THE EFFECTS OF SOME INHIBITORS ON THE RATES OF PHOTOSYNTHESIS AND RESPIRATION BY CHLORELLA

By EDWARD LUNG YUAN and FARRINGTON DANIELS

(From the Department of Chemistry, University of Wisconsin, Madison)

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The rates of photosynthesis and respiration of most plants are readily affected by the presence of added chemicals. The action of some substances that have strong affinities for certain components of the photosynthetic and respiratory apparatus is specific and effective. The skillful use of such substances may help to differentiate the component reactions of photosynthesis. Warburg initiated the work by using potassium cyanide on the green algae, *Chlorella pyrenoidosa* (1). Gaffron has used many inhibitory effects to explain the complex chemistry of photosynthesis (2–4). Greenfield has made a detailed study of the inhibitory effects of inorganic compounds on photosynthesis in *Chlorella* (5). Except for a few specific cases, little is known concerning the various ways in which the different inhibitors affect the separate processes of photosynthesis and respiration. The present work was intended as a qualitative exploration of the effects of some common inhibitors on the rates of photosynthesis and respiration in *Chlorella pyrenoidosa*. Some less studied inhibitors were included in the study while taking the common inhibitor, KCN, as a comparative measure. No attempt will be made to offer theoretical explanations of the results. The inhibitors used in this study include potassium cyanide, sodium azide, hydroxylamine hydrogen chloride, and dinitrophenol.

EXPERIMENTAL

The apparatus used in this study was essentially the same as that described in a previous report (6). A carbon dioxide infrared gas analyzer was used to follow the exchange of carbon dioxide gas. The magnetic, oxygen meter was not used because the addition of liquid solutions of the inhibitor caused unstable readings. The rate of change of carbon dioxide gas was recorded on an automatic Brown recorder.

The reaction vessel had a liquid volume of 70 ml. The volume of the circulating system was 125 ml. The solution containing the inhibitor was added to the *Chlorella* suspension by means of a burette fitted to the reaction vessel. Equilibrium between gas and liquid was usually established within 3 minutes after each addition of the inhibitor solution. All experiments were carried out at room temperature, 20–23°C.

The light source was a mercury AH-6 lamp. Monochromatic beams of light at 5460 A and 4360 A, were obtained by means of interference filters supplied by the

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Baird Associates, and Co. The light intensity varied from 1.0×10^{-8} einstein per cm.^{2} per minute to 5.0×10^{-8} einstein per cm.^{2} per minute (1 einstein = 6.02×10^{23} photons).

The culture used was a strain of algae, *Chlorella pyrenoidosa*, maintained in pure culture by the Botany Department of the University of Wisconsin. The culturing of algae has been described in a previous paper (6). About 300 to 700 microliters of the freshly cultured cells were suspended in 20 ml. of culture medium, at pH about 5.0. The suspension was saturated with 2 per cent carbon dioxide in air. The absorption of incident light by the algal cells was generally more than 70 per cent.

The addition of the inhibitor solution was carried out by means of a 10 ml. burette. During each experiment, the normal rates of respiration in the dark and photosynthesis in the light were measured. A small measured amount of the inhibitor solution was then introduced through the burette, and rates of respiration and photosynthesis were

TABLE I

Effects of KCN on Respiration

Experiment No.	KCN concentration	Respiration (changes in micromoles CO_2 per 10 min.)		Percentage change in respiration
		Normal	With KCN	
	moles/liter			
1	4.4×10^{-5}	2.38	2.40	+1.3
2	5.5×10^{-5}	1.11	1.14	+2.9
1	7.7×10^{-5}	2.38	2.44	+2.7
3	11.0×10^{-5}	1.35	1.91	+41.2
2	16.5×10^{-5}	1.11	1.37	+22.9
3	22.0×10^{-5}	1.35	1.59	+17.7
2	27.5×10^{-5}	1.11	1.03	−7.2
4	31.8×10^{-5}	2.34	2.39	+2.0
4	45.0×10^{-5}	2.34	2.04	−12.5
4	63.6×10^{-5}	2.34	1.91	−19.7

again recorded at the same light intensity. Calculations were made directly from the readings on the recording chart.

RESULTS

Inhibition by Potassium Cyanide.—

The poisoning effect of potassium cyanide on plants has been studied quite thoroughly. Our experimental results are presented here first in order that a comparison can be made with the literature. Generally our results agreed with those of other workers in this field.

The poisoning effect of potassium cyanide on photosynthesis is due to the formation of a complex with metal atoms contained in the prosthetic groups of many enzymes. The molecular species of the poisoning agent penetrates the cells as the non-dissociated acid, HCN. At pH values below 8.5, the concentration of HCN molecules in a solution is approximately equal to the concentration of the cyanide ion. Tables I and II show the general results of the ef-

fect of cyanide. The light intensities used in these experiments were about 1.0×10^{-8} einstein per cm.^{2} per minute.

The effects of potassium cyanide on respiration and photosynthesis by *Chlorella* come immediately after its addition. Respiration is stimulated at small concentrations of KCN (10×10^{-5} moles per liter), and is only slightly inhibited at high concentrations of KCN. The rate of photosynthesis is strongly inhibited by the addition of KCN.

The inhibition of photosynthesis by cyanide is only effective above the compensation point—the intensity of light at which photosynthesis is enough to just balance respiration. In weak light where measurements are made under the compensation point, and respiration is greater than photosynthesis, no inhibition of photosynthesis is observed.

TABLE II

Effects of KCN on Photosynthesis

Experiment No.	KCN concentration	Photosynthesis (changes in micromoles CO_2 per 10 min.)		Percentage change in photosynthesis
		Normal	With KCN	
	moles/liter			
1	4.4×10^{-5}	6.25	5.70	−9.0
2	5.5×10^{-5}	4.64	3.49	−24.6
1	7.7×10^{-5}	6.25	5.58	−10.7
3	11.0×10^{-5}	5.79	4.34	−30.0
2	16.5×10^{-5}	4.64	2.20	−52.5
3	22.0×10^{-5}	5.79	3.28	−43.3
2	27.5×10^{-5}	4.64	1.59	−65.7
4	31.8×10^{-5}	6.83	2.96	−56.5
4	45.0×10^{-5}	6.83	1.96	−71.0
4	63.6×10^{-5}	6.83	1.91	−72.0

This residual photosynthesis, the amount of photosynthesis under the compensation point, was observed first by Warburg (1).

Inhibition by Sodium Azide.—

The results are summarized in Tables III and IV. The light intensities were about 1.0×10^{-8} einstein per cm.^{2} per minute.

The most interesting feature of this inhibitor is its remarkable effectiveness in the poisoning of photosynthesis. Complete inhibition of photosynthesis can be achieved with the addition of only 40×10^{-6} moles per liter of sodium azide. It is more than ten times as effective as potassium cyanide. The inhibition of photosynthesis is also effective under the compensation point, and there is no residual photosynthesis.

The response after the addition of sodium azide is fast. Usually a steady rate is observed in less than 5 minutes.

The rate of respiration is stimulated at very low concentrations of sodium azide. The inhibition of respiration at higher concentrations of sodium azide does not seem to exceed 20 per cent.

TABLE III

Effects of Sodium Azide on Respiration

Experiment No.	NaN_3 concentration	Respiration (changes in micromoles CO_2 per 10 min.)		Percentage change in respiration
		Normal	With NaN_3	
	moles/liter			
1	1.6×10^{-6}	2.07	2.75	+33.0
1	3.6×10^{-6}	2.07	2.96	+43.0
1	5.9×10^{-6}	2.07	2.83	+36.0
3	6.6×10^{-6}	2.54	3.43	+35.0
2	7.9×10^{-6}	3.02	3.74	+23.7
2	10.5×10^{-6}	3.02	3.15	+4.2
3	19.6×10^{-6}	2.54	2.45	−4.0
3	35.0×10^{-6}	2.54	2.04	−20.0
3	45.9×10^{-6}	2.54	2.00	−21.3

TABLE IV

Effects of Sodium Azide on Photosynthesis

Experiment No.	NaN_3 concentration	Photosynthesis (changes in mieromoles CO_2 per 10 min.)		Percentage change in photosynthesis
		Normal	With NaN_3	
	moles/liter			
1	1.6×10^{-6}	5.20	4.83	−7.0
1	3.6×10^{-6}	5.20	3.67	−29.4
1	5.9×10^{-6}	5.20	2.88	−41.5
3	6.6×10^{-6}	6.28	3.13	−50.0
2	7.9×10^{-6}	5.96	3.02	−49.4
2	10.5×10^{-6}	5.96	2.10	−64.9
3	19.6×10^{-6}	6.28	1.54	−75.6
3	35.0×10^{-6}	6.28	0.19	−97.0
3	45.9×10^{-6}	6.28	0.00	−100.0

Inhibition by Hydroxylamine Hydrogen Chloride.—

Shibata and Yakushiji (7), and Nakamura (8) reported that the hydroxylamine-sensitive enzyme participates only in the oxygen-liberating stage of the photosynthetic process. The results are summarized in Tables V and VI. The light intensities used in these experiments were about 1.0×10^{-6} einstein per $cm.^2$ per minute.

Hydroxylamine causes no stimulation of respiration. There is also no large

inhibition of respiration at higher concentrations of hydroxylamine. The effect on photosynthesis is of the same magnitude as that of potassium cyanide. The residual photosynthesis below the compensation point is not inhibited, unless very high light intensity is used.

A delay in the action of inhibition is observed on the addition of hydroxylamine hydrogen chloride. As much as 20 minutes was required in some cases before the steady rate was reached.

TABLE V

Effects of Hydroxylamine on Respiration

Experiment No.	NH_2OH concentration	Respiration (changes in micromoles CO_2 per 10 min.)		Percentage change in respiration
		Normal	With NH_2OH	
	moles/liter			
1	3.0×10^{-5}	3.25	3.12	−3.9
1	5.9×10^{-5}	3.25	2.78	−14.2
1	8.9×10^{-5}	3.25	2.07	−36.2
2	11.8×10^{-5}	2.37	2.04	−14.1
3	14.8×10^{-5}	1.81	1.49	−17.5
2	17.7×10^{-5}	2.37	2.00	−15.4
3	20.7×10^{-5}	1.81	1.53	−15.8
2	23.6×10^{-5}	2.37	2.15	−9.4
3	26.6×10^{-5}	1.81	1.40	−22.8
4	29.6×10^{-5}	2.23	2.15	−3.6
4	35.5×10^{-5}	2.23	2.11	−5.0
4	41.5×10^{-5}	2.23	1.64	−25.7

TABLE VI

Effects of Hydroxylamine on Photosynthesis

Experiment No.	NH_2OH concentration	Photosynthesis (changes in micromoles CO_2 per 10 min.)		Percentage change in photosynthesis
		Normal	With NH_2OH	
	moles/liter			
1	3.0×10^{-5}	5.87	6.04	+3.3
1	5.9×10^{-5}	5.87	5.84	0.0
1	8.9×10^{-5}	5.87	4.77	−18.5
2	11.8×10^{-5}	5.20	4.88	−6.4
3	14.8×10^{-5}	4.99	3.95	−20.8
2	17.7×10^{-5}	5.20	3.65	−30.0
3	20.7×10^{-5}	4.99	2.84	−43.0
2	23.6×10^{-5}	5.20	2.99	−45.6
3	26.6×10^{-5}	4.99	2.59	−48.0
4	29.6×10^{-5}	4.49	2.57	−42.5
4	35.5×10^{-5}	4.49	2.04	−56.7
4	41.5×10^{-5}	4.49	1.70	−62.1

Inhibition by Dinitrophenol.—

Dinitrophenol is known to affect both photosynthesis and respiration. It is thought to act on enzymatically active proteins. Catalytically active protein

TABLE VII

Effects of Dinitrophenol on Respiration

Experiment No.	DNP concentration	Respiration (changes in micromoles CO_2 per 10 min.)		Percentage change in respiration
		Normal	With DNP	
	moles/liter			
1	1.9×10^{-5}	4.85	5.41	+11.8
2	3.8×10^{-5}	3.56	4.40	+23.2
3	5.7×10^{-5}	4.17	5.05	+21.4
1	7.6×10^{-5}	4.85	6.20	+27.9
3	9.5×10^{-5}	4.17	5.00	+20.2
1	17.1×10^{-5}	4.85	5.10	+5.3
4	19.0×10^{-5}	9.65	8.10	−15.8
5	28.5×10^{-5}	9.25	5.96	−35.5
4	38.0×10^{-5}	9.65	4.55	−53.0
4	57.0×10^{-5}	9.65	3.81	−60.5
6	66.5×10^{-5}	9.89	3.78	−62.1

TABLE VIII

Effect of Dinitrophenol on Photosynthesis

Experiment No.	DNP concentration	Photosynthesis (changes in micromoles CO_2 per 10 min.)		Percentage change in photosynthesis
		Normal	With DNP	
	moles/liter			
1	1.9×10^{-5}	8.79	8.34	−4.9
2	3.8×10^{-5}	6.61	6.37	−4.1
3	5.7×10^{-5}	7.55	7.38	−2.3
1	7.6×10^{-5}	8.79	8.21	−6.4
3	9.5×10^{-5}	7.55	7.31	−3.0
1	17.1×10^{-5}	8.79	7.62	−13.1
4	19.0×10^{-5}	20.30	6.90	−66.0
5	28.5×10^{-5}	19.75	4.42	−77.0
4	38.0×10^{-5}	20.30	3.01	−85.5
4	57.0×10^{-5}	20.30	2.63	−87.1
6	66.5×10^{-5}	19.00	3.78	−81.0

may be used to transfer hydrogen atoms; therefore, the inhibition effect of dinitrophenol on photosynthesis probably arises from inhibition of the transfer of hydrogen atoms from an intermediary reduction product to carbon dioxide.

The results are summarized in Tables VII and VIII. The light intensities

used in Experiments 1 to 3 were about 1.0×10^{-8} einstein per cm.^{2} per minute, and in Experiments 4 to 6, about 5.0×10^{-8} einstein per cm.^{2} per minute.

The effects of dinitrophenol on the rates of respiration and photosynthesis in *Chlorella* are quite different from those of the previous inhibitors. The rate of respiration is stimulated at low concentrations, but decreases sharply when the concentration of dinitrophenol reaches 10×10^{-5} moles per liter. The inhibition of respiration seems to level off near -60 per cent. The rate of photosynthesis, on the other hand, is not affected until dinitrophenol concentration reaches about 10×10^{-5} moles per liter. Then, with increasing dinitrophenol concentration, the rate of photosynthesis decreases rapidly. Although the curve seems to level off near 90 per cent inhibition, complete inhibition of photosynthesis, even below the compensation point, can undoubtedly be obtained at high concentrations of dinitrophenol.

A delay of about 10 minutes in the rates of respiration and photosynthesis was also observed in the action of dinitrophenol.

It has been suggested that dinitrophenol affects the rates of respiration and photosynthesis by reversibly inhibiting the coupling of phosphorylation. In the process, it apparently replaces inorganic phosphate, which is a necessary component of the system. Our results, however, do not fully agree with this suggestion. Although respiration is stimulated considerably at low concentrations of dinitrophenol, the rate of photosynthesis does not seem to be affected at this low concentration. When the rate of photosynthesis is decreasing sharply at a dinitrophenol concentration of about 10×10^{-5} moles per liter, the rate of respiration is decreasing sharply, too. Should the reversible inhibition of the coupling of phosphorylation be true, the maximum decrease in the rate of photosynthesis should occur at maximum stimulation of respiration.

DISCUSSION

The data presented in the previous sections are the results of at least three experiments on each inhibitor. During the measurements for each inhibitor, the concentration and age of the *Chlorella* cells were kept approximately the same in order to obtain nearly constant respiration rates. In the study of photosynthesis, about the same amount of light intensity was used in all the experiments. High light intensity was introduced only when needed to reveal the maximum amount of inhibition, as in the experiments with dinitrophenol. Usually, the light intensity was one to three times that required for respiration compensation.

Quantitative measurements of the effects of different inhibitors on photosynthesis and respiration are difficult. Furthermore, it is not possible to determine normal respiration rates for the correction of photosynthesis rates, after the inhibitor has been added. The accuracy is not limited by instrumentation, but mainly by the condition and concentration of the algal suspension. It is

difficult to have algal suspensions in different experiments with the same concentrations of cells and the same respiration rates. The results presented here, however, are self-consistent and give definite information concerning the effects of each inhibitor.

The intermediate steps in photosynthesis and in respiration are complicated and they include several enzymatic reactions. The amounts of inhibiting and accelerating materials added are so small that the effects which they produce must involve catalytic agents such as enzymes. These effects are shown to be quite specific and since they often are not the same for photosynthesis and respiration, it is hoped that it may be possible eventually to contribute to our understanding of these phenomena and to distinguish between the mechanisms which are common to both and the steps which involve photosynthesis alone or respiration alone.

SUMMARY

The effects of several inhibitors on the rates of photosynthesis and of respiration by *Chlorella pyrenoidosa* have been studied. The inhibitors used in this study include potassium cyanide, hydroxylamine hydrogen chloride, dinitrophenol, and sodium azide. Each inhibitor seems to have its own characteristic action in photosynthesis and in respiration. With the exception of hydroxylamine, all the inhibitors show a stimulation of respiration at low concentrations of the inhibitors. Only dinitrophenol inhibits respiration to a marked extent. Potassium cyanide, hydroxylamine, and dinitrophenol are about equally effective in inhibiting photosynthesis, but sodium azide is nearly ten times as effective.

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