthophosphate (2.5 μM) pH 6.8. Total volume: 250 μl. Temperature: 20°. Incubation time: Expt. 1, 10 min; Expt. 2, 3, 15 min. Values expressed in counts per minute fixed on paper.

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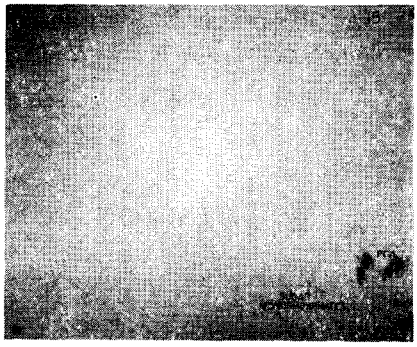

Fig. 8. RuDP-^{14}C + HCO_3^- + carboxydismutase.

tose monophosphates appear at the same concentration as in the control experiment (Table IV, Expt. 3). The carboxylation is directly proportional to the time and to the respective concentrations of carboxydismutase, RuDP and HCO_3^-; this is shown clearly by the kinetic study of the carboxylation.

The influence of enzymic concentration on carboxylation has been studied by introducing increased quantities of from 2 to 20 μl of carboxydismutase solution respectively in tubes, each containing orthophosphate (2.5 μM), pH 6.8, $NaH^{14}CO_3$ (1.2 μM) and RuDP ($\sim$0.1 μM); the final volume being increased to 200 μl. After an incubation time fixed at one minute, the reaction was stopped by heating and the solution was spread on an aluminum plate to determine the activity of the fixed $^{14}CO_2$.

It follows (Fig. 9) that the $^{14}CO_2$ fixation is directly proportional to the enzyme added when RuDP and $H^{14}CO_3^-$ were unlimited. Fig. 10A shows that RuDP and $H^{14}CO_3^-$ being unlimited, the carboxylation is linear with time for at least sixteen

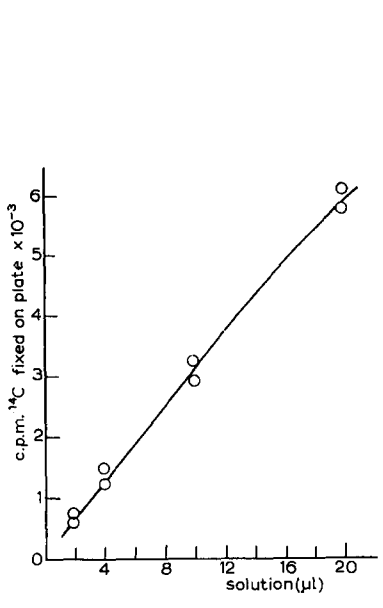

Fig. 9.

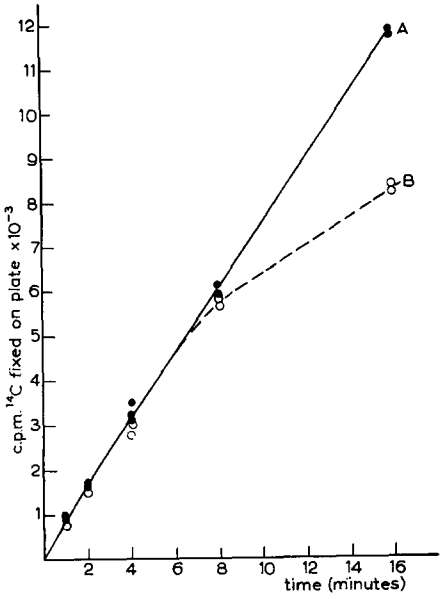

Fig. 10.

Fig. 9. Linear relationship between the carboxydismutase concentration and the rate of the carboxylation reaction. Carboxydismutase solutions: quantities varying from 2 to 20 μl as indicated. Each experiment: RuDP($\sim$0.1 μM); $NaH^{14}CO_3$ (1.2 μM); orthophosphate (2.5 μM) pH 6.8. Total volume: 200 μl. Temperature: 20°. Incubation time: 1 min. Values expressed in ^{14}C fixed on aluminum plate.

Fig. 10. Linear relationship between the time and the rate of the carboxylation reaction. A. RuDP ($\sim$0.1 μM); B. RuDP ($\sim$0.05 μM). Each experiment: 10 μl carboxydismutase solution. $NaH^{14}CO_3$ (1.2 μM); orthophosphate (2.5 μM) pH 6.8. Total volume: 200 μl. Temperature: 20°. Incubation time varying as indicated from 1 to 16 min. Values expressed in ^{14}C fixed on aluminum plates.

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minutes. In examining Curves A and B (Fig. 10) it is seen that at a given concentration of carboxydismutase and unlimited $H^{14}CO_3$, the rate of carboxylation is independent of RuDP concentration down to very low levels. The dependence of rate on HCO_3^- concentration is shown in Fig. 11, in which the concentration of $H^{14}CO_3^-$ is varied from 0.2 to 1.2 μM, the carboxydismutase concentration being fixed and RuDP unlimited.

The amount of carboxylation is thus dependent on the presence of the three factors: carboxydismutase, RuDP and HCO_3^-. The reaction ceases as soon as one of the factors is missing. Knowing that PGA is the only product formed, these results show that reaction (1) is unequivocal.

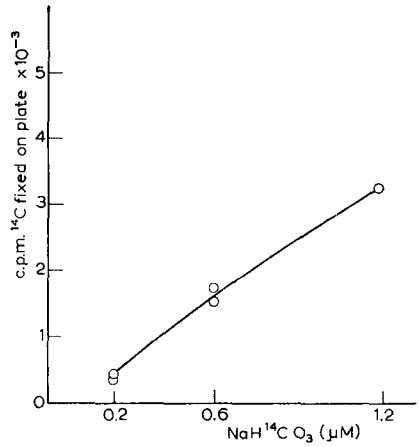

Fig. 11. Influence of $H^{14}CO_3^-$ concentration and the rate of the carboxylation reaction. $NaH^{14}CO_3$: Quantities varying as indicated from 0.2 to 1.2 μM. Each experiment: 10 μl carboxydismutase solution; RuDP ($\sim$0.1 μM); orthophosphate (2.5 μM) pH 6.8. Total volume: 200 μl. Temperature: 20°. Incubation time: 1 min. Values expressed in ^{14}C fixed on aluminum plate.

3. Functional groups of carboxydismutase: Table V, A and B, shows that at the high concentration of 10^{-2} M sodium azide (an inhibitor of phosphorylation reactions) scarcely inhibits carboxylation. On the other hand, iodoacetamide (10^{-2} M) after twelve hours of incubation, and *p*-chloromercuribenzoate ($5 \cdot 10^{-5}$ M) after ten minutes inhibit the reaction up to 60%. This last reaction was completely reversed by addition of cysteine. It is thus indicated that in the carboxydismutase –SH groups may be functional. Let us note that the inhibition by *p*-chloromercuribenzoate has been equally found in enzymic extracts of *Chlorella*[10].

TABLE V

THE EFFECT OF SODIUM AZIDE, *p*-CHLOROMERCURIBENZOATE AND IODOACETAMIDE ON CARBOXYLATION REACTION*

Compounds	*Fixed CO_2*	*Inhibition*
A.		
Control	6,150	—
Sodium azide 10^{-2} M	4,291	30
p-Chloromercuribenzoate $5 \cdot 10^{-5}$ M	2,843	53
p-Chloromercuribenzoate $5 \cdot 10^{-5}$ M cysteine 10^{-3} M	7,012	—
Cysteine 10^{-3} M	7,529	—
Iodoacetamide 10^{-2} M after 10 min contact	5,150	—
B.		
Control	2,333	—
Iodoacetamide 10^{-2} M after 12 h contact	990	58

* Each experiment: 50 μl carboxydismutase solution; RuDP ($\sim$0.1 μM); $NaH^{14}CO_3$ (1.2 μM); orthophosphate (2.5 μM) pH 6.8. Other reactants added as indicated. Total volume: 250 μl. Temperature: 20°. Incubation time: 10 min. Values expressed in counts per minute fixed on paper.

References p. 351.

The properties found by FAGER[11] for the activity of carboxylation associated with his suspension of chloroplasts approach those reported here.

SUMMARY

1. Using *Tetragonia expansa* leaves, a carboxylation enzyme system, carboxydismutase, capable of fixing CO_2 with RuDP to give two molecules of phosphoglyceric acid is found free from other enzymes.

2. The enzymic activity is not sensitive to dialysis with certain conditions. It seems to act as an independent system.

3. The enzymic activity is sensitive to *p*-chloromercuribenzoate; this last inhibitor being reversible by addition of cysteine, suggests the participation of –SH groups in the CO_2 fixation.

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BIOLOGICAL EFFECTS OF THE INCORPORATION OF THIOURACIL INTO THE RIBONUCLEIC ACID OF TOBACCO MOSAIC VIRUS

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INTRODUCTION

In our previous work[1], we have shown that thiouracil marked with ^{35}S could be incorporated into the ribonucleic acid of growing tobacco mosaic virus. The chromatographic study of hydrolysates of ribonucleic acid modified in this way indicated that the incorporated thiouracil was probably present in the form of thiouridylic acid. MATTHEWS repeated these experiments with a different method, and at first could not verify our results[2,3]. More recently, however, he confirmed and extended them[4].

This confirmation encourages us to publish in more detail some experiments[5] on the biological effects of the structural modifications undergone by the virus ribonucleic acid when thiouracil is incorporated.

References p. 361.