PHOTOSYNTHETICA 37 (4): 519-527, 1999

Relationship between carbon and nitrogen metabolisms in photosynthesis. The role of photooxidation processes

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Abstract

$^{14}CO_2$ uptake in leaves of wheat plants (*Triticum aestivum* L.) fertilized by urea or $Ca(NO_3)_2$ (25 mol m^{-3}) was investigated. The Warburg effect (inhibition of $^{14}CO_2$ uptake by oxygen) under 0.03 vol. % CO_2 concentration was observed only in non-fertilized plants. Under 0.03 vol. % CO_2, the Warburg antieffect (stimulation of $^{14}CO_2$ uptake by oxygen) was detected only in plants fertilized by $Ca(NO_3)_2$. Under saturating CO_2 concentration (0.30 vol. %), the Warburg antieffect was observed in all variants. Under limitation of ribulose-1,5-bisphosphate carboxylase/oxygenase activity (0.30 vol. % CO_2 + 1 vol. % O_2), the rate of synthesis of glycollate metabolism products decreased in control and urea-fertilized plants but was enhanced in nitrate-fed plants. Hence, there was an activation of glycollate formation *via* transketolase reaction in fertilized plants, and the products of nitrate reduction function were oxidants in nitrate-fertilized plants whereas the superoxide radical played this role in urea-fertilized plants.

Additional key words: alanine; aspartate; glycine; glycollate; malate; nitrate; serine; sugars; *Triticum aestivum*; urea; wheat.

Introduction

In a previous paper it was shown (Chikov *et al.* 1988) that the change of PCM under a rapid increase (on the eve of the experiment day) of root nitrogen nutrition depends on the form of N used (oxidized or reduced one). Using the reduced N form (urea) the high rate of saccharide synthesis and high radioactivity ratio of sucrose/hexoses were maintained, but when introducing an oxidized N form [$Ca(NO_3)_2$] the sucrose

Received 16 August 1999, *accepted* 16 November 1999.

Abbreviations: ETC - electron transport chain; Fd - ferredoxin; PCM - photosynthetic carbon metabolism; PEP - phosphoenolpyruvate; PES - phosphorous esters of sugars; 3-PGA - 3-phosphoglycerate; PS - photosystem; "reducing power" - fund of photosynthetically formed NADP and ATP; RuBP - ribulose 1,5-bisphosphate; RuBPCO - ribulose-1,5-bisphosphate carboxylase/-oxygenase.

synthesis sharply decreased and the ^{14}C incorporation in the products of glycollate pathway relatively increased.

Since glycollate pathway is the main channel of nitrogen assimilation during photosynthesis (Wallsgrove *et al.* 1983) and the glycollate formation depends on the concentration ratio of CO_2/O_2 in chloroplast (Moyse 1980), we tried to elucidate how the concentration and form of N nutrition affect PCM under changes in photooxidized reactions (irradiance, concentrations of CO_2 and O_2).

Materials and methods

Wheat plants (*Triticum aestivum* L.) were grown in field conditions, 15 plants per pot (7 kg air dry soil) under high concentration of mineral nutrition (1 g of N, P, and K each per pot), optimum humidity, and natural irradiance. Plants were used in the reproductive stage of development when the export function of leaves increased (Chikov *et al.* 1984). At this time, the leaf growth is finished and leaves are the typical exporters of assimilates. $^{14}CO_2$ fixation was studied at two concentrations of CO_2 (natural 0.03 vol. % and saturating 0.30 vol. %) and O_2 (21 and 1 vol. %) in a leaf chamber. Irradiance in the chamber was 440 (saturating) or 110 W m^{-2} (limiting). The additional fertilizing by N compounds (25 mol m^{-3}) was done on the eve of the ^{14}C-experiment day. The $^{14}CO_2$ fixation was tested on the top leaf of wheat plants using a thermostated leaf-chamber (2.0×2.5 cm). To study the ^{14}C distribution among of the water-ethanol-soluble photosynthates we used paper chromatography and radiography (see Chikov *et al.* 1988).

Results

The rate of $^{14}CO_2$ uptake by experimental and control plants showed that the so-called Warburg effect was observed only in control plants at limiting CO_2 concentration (Fig. 1). The decrease in O_2 concentration in such conditions did not change the $^{14}CO_2$ uptake in urea-fertilized plants, whereas the nitrate-fertilized plants showed the Warburg antieffect. The Warburg antieffect was observed in all variants under saturating CO_2 (in all experiments the gas composition was altered only in the leaf chamber and only in the moment of $^{14}CO_2$ uptake).

For the analyses of ^{14}C distribution among photosynthates it is convenient to classify them in the following groups: (*1*) free sugars (sucrose, glucose, fructose) which display the export function of leaf; (*2*) glycollate and products of its metabolism (serine, glycine, glycollate); (*3*) 3-PGA and the products of its non-reduced metabolism such as alanine and (after β-carboxylation of PEP) malate and aspartate. The last two groups characterize the channels of outflow of carbon that has been assimilated in the Calvin cycle in the form of non-saccharides (organic acids and amino acids).

The ^{14}C distribution among labelled photosynthates showed (Table 1) that under the intensive N supply (both variants), ^{14}C was metabolized to a great extent *via* glycollate pathway and non-reduced turnover of 3-PGA. Under CO_2-saturated photosynthesis, the ^{14}C incorporation into products of glycollate pathway relatively decreased, but the formation of free sugars increased.

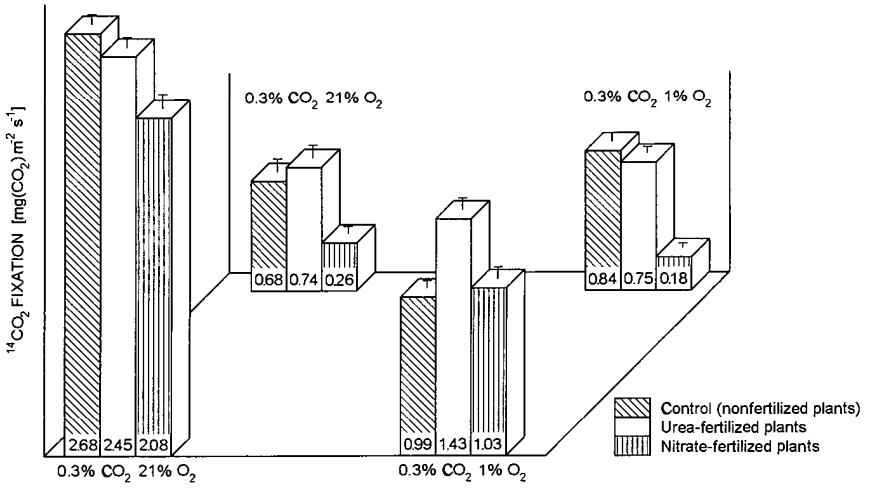

Fig. 1. The influence of CO_2 and O_2 concentrations [vol. %] on the rate of $^{14}CO_2$ fixation [mg m^{-2} s^{-1}].

Under the saturating CO_2 concentration the RuBP oxygenase reaction is suppressed, but the CO_2 uptake (result of reduction of leaf resistance of CO_2 diffusion and inhibition of oxygenase) is stimulated. Accordingly, in these conditions the glycollate formation must be suppressed. In fact, the stimulation of formation of glycollate and products of its metabolism takes place. Under saturating CO_2 concentration, the rate of formation of glycollate metabolites was, in absolute values, markedly greater than under natural CO_2 concentration (Fig. 2*A*). In urea-fertilized plants, this difference was large. Therefore, in these conditions, glycollate is formed in a reaction different from RuBP-oxygenation (*e.g.*, by transketolase mechanism of glycollate synthesis - Takabe *et al.* 1980).

In differently fed plants the formation of glycollate pathway products had various sensitivity to the decrease in O_2 concentration (Fig. 2*B*). Under maximum suppression of RuBP-oxygenase activity (high CO_2 concentration and low O_2 concentration), a 2.0-2.5 fold decrease in the rate of synthesis of these compounds was observed in control plants and urea-fertilized plants. On the contrary, in nitrate-fertilized plants the syntheses of glycollate, serine, and glycine were 2-fold higher. This means that the glycollate formation in these plants was not suppressed by high CO_2 concentration, but it was observed in plants of other variants under low O_2 concentration. Consequently, under high concentration of nitrates the glycollate synthesis is slightly conected with the presence of oxygen.

Table 1. The influence of irradiance and various CO_2 and O_2 concentrations and nitrogen fertilizers on the distribution of ^{14}C among some labelled products after a 2-min $^{14}CO_2$ uptake [% to radioactivity of compounds of water-ethanol-soluble fraction].

Variant	Free sugars	Phosphorus esters of sugars	Serine, glycine, glycollate	Alanine, malate, aspartate
440 W m^{-2}; 21 vol. % O_2; 0.03 vol. % CO_2				
Control	42.2±1.0	27.6±1.1	11.8± .1	7.7±0.4
Urea	44.5±0.8	24.1±0.9	11.3±1.0	12.2±1.1
Nitrates	40.1±1.5	16.3±2.6	18.9±2.2	11.8±0.4
440 W m^{-2}; 21 vol. % O_2; 0.30 vol. % CO_2				
Control	44.5±1.7	27.8±1.0	6.4±0.6	12.8±0.4
Urea	45.1±1.3	18.9±1.4	8.2±0.5	20.2±1.0
Nitrates	44.6±1.8	14.9±1.6	9.5±0.6	17.9±1.1
110 W m^{-2}; 21 vol. % O_2; 0.30 vol. % CO_2				
Control	48.8±1.7	23.6±1.6	4.3±0.3	14.7±0.3
Urea	44.5±1.1	17.9±1.6	5.5±0.4	22.7±0.5
Nitrates	34.8±1.5	16.5±1.1	14.4±1.2	24.7±1.3
440 W m^{-2}; 1 vol. % O_2; 0.03 vol. % CO_2				
Control	51.7±2.8	20.1±1.7	6.5±0.3	13.9±0.9
Urea	43.1±1.2	22.8±0.8	6.8±0.8	18.1±0.4
Nitrates	45.3±2.2	11.4±1.1	15.4±1.3	20.9±1.3
440 W m^{-2}; 1 vol. % O_2; 0.30 vol. % CO_2				
Control	44.9±1.0	28.9±1.4	4.0±0.6	13.2±0.6
Urea	47.3±1.1	14.1±2.5	2.4±0.4	22.0±0.8
Nitrates	46.8±2.0	14.2±1.6	9.6±0.5	22.5±1.0

Since the increase in CO_2 concentrations in leaf chamber occurred only at the moment of $^{14}CO_2$ uptake, we suggest that labelled carbon had to be incorporated, first of all, in PES, and the radioactivity of these compounds had to be increased both absolutely and relatively to other compounds. However, in control plants the ^{14}C incorporation in PES almost did not change, but in fertilized plants, the part of ^{14}C incorporated in these compounds even decreased. Extremely low content of ^{14}C in PES and its intense incorporation into products of the glycollate pathway point to the intensity of PES "burning" in photooxidation processes. As a result, in nitrate-plants the ratio of radioactivity of the glycollate pathway products was 1.5-2.5-fold higher than in plants of other variants (Table 1).

Under saturation of photosynthesis by CO_2 and intense formation of ^{14}C-PGA, full extent of its subsequent reduction was difficult and PGA was more intensively metabolized *via* the non-reduced pathway forming alanine, malate, and aspartate. The amount of labelled carbon in these compounds increased markedly with the increase in CO_2 concentration (Table 1, Fig. 2*C*). The increase in formation of four-carbon compounds reflects (to a great extent) an intensification of cytoplasmic processes.

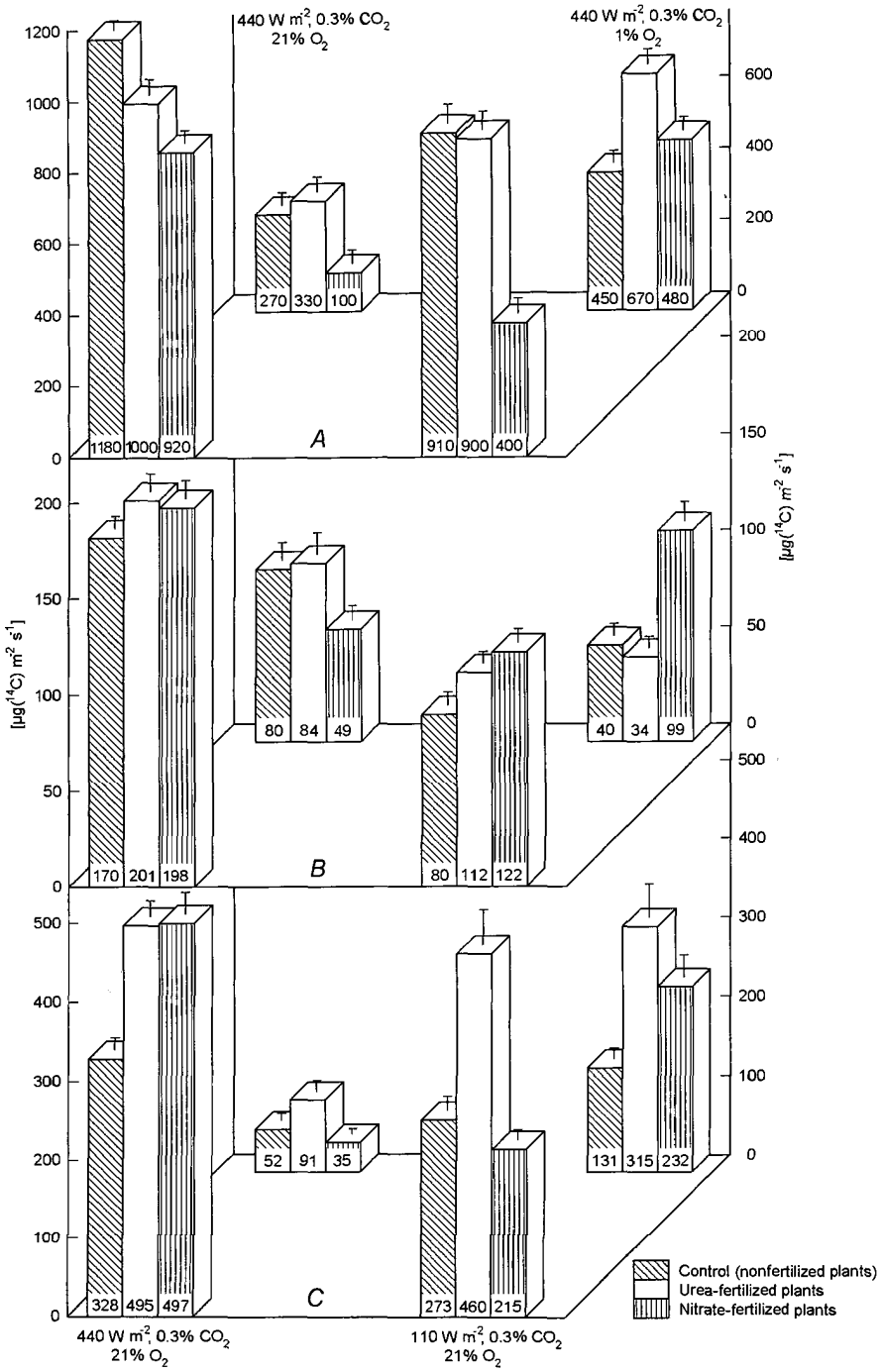

Fig. 2. The influence of irradiance, various CO_2 and O_2 concentrations [vol. %], and nitrogen fertilization on the synthesis of some labelled products (*A* - free sugars; *B* - serine + glycine + glycollate; *C* - alanine + aspartate + malate) in 2 min photosynthesis in wheat leaves [μg(^{14}C) m^{-2} s^{-1}].

Since PEP-carboxylase functions in the cytoplasm, the products of reaction (oxaloacetate, malate, and aspartate) may be used later, in the course of respiration. Thus, the ^{14}C amount in PES is essentially limited by outflow of freshly fixed carbon *via* the channel of non-reduced metabolism of PGA, and this process was more active in fertilized plants (Table 1, Fig. 2*C*).

The metabolism of PGA was sensitive not only to increase in CO_2 concentration but also to any other alteration of conditions for photosynthesis. For example, if the decrease in O_2 concentration alone occurred, the RuBP carboxylation and generation of redundant PGA (in consequence of decrease of oxygenation) were enhanced (Table 1, Fig. 2*C*). This phenomenon was more pronounced in fertilized plants.

Although in plants of both fertilized variants the ^{14}C portion in PES decreased, the reasons of this phenomenon were different in urea-fertilized and $Ca(NO_3)_2$-fertilized plants. This was confirmed by the ratio of the channels through which the labelled carbon was metabolized into the non-saccharide photosynthates. In nitrate-plants, the glycollate pathway of carbon out-flow from the Calvin cycle was more pronounced, while in urea-fertilized plants the non-reduced metabolism of PGA was enhanced. As a result, in nitrate-fed plants the ratio of radioactivity of glycollate pathway products to the compounds of non-reduced metabolism was higher than that in urea-fertilized plants (Table 1).

Discussion

The decrease in PES radioactivity may occur for two reasons (see scheme in Fig. 3). Firstly, in fertilized plants, PES pools seem to metabolize more actively, not only *via* saccharide channel, but also *via* other channels forming organic acids and amino acids. High radioactivity of these compounds relatively decreases a part of labelled carbon, contained in PES. Secondly, the amount of ^{14}C entering into PES possibly decreases due to diminution of ^{14}C-PGA reduction.

One of the channels removing carbon from the Calvin cycle pools is the glycollate pathway. Moyse (1980) and other researchers connected the glycollate formation during phosynthesis, first of all, with the function of RuBPCO (EC 4.1.1.39) (see also Schnarrenberger and Martin 1997). The ratio of carboxylation to oxygenation is ultimately regulated by the ratio of O_2 and CO_2 concentrations, and O_2 and CO_2 compete for coupling with the reaction centre of enzyme.

Glycollate may originate in transketolase reaction of Calvin cycle (Gibbs 1972, Eickenbusch 1973) which needs for its realization a superoxide radical (Aufhammer and Solansky 1976) formed in the Mehler reaction (O_2 photoreduction in electron transport chain of chloroplast - Takabe *et al.* 1980). In the transketolase reaction the phosphorous esters of early precursors of sugars are oxidized. Photooxidation of these sugars is a principal reason of the radioactivity decrease in fertilized plants. This hypothesis is in accordance with the results of Robinson and Gibbs (1982) who found a stimulation of O_2 photoabsorption in the presence of reduced N in spinach chloroplasts.

The increase in CO_2 concentration influences not only the process of its assimilation as a substrate, but intensifies also the electron flow in ETC of chloroplasts (Govindjee 1982). *In vivo,* the reaction of bicarbonate ion may be directed at plastoquinone pool in its donor part, increasing the connection between photosystems 1 and 2. It enhances the electron flow for the reduction of Fd and then NADP (Fig. 3). Hence upon the increase of electron flow in ETC and additional formation of "reducing power" the latter could be used more intensively for the reduction of PGA to sugars or for the reduction of nitrates. This is confirmed by the present results (Fig. 2, Table 1).

The increase in integration of PS1 and PS2 and the intensification of electron transport upon the enhancement of CO_2 concentration allow even to decline the quantum flow without a decrease (or even upon increase) of the activity of NADP and ATP formation that intensify photosynthesis. Since the sugar formation from CO_2 needs obligatory assimilation of "reducing power", the rate of ^{14}C incorporation into sugars can, to some extent, characterize the activity of photochemical reaction of chloroplasts *in vivo*. As shown in Fig. 2, the decrease in irradiance and simultaneous increase in CO_2 concentration enhanced the intensity of sugar formation as compared with that under saturating irradiance and natural CO_2 concentration.

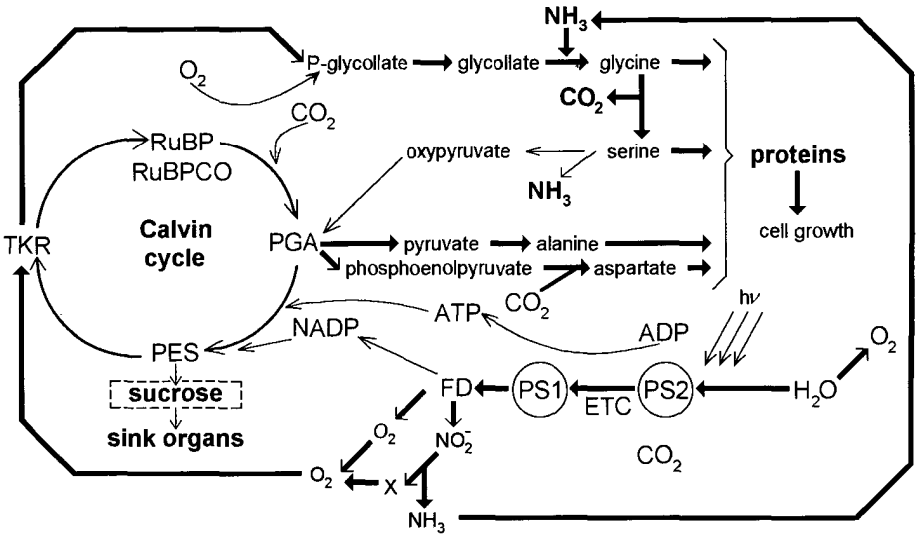

Fig. 3. Scheme of regulation of carbon photosynthetic metabolism (*thick arrows*: reactions activated by nitrogen). X - unknown oxidizer. Sucrose transport fund in apoplast and phloem is shown.

According to Moyse (1980), carbon of P-glycollate generated in oxygenase reaction after the turnover *via* the glycollate pathway (P-glycollate → glycollate → glyoxylate → glycine → serine → oxypyruvate → glycerate →PGA) returns into the Calvin cycle where it is reduced into sugars (Fig. 3). Therefore we conclude that the glycollate pathway is a cyclic one, and it shunts carboxylase reaction of the Calvin cycle. However, amino acids generated *via* the glycollate pathway can

obviously be used for synthetic processes in cells and, in that case, the glycollate pathway becomes partially open and the unloading of pools of the Calvin cycle takes place.

The Warburg antieffect is usually observed upon saturating CO_2 concentration and saturating irradiance (Laisk 1977), but a convincing explanation of this phenomenon is still lacking. According to our hypothesis (Chikov 1987), the rate of photosynthesis is determined not only by the rate of carboxylase reaction but also by the rate of carbon outflow from the Calvin cycle. This outflow is carried out mainly through the saccharide channel (sucrose synthesis) which can be limited by the export possibilities of mesophyll cells. In this case sucrose is accumulated in apoplast and phloem (Fig. 3). With increasing CO_2 concentration, PES are accumulated in large amounts, and thus their conversion to sucrose and subsequent transport become difficult. The increase in PES metabolism *via* the glycollate pathway in the presence of O_2 creates additional possibilities for the unloading of Calvin cycle pools.

Amino acids additionally generated during photorespiration and mitochondrial respiration as well as the products of PGA turnover *via* the non-reduced pathway are used in synthetic processes in leaf which is a donor of photosynthates. Hence, the rate of photosynthesis can be increased by the outflow of carbon from the Calvin cycle *via* the non-saccharide pathway if the compounds formed will be later used in synthetic processes in the cell. Also leaf area density rises under high N nutrition of plants.

The basic difference between the utilization of reduced nitrogen and nitrates is that ammonium may be incorporated in amino acids immediately, while the nitrates have to be preliminary reduced. In our experiments with high dose of nitrates and short time from the additional fertilization till $^{14}CO_2$ application, the leaves seem to contain large amounts of non-reduced nitrates, but the process of their reduction requires a great quantity of photons. The final step of nitrate reduction is the reduction of nitrite (Fig. 3) *via* electron transport chain of chloroplast. This process is also called the "nitrite photosynthesis". One of intermediates of this reaction is hydroxylamine which can interact with aldehydes and ketones. If we suggest that glycollate is derived from such interaction with sugar phosphates of the Calvin cycle, the low dependence of ^{14}C incorporation into products of the glycollate pathway on the decrease in O_2 concentration becomes clear. In nitrate-plants, oxygen contained in nitrites plays a role of a superoxide radical.

Thus, in ETC of chloroplasts (Fig. 3) there are three final electron acceptors: (*1*) NADPH used in CO_2 reduction, (*2*) oxygen forming superoxide radical involved into glycollate formation *via* the transketolase reaction, and (*3*) the nitrite-ion. The later, in the course of its reduction, seems to produce the oxidizer able to activate the transketolase mechanism of glycollate formation. Thus, the glycollate formation is differently stimulated (Fig. 3): (*a*) in urea-fertilized plants by the participation of superoxide radical derived from Mehler reaction; (*b*) in nitrate-fertilized plants by the participation of some agent "X", formed in the process of nitrite-ion reduction.

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