EXPERIMENTAL STUDIES ON SO_2 INJURIES IN HIGHER PLANTS

III: *Inhibitory Effect of Sulfite Ion on* $^{14}CO_2$ *Fixation*

HIROFUMI TANAKA, TOSHIHARU TAKANASHI*,

and

MICHIHIKO YATAZAWA

Dept. of Agricultural Chemistry, Nagoya University, Chikusa, Nagoya, Japan

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Abstract. Photosynthesis decreases reversibly in plants exposed to SO_2. Photosynthesis recovers when the exposure to SO_2 is discontinued. Inactivation of a photosynthetic enzyme, ribulose-1,5-diphosphate carboxylase, by sulfonation of its SH groups was investigated as a cause of the reversible reduction of photosynthesis. The relationship between the sulfite ion concentration in the reaction mixture and $^{14}CO_2$ fixation catalized by the enzyme which was prepared from alfalfa leaves was explored by using radioactive $NaHCO_3$.

About 50 % and 85 % inhibitions of $^{14}CO_2$ fixation were observed at 3×10^{-3} M and 3×10^{-2} M concentration of sulfite ion in the reaction mixture, respectively. The accumulation of 3×10^{-4} M sulfite ion on the reaction site of the enzyme involved in the plants which were exposed to SO_2 could considerably reduce the CO_2 assimilation of the plant.

1. Introduction

Decreased photosynthesis and increased respiration in plants exposed to low concentration of SO_2 were reported by Thomas and Hill (1937) and by Katz (1949). They mentioned that high concentrations of the gas inhibited CO_2 assimilation almost immediately after the fumigation. Though the extensive acute leaf destruction reduced photosynthesis to a low level, the affected photosynthesis recovered to the normal level and sometimes greater than normal level in a few hours, corresponding to the leaf tissue remaining uninjured.

The mechanism of the reduction in photosynthesis is still obscure. The formation of glyoxylate bisulfite reported by Tanaka *et al.* (1972a) could be a key mechanism. They mentioned that glyoxylate bisulfite, one of the sulfite additive products of aldehydes, was formed in plants exposed to SO_2 and this compound inhibited glycolic oxidase of the glycolic acid pathway which plays an important role in photosynthesis of the plant.

Recently, Matsuoka *et al.* (1969) also observed both reversible and irreversible inhibitions in the effect of SO_2 on CO_2 assimilation of rice plants. The reduction of the assimilation occurred immediately after the exposure of the plant to a low concentration of SO_2 followed by an immediate recovery when the fumigation was discontinued. This immediate appearance and disappearance of SO_2 effects suggest that the reaction occurs at an earlier stage in CO_2 assimilation.

* Present address: The Environmental Pollution Research Center, Aichi Prefecture Government, Kita-ku, Nagoya, Japan.

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Tanaka *et al.* (1972b) reported that an inclusive reduction of ^{14}C incorporation occurred in plants exposed to SO_2, and suggested the presence of other inhibitory mechanisms than that caused by the formation of glyoxylate bisulfite.

This report deals with the effect of sulfite ions on ribulose-1,5-diphosphate carboxylase, an enzyme catalizing the incorporation of CO_2 into 3-phosphoglyceric acid in the photosynthetic pathway.

2. Materials and Methods

A. PREPARATION OF ENZYME SOLUTION

Alfalfa plants were raised on a sand bed with Arnon's medium (Arnon, 1938) for 1 month. Two grams of fresh leaves were homogenized by mortar and pestle with cold 0.05 M Tris-HCl buffer solution (containing 0.1 M ethylenediaminetetraacetic acid). The homogenate was centrifuged at 10000 G and the supernatant was used as an enzyme solution.

B. RIBULOSE-1,5-DIPHOSPHATE

Eighty mg of ribulose-1,5-diphosphate (barium salt) were suspended in 1.2 ml of cold water, and 1.2 ml of 0.1 N HCl and 2.4 ml of 0.1 M Na_2SO_4 were added. The mixture was stirred, and allowed to stand. After 10 min, 1.2 ml of 0.1 M NaOH solution was added, and left for 15 min. The barium sulfate formed was centrifuged off at 2000 rpm and the supernatant, sodium salt of ribulose-1,5-diphosphate, was used as a substrate of the enzyme reaction (14 μM ribulose-1,5-diphosphate ml^{-1}). All treatments were carried out at 0 °C.

C. ENZYME REACTION AND MEASUREMENT

The composition of standard reaction mixture is shown in Table I. Each component of the reaction mixture was mixed in a small test tube, and incubated at 25 °C. After the incubation period, the reaction was stopped by adding 0.05 ml of acetic acid, and simultaneously the excess $H^{14}CO_3^-$ was expelled. Two-tenth ml aliquot of the reaction mixture was taken in a planchet for radioactivity counting. After evaporation under an infra-red lamp, the radioactivity was measured by a Nuclear Chicago windowless gas flow counter.

TABLE I

Composition of the standard reaction mixture

1.0 M Tris-HCl buffer solution (pH 7.0)	0.1 ml
0.1 M $MgCl_2$	0.05
Ribulose-1,5-diphosphate (14 μM/ml)	0.05
0.5 M $NaH^{14}CO_3$	0.05 (2.0 μCi)
Enzyme solution	0.1
Water	0.15
Total	0.5

In the experiments for examining the effect of sulfite or sulfate ions on the enzyme reaction, a solution of each ion was added to the reaction mixture (Table I) from which 0.15 ml water was renewed. The final concentrations of sulfite or sulfate ions in the reaction mixture were 0, 3×10^{-5}, 3×10^{-4}, 3×10^{-3}, and 3×10^{-2} M. Other procedures were the same as that of the standard reaction.

3. Results and Discussion

A. $^{14}CO_2$ FIXATION BY STANDARD REACTION MIXTURE

The temporal behavior of $^{14}CO_2$ fixation in a standard reaction mixture under the condition used is illustrated in Figure 1. The level of radioactivity, which reflects the amount of ^{14}C fixed by 0.2 ml of the reaction mixture, became almost constant by 20 min.

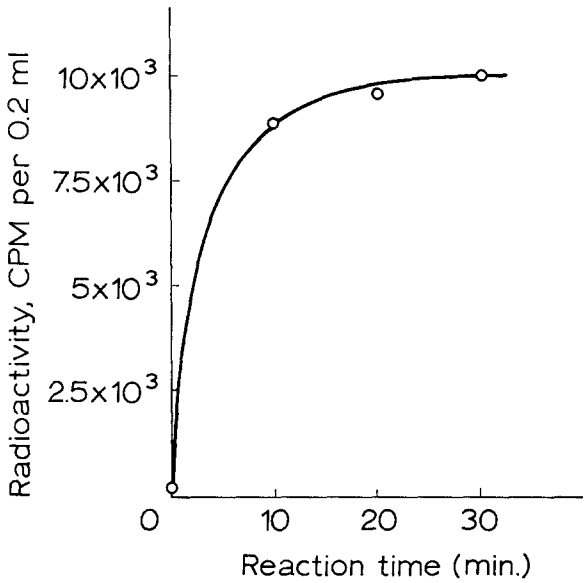

Fig. 1. Time course of $^{14}CO_2$ fixation in standard reaction mixture.

Only 2 cpm of radioactivity were counted in the reaction mixture containing no ribulose-1,5-diphosphate substrate during 20 min, therefore, it is obvious that no $^{14}CO_2$ was fixed without added substrate. Therefore, Figure 1 shows the progress of the $^{14}CO_2$-fixation by the ribulose-1,5-diphosphate carboxylase.

B. EFFECT OF SULFITE OR SULFATE IONS ON THE FIXATION OF $^{14}CO_2$

In this experiment, sodium sulfite (neutralized with HCl, pH 7) or a sodium sulfate solution was used instead of water in the standard reaction mixture shown in Table I. The final concentration of each ion is indicated on the abscissa in Figures 2 and 3. Figure 2 shows the inhibition of $^{14}CO_2$ fixation by sulfite ion in the reaction mixture. The effect of a sulfate ion on $^{14}CO_2$ fixation is shown in Figure 3. The ordinates of

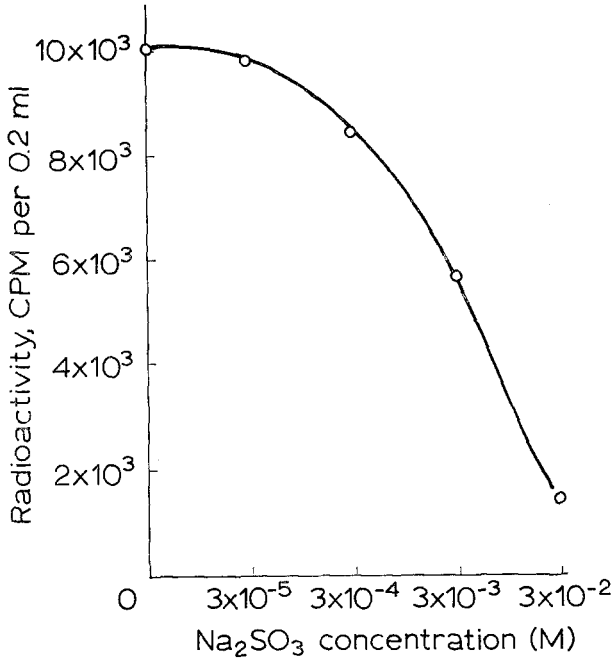

Fig. 2. Inhibition of $^{14}CO_2$ fixation by sulfite ion (reaction time: 20 min).

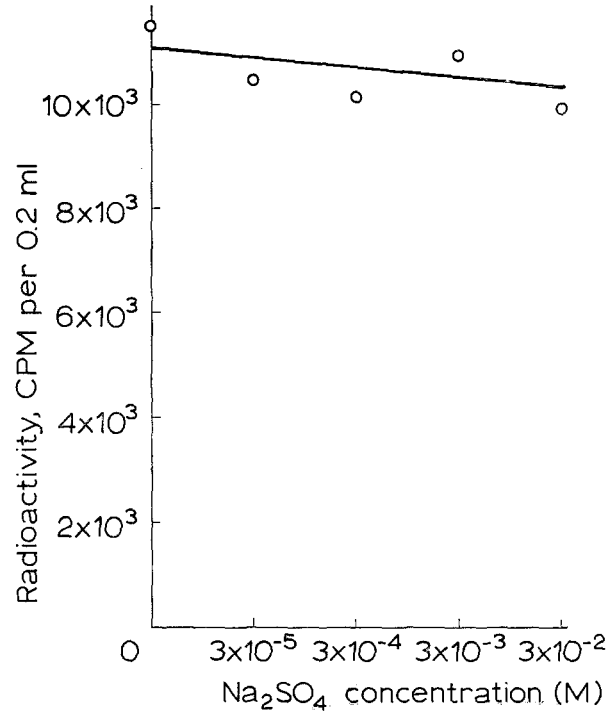

Fig. 3. Effect of sulfate ion on $^{14}CO_2$ fixation (reaction time: 20 min).

both figures show the radioactivity of ^{14}C in 0.2 ml of the reaction mixture after 20 min incubation.

It is clearly shown in Figure 2 that $^{14}CO_2$ fixation decreased as the concentration of sulfite ion increased; about 50% and 85% decrease at 3×10^{-3} and 3×10^{-2} M, respectively. On the other hand, almost no effect was seen up to 3×10^{-2} M sulfate ion as shown in Figure 3. From these results, it is seen that the sulfite ion used in this study reacted without having been oxidized during the experimental period and the phenomena shown in Figure 2 are due to the effect of sulfite, not to the sodium ion. The experiments were repeated and similar results were obtained.

Results shown above apparently indicate the inhibition of ribulose-1,5-diphosphate carboxylase by sulfite ion and the CO_2 assimilation may be inhibited considerably when sulfite ion concentration reaches 3×10^{-4} M at the reaction site of the enzyme of the SO_2 exposed plants.

According to Puckett *et al.* (1973) experimenting on the effect of SO_2 on ^{14}C fixation in lichens, quoted the estimation of Saunders (1970) that a concentration of 100 μg SO_2 m^{-3} (0.035 ppm) in air can give rise to 35 ppm SO_2 in solution. They obtained 40 to 100% inhibition of ^{14}C fixation in various lichens incubated in solutions of 75 ppm SO_2 buffered at pH 4.4. This concentration was considered to be equivalent to the atmospheric concentration of 0.075 ppm SO_2. Applying this relationship to the results in this paper, the sulfite ion concentration of 3×10^{-2}, 3×10^{-3}, and 3×10^{-4} M would be equivalent to an atmospheric concentration of about 2, 0.2, and 0.02 ppm SO_2, respectively.

General properties of the enzyme used in this investigation have been reviewed by Kawashima and Wildman (1970). An interpretation on the mechanism of this inhibiting reaction could be possible by referring to Chan's experiment (Chan, 1968) on sulfonation of aldolase and experiments conducted by Sugiyama *et al.* (1968) on the properties of soluble leaf protein (fraction 1 protein). According to Sugiyama *et al.* fraction 1 protein from spinach leaves has 96 SH groups in one protein molecule and the activity of this enzyme falls to zero when about half of the SH groups were blocked. Therefore, the inhibition of CO_2 assimilation occurs when SH groups of this enzyme are blocked by SO_2 addition as have been observed by Chan in aldolase.

The enzyme protein, whose activity was reduced by SO_2, may release sulfite ions in changing biological conditions and recover their activity. Chan also reported that inactivated aldolase by sulfonation completely recovered its activity by the treatment with excess thiol: cystin. This agrees with the fact that the reduced CO_2 assimilation due to the exposure to low concentration of SO_2 was recovered after the exposure was discontinued.

Acknowledgments

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