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ROLE OF THIOCTIC ACID IN THE HILL REACTION

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Berkeley, California

THE PATH OF PHOTONS IN PHOTOSYNTHESIS. I
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It has recently been proposed[1] that the primary conversion of electromagnetic energy to chemical bond energy in photosynthesis is mediated by 6-thioctic acid (6,8-dithiooctanoic acid, 6 T).**** Because of the strain in its five-membered ring, this molecule provides a chemical bond which might be broken by a ca. 30 kcal/mole quantum transferred to it from the excited state of chlorophyll. The resulting diradical then abstracts hydrogen from a donor derived from water, forming a dithiol, 6 DT, and a moiety whose oxidizing power is ultimately the source of molecular oxygen. The dithiol may subsequently transfer its hydrogen to a suitable acceptor such as DPN[2] or FAD, distributing the reducing power and regenerating the cyclic disulfide, 6 T. Energy of the quantum is conserved in this process provided that the reduction potential of the hydrogen acceptor exceeds that of the original donor. From established thermodynamic considerations, at least four of these 6 T "cycles" must occur for the net reduction to carbohydrate of a molecule of carbon dioxide, and it is therefore probable that subsequent reactions in the sequence would involve reoxidation of the reducing hydrogen and storage of the energy in some chemical form such as high energy phosphate.[2,3,4]

If 6 T is operating in the manner described, it should be possible to realize conditions under which synthetic 6 T added to the plant could be utilized to stimulate the quantum conversion, resulting in a net higher rate of photosynthesis. The demonstration of such a stimulation in the Hill reaction in which the photochemical apparatus is experimentally separated from the carbon dioxide reduction system would provide even more satisfactory confirmatory evidence for the proposed mechanism of quantum conversion.

This paper reports a stimulation of the Hill reaction by 6 T in cellular chloroplast preparations[5] of the unicellular alga Scenedesmus under conditions which are highly specific with respect to 6 T, the organism involved, and the physical conditions of the experiment. These conditions are completely consistent with what would be expected from the theory described above.

Materials and Methods

Scenedesmus obliquus, Chlorella pyrenoidosa and Synechococcus cedorum were grown in continuous culture under conditions which have been described previously,[6,7] and were harvested daily. The algae were centrifuged (1900 x g) and washed with distilled water four times, and resuspended in M/15 K-phosphate + M/100 KCl buffer (pH = 6.7)[8,9] to the desired suspension density, generally 20 mm^3 wet packed cells/ml. The pH of the resulting suspension was identical with that of the original buffer. Scenedesmus which were stored at 6° C. in the dark for 48 hours suffered no loss in the rate of quinone-dependent, photochemical oxygen production.

Quinone was purified by sublimation (m.p. = 113-5) and stored at -10° C. in the dark. Solutions of 1-12 mg/ml in distilled water were stored in red glass vessels in the dark and used within four hours after preparation. Solutions of dl-6 T (cryst.), dl-5 T (cryst.), dl-4T (cryst.), 6 DT (oil), and 6 MO (oil) were prepared in M/3 phosphate buffer (pH = 6.7) in concentrations of 0.25-1.0 mg/0.05 ml.*****

Standard double side arm Warburg flasks were used. All reactions were carried out at 15.7° C. The light field was supplied by seven General Electric photospots and was homogenized by passing the beams through a water-cooled layer of 2 1/2 mm. glass beads. The resulting field (26,000 lux) varied by less than 4% throughout the entire thermostat.

Light screens were made from brass screening which was wrapped around the vessels. The screens were calibrated against both a Cary Recording Spectrophotometer and a General Electric Photovoltaic cell for relative transmission. There was no selective transmission and the results of the two methods agreed within 2%.

Chlorophyll content was determined by centrifuging aliquots of the suspension and extracting the centrifugate with aqueous ethanol. The visible absorption spectrum of the extract was determined on a Cary Spectrophotometer, and the concentration of chlorophyll (chl a) calculated from the transmission at the 662 mμ. absorption maxima (ϵ_{max} assumed 9×10^4 liter $mole^{-1}$ cm^{-1}), mean deviation of duplicate runs being 1.1%.[10]

The standard pattern for the experiments was as follows: 2.0 ml. of the algal suspension was transferred to each manometer flask, which contained 0.2 ml. 20% KOH and a pleated filter strip in the center well. The vessels were then allowed to stand aerobically in the dark for one hour, at which time 0.5 ml. quinone solution was added to a side arm of each vessel, 0.05 ml. 6 T solution to each experimental sample of algae, and 0.05 ml. M/3 phosphate buffer to each control algal sample. The vessels were then shaken for ten minutes aerobically in the dark, followed by 10 minutes of flushing with commercial N_2. Ten minutes later, when pressure equilibrium had been established, the quinone solutions were tipped into the algae, and after fifteen minutes of anaerobic dark reaction the lights were turned on.[8]

Experimental

Illuminated Scenedesmus cells consistently produced photochemical oxygen with quinone as oxidant, corresponding to 83.4 ± 3% of the stoichiometric yield.[8,11] The rate curves were qualitatively similar to those obtained by previous investigators with Q values (mm^3O_2/hr/mg chl a) of approximately 2000.[11] The cells were completely unable to evolve oxygen with carbon dioxide as oxidant subsequent to completion of the Hill reaction.[8] The pH of the suspensions were changed neither by completion of the Hill reaction nor addition of 6 T solution, M/3 phosphate buffer, or quinone by more than 0.02.[11]

When 0.25-0.50 mg. of 6 T was incubated with the algae prior to quinone addition the effect (6 T effect) on the subsequent rate varied from two-fold stimulation to nearly complete inhibition. These variations could be correlated with the dependence of the control rate on the concentration of quinone. At low values, when the rate increased with increasing quinone concentration, the 6 T effect was consistently stimulatory (44 experiments). At high concentrations, when the rate was self-inhibited by quinone and/or its photoproducts, the 6 T effect was an increase in the inhibition (17 exp.).[8,12] At intermediate concentrations no effect (rate within 5% of control) was observed. The numerical concentrations at which each of these effects appeared in turn depended upon the light intensity, suspension density, chlorophyll content, incubation conditions, and culture conditions as described below.

There was no significant alteration in the yield of oxygen in the presence of 6 T. Table I which summarizes the data on Scenedesmus in all experiments where quinone was not self-inhibitory indicates that the amount of oxygen attributable to 6 T is less than 5% of what would be expected if it were acting as a Hill oxidant (54 mm^3/mg 6 T). Such a result is consistent with separate

experiments in which 1 mg. 6 T was incubated with algae (in the absence of quinone to inactivate them photosynthetically) and also with quinone in the absence of algae. The subsequent yields of photochemical oxygen were 12 and 3 mm^3O_2/mg 6 T respectively.

Chemical Specificity

If the observed rate effects are the result of a change in the concentration of the 6 T quantum converter within the cell, and not merely a chemical artifact, addition of the 4 T and 5 T which do not possess the ring-strain energy required for quantum conversion should have no effect.[1] 6 MO and 6 DT which might be easily converted to 6 T biosynthetically,[13] however, might behave in a manner similar to 6 T. In a standard dark incubation experiment, under conditions in which 6 T gave 50% stimulation, neither 5 T, 4 T, 6 MO nor 6 DT in the same molar concentration (0.0005 M) produced any effect. (Figure 1A.)

To test whether more active metabolism would facilitate conversion of 6 DT and 6 MO to photoactive 6 T, incubation was carried out aerobically in light (26,000 lux) for 1/2 hour. The subsequent rates were essentially identical with the dark incubated rates, with the exception of a small stimulatory effect by 6 DT after light incubation. (Figure 1B.)

Although there was no appreciable alteration of the rate by 6 DT, the average yield was 13 ± 3 mm^3O_2/0.25 mg. 6 DT less than in the controls (4 exp). The decrease calculated for a one-to-one reaction of 6 DT and quinone, such as a dark reduction of the latter, is 13.4 mm^3O_2/0.25 mg. 6 DT. A non-reversible reaction with quinone which inactivates the dithiol-disulfide system may explain the lack of Hill activity of 6 DT.

Thioctic Acid Concentration and Metabolic Requirement

The total of free and combined forms of 6 T in Scenedesmus is quite small, approximately 10^{-8} gms/40 mm^3 cells,[14] and presumably the photactive form in the "photosynthetic unit"[15,16] is only a small fraction of that amount. Although in principle it should require only a few-fold increase in the amount of the photoactive form to give rise to the observed effects on the Hill reaction, the stimulation in a typical experiment drops off rapidly when the amount of added 6 T is less than 10^{-4} gm/40 mm^3 cells. (Figure 2.)

There is evidence that in its other metabolic functions, 6 T is present in specific bound forms, such as lipothiamide pyro-phosphate.[17] This may be the case when it functions in the quantum conversion process, and the biosynthesis of such a form from externally supplied 6 T might be slow and require both oxidative metabolism and the relatively high concentration of exogenous 6 T actually observed.

To test the possible dependence of the 6 T effect upon oxidative metabolism equal aliquots of 6 T solution were added to algae at the beginning of the dark adaptation period, just before the dark aerobic shaking, just after the N_2 flush, mixed with quinone, and ten minutes after quinone addition. The preparatory reactions are essentially complete within 10 minutes in air, but are not accomplished in N_2 or by quinone killed algae, supporting the concept that the molecule must be metabolically prepared for its photactive function.

Quinone Concentration

Although the control rates varied considerably with the particular harvest of algae, certain characteristics of the effect of the quinone concentration on the 6 T effect were common to most samples tested. (Figure 4.) At sufficiently low quinone concentrations the control rate varied directly though not

linearly with the quinone concentration. It is in this concentration range that the 6 T effect is stimulatory, lower concentrations yielding higher percentage but less accurately measured stimulations. As the concentration of quinone in the control is increased, a maximum rate is obtained; higher concentrations of quinone result in lower rates.[8] When the quinone concentration is increased in 6 T incubated algae, a maximum rate is also obtained, but at a somewhat lower quinone concentration and with a more rapid decrease in rate at higher concentrations than in the control. An important characteristic of the 6 T effect is that the maximum rate with 6 T is nearly identical to the maximum without it.

The existence of quinone-saturated rates indicates that there are one or more quinone independent rate-determining steps in the Hill reaction sequence. If 6 T were to act on one or more of these other slow steps, then it should yield, assuming microscopic reversibility of the reactions, a maximum stimulated rate considerably higher than that obtainable by saturating the quinone-dependent rate-determining reaction alone. If on the other hand 6 T acts upon the quinone dependent step, the maximum stimulated and maximum control rate should be nearly identical, the former possibly slightly higher if at these concentrations the quinone partially inhibits some other reaction in the sequence. The available evidence therefore supports the idea that 6 T acts upon the quinone dependent step.

Mechanism of Inhibition

Although the inhibitory effects of high quinone concentrations and pre-illuminated quinone are established observations,[8,11,12] there is at present little information as to the chemical species involved. If 6DT is the direct reductant of quinone in the Hill reaction, a photolysis product of quinone of

somewhat similar structure might enter into a non-reversible reaction with the natural 6 T destroying the quantum conversion mechanism and possibly inhibiting other reactions in the Hill reaction sequence.

To test the effect of photolytic products of quinone on the 6 T effect, aliquots of quinone solution and quinone plus 6 T were illuminated in nitrogen for thirteen minutes before use in the standard experiment (Figure 5). Illumination of the quinone produced the previously-described inhibition.[8,11] While the concentration of quinone was low enough to yield a two-fold 6 T stimulation in the control preillumination of the quinone changed the effect to nearly complete inhibition. When quinone and thioctic acid were preilluminated together, however, the inhibition was less than with quinone preilluminated alone. Incubation with 6 T thus does sensitize the cells in some way to inhibition by photolyzed quinone and it is probable that the observed instances of an inhibitory 6 T effect at high quinone concentrations result from such an interraction.

It may be well to point out that the observed inhibitions were not caused by a net destruction of the quinone. When the latter was illuminated a broad u.v. absorption band slowly appeared whose optical density increased approximately linearly with time. The optical density after 13 minutes was found to be less than 1% of the value after 90 minutes in the standard light field followed by 90 minutes in the light from a bank of black lights (u.v.). The most obvious conclusion is that the fraction of quinone destroyed in the preillumination period was also less than 1%. The photoproducts would then be toxic in a concentration of $<4 \times 10^{-5}$ M.

Light Intensity

The effect of light intensity on the 6 T effect is closely related to its role in the photolysis of quinone and as a partially rate-determining step in

the Hill reaction sequence.[18] The former effect results in inhibitory 6 T effects at very high intensities.[12,19]

The second effect is exactly what would be expected from a partially rate-controlling step which is independent of 6 T. An increase in the quantum capture rate induced by an increase in the light intensity will increase the dependence of the net Hill rate on the quinone concentration. Saturation of the capture process at higher light intensities corresponds to the greatest dependence of the Hill rate upon quinone concentration and therefore coincides with the greatest percentage stimulations by 6 T. In several experiments a third rate-determining step, as yet unidentified, proved slower than either the quinone-dependent or quantum capture steps and since in these instances light saturation did not correspond to quinone dependence no stimulatory 6 T effect was observed.

Although saturation of the capture process was not a sufficient condition for stimulation, it was a necessary one. After conditions were found which yielded a stimulatory 6 T effect, the incident light intensity was reduced to 33%, resulting in lower percentage stimulations or slight inhibition. The existence of these inhibitions indicates that the net 6 T effect is the resultant of both a stimulatory and an inhibitory effect which have different dependences on the quinone concentration and light intensity (Figure 6).

Suspension Density

The quantity of cells present in the manometer vessels determines the quantitative dependence of the control rate on light intensity and quinone concentration, and therefore the 6 T effect, in several ways.[18] At low suspension densities the Hill rate becomes saturated with respect to both quinone and light, and there is no 6 T stimulation. If the concentration of quinone is sufficiently

high the photolysis products formed result in 6 T inhibition. As the density is increased, an increasing fraction of the incident light is captured, the average light absorbed per alga decreased, the dependence of the net Hill rate on light increased, and the net effect is to decrease the possibility of a stimulatory 6 T effect. On the other hand, while at a particular algae density the relative quinone concentration determines quinone dependence, the quinone-algae ratio determines the actual concentration range of this dependence. Hence the dependence of the control rate on quinone increases with the suspension density, and an increase in the latter increases the possibility of a stimulatory 6 T effect.

The observed 6 T effect is the resultant of these two opposing effects whose magnitudes are sensitive functions of the physiological state of the algae. Fortunately, under the present culture conditions, Scenedesmus becomes quinone dependent before light dependent as the suspension density is increased, and a region of net stimulatory 6 T effect is observed at intermediate densities, with no effect at higher and lower densities (Figures 7A and B).

Culture Conditions and Chlorophyll Content

The physical conditions under which the algae are cultured in part determines the relative contributions of the rate determining steps in the Hill reaction. As these conditions are adjusted to produce algae with a relatively low chlorophyll content, it becomes more difficult to saturate the quantum capture step, the algae become light dependent more rapidly than quinone dependent, and the net effect is that at no algae density is a stimulatory 6 T effect observed. Such "pale algae" (30-75% of the standard, ca. 6×10^{-3} mg. chl a/mm^3 cells) were developed by decreasing the innoculum size (to ca. 10%) following each harvest, and not allowing the culture cell density to become large (standard

ca. 4 mm^3/ml.). Under these conditions the algae developed at a higher average light intensity because of a decrease in the light shielding by other algae in the light path. Such algae appeared to adjust to their environment by developing an efficient quantum conversion system at the expense of the capture system and failed to exhibit either light saturation or 6 T stimulation of the Hill reaction under the present experimental conditions.[20]

When the algae were cultured under low incident light intensities (33% standard) they appeared to adapt to a lower rate of photosynthesis and become saturated with respect to both quinone and light at the lowest convenient concentration of the former (0.8 mg/40 mm^3 cells), and thus exhibit no stimulatory 6 T effect.

Other Biological Systems

If the quantum conversion theory possesses the generality which it claims, it should be possible to realize stimulatory 6 T effects in other plants. However, Chlorella pyrenoidosa cultured under the same light and gas phase conditions as Scenedesmus repeatedly failed to exhibit any but inhibitory or nil 6 T effects (Figure 8). These algae developed considerably less chlorophyll/mm^3 cells than Scenedesmus under the same culture conditions (ca. 4×10^{-3} mg chl a/mm^3 cells), and behaved in a manner similar to "pale" Scenedesmus, being quinone independent and, generally, highly light dependent. Stimulatory thioctic effects are not to be expected under these conditions, and we have as yet been unable to find growth conditions which develop Chlorella with a sufficiently high chlorophyll content to obtain quinone dependence and light saturation under the present Hill conditions.

Several experiments with Synechococcus cedorum which were cultured under lower light intensities also resulted in inhibitory 6 T effects. As in the

case of "pale" Scenedesmus and Chlorella the control rates were quinone saturated and light dependent.

Discussion

There are many a priori possible mechanisms by which 6 T may affect the Hill reaction. Fortunately it is possible to eliminate a number of them on the basis of the data available. The experiments with illuminated quinone solutions give strong support to the idea that an interaction between one or more quinone photoproducts and 6 T to produce an extremely toxic material, possibly the same moiety which is active in ordinary "self-inhibition" by quinone, is primarily responsible for the observed instances of 6 T inhibition.

It is also possible to draw rather general conclusions as to the mechanism responsible for the observed instances of stimulation. The failure to observe oxygen evolution when 6 T is incubated separately with either algae or quinone rules out the possibility of any simple two-component process. The fact that 6 T does not contribute to the oxygen yield of quinone further indicates that it is not acting as a Hill oxidant in the ordinary sense, and that the stimulatory effect is not simply the result of an increase in the total concentration of oxidants at the site of reduction. In addition, it is unlikely that the effect involves either a pH change, or a reversal of self-inhibition by quinone since under conditions in which the latter is considerably self-inhibitory 6 T is more so.

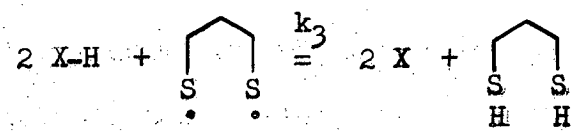
As has been discussed previously, the fact that the maximum thioctic stimulated rate is nearly identical with the maximum control rate indicates that 6 T acts on the quinone dependent step in the Hill sequence. Presumably, the only reactions which are quinone dependent involve either transport of

the quinone to the reduction site or the actual reduction process itself. It seems unlikely, for example, that the systems for evolving oxygen or transporting hydrogen to the chloroplast would depend on the quinone concentration. It seems equally improbable that 6 T would stimulate the transport of quinone to the reduction site, which appears to be very close to the chlorophyll.[21] If then, the 6 T effect is on the reduction process itself, either a change in the actual mechanism or an increase in the concentration of the reductant at the reduction site may be responsible for the observed stimulation. Such an increase in reductant concentration could be brought about if 6 T acted as a hydrogen transport system from a primary reductant associated with the chloroplast to the quinone reduction site. Receiving hydrogen from reduced DPN, carrying it to the reduction site, and reducing the quinone directly might be the 6 T function.[2] Such a function would depend upon the unique character of the 6 T five-membered ring since 4 T and 5 T are inactive. It is difficult, however, to reconcile this mechanism with the apparent resistance of the 6 T to net reduction as a Hill oxidant, together with the experimental observation that when it acts in pyruvic acid oxidation it functions in the reverse manner, i.e. the 6 DT reduces oxidized DPN.[22] Finally, the apparent absence of soluble reduced DPN in illuminated washed chloroplasts which reduce quinone is evidenced by their failure to activate the malic enzyme system.[23,24,25] The latter evidence indicates that pyridine nucleotides are not required for reduction of quinone in chloroplasts.

The quantum conversion theory provides a unique mechanism by which the quinone reductant concentration may be increased by 6 T. The process involves a sequence of reactions such as:

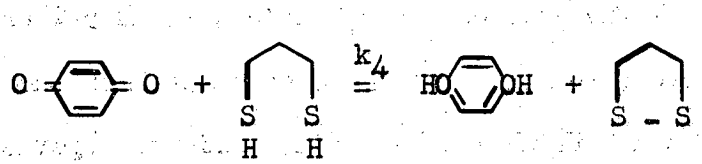
$$\mathrm{Chl} + h\nu \overset{k}{=} \mathrm{Chl}^{*}$$ (light absorption and quantum transformation and migration.)

$$\mathrm{Chl}^{*} \overset{k_2}{=} \mathrm{Chl}$$ (non-productive decay)



$$X \longrightarrow Y \longrightarrow Z \longrightarrow \ldots\ldots = O_2$$

$$H_2O \longrightarrow A \longrightarrow B \longrightarrow C \longrightarrow \ldots\ldots = XH$$



in which the hydrogen donor, XH, is produced by a series of reactions from water; the oxidized product, X, is ultimately the source of oxygen evolved; and non-productive decay includes fluorescence, non-radiative thermal decay to the ground state, etc. 6 T provides the rather unique function of increasing the reduction level of the transferred hydrogen by an amount corresponding to some fraction of the energy difference between the oxidized form and the diradical, the remaining fraction being used to raise the oxidation level of the oxygen-containing residuum, X.

The rate laws associated with this process may be formulated as:

$$\frac{d\ chl^*}{dt} = k(I)(chl) - k_1(chl^*)(S\text{-}S) - k_2(chl^*)$$

$$\frac{d\ S\text{-}S}{dt} = k_4(SH)(Q) - k_1(chl^*)(S\text{-}S)$$

$$\frac{d\ S\cdot}{dt} = k_1(chl^*)(S\text{-}S) - k_3(X\text{-}H)^2(S\cdot)$$

$$\frac{d\ SH}{dt} = k_3(X\text{-}H)^2(S\cdot) - k_4(Q)(SH)$$

$$\frac{d\ Q}{dt} = -k_4(Q)(SH)$$

where (I) is the incident light intensity, (S-S) the concentration of 6 T, (SH) that of 6 DT, (S·) that of the 6 TR, (Q) that of quinone. For convenience, $m \equiv (S\text{-}S) + (S\cdot) + (SH)$.

The observed dependence of 6 T stimulation effect upon light intensity and quinone concentration is consistent with the proposed model. The general form of this dependence may be seen without resorting to an exact solution of the differential rate equations in the restricted case of the steady state. Under this restriction the concentration of all intermediates remains constant throughout the course of the reaction, and in addition $dQ/dt = -2dO_2/dt$. Since the concentration of quinone decreases continuously as the reaction proceeds, this steady state assumption cannot be completely valid. However, since m is several orders of magnitude smaller than the quinone concentration, the system will adjust itself to changes in quinone concentration extremely rapidly, and conclusions deducted on the steady state model will apply for each manometer reading, although not a sequence of them.

The effect of added 6 T, i.e. an increase in m, depends upon the value of K, defined as

$$\frac{k_1(S\text{-}S)}{k_2 + k_1(S\text{-}S)} \equiv K$$

and which corresponds to the fraction of quanta used productively. Whenever K is significantly less than unity additional 6 T will stimulate the net reaction. At sufficiently low light intensities the net rate will become completely light dependent, and the steady state values of (S•) and (SH) will become small so that (S-S) ~m. (A quantitative analysis may be found in the Appendix.) If $k_1m \gg k_2$ then, K is nearly unity and there will be no 6 T effect. If $k_1m \ll k_2$, K will approach zero, and the 6 T effect will be stimulatory. As the light intensity is increased indefinitely, some other reaction such as quinone reduction will become partially rate determining, (S•) and (SH) will become appreciable and K will decrease. Increasing m by added 6 T will increase (S-S) by a fraction of m which depends on the actual values of the rate constants involved, K will increase and the thioctic effect will be stimulatory. 6 T increases (S-S) by speeding up the assumed rate determining step, k_4(SH)(Q) and ∴ an increase in quinone will have the same effect.

If K at quinone saturation is unity then the maximum thioctic stimulated rate will equal the maximum quinone stimulated rate. If not, then 6 T will result in a higher maximum rate, corresponding to the direct stimulation of two partially rate determining steps, quantum conversion and quinone reduction. The observed correspondence of the two rates indicates that it is only the latter reaction which is being stimulated directly and that therefore at quinone saturation there is negligible non-productive decay.

As there is no a priori reason that the quinone reduction be a slower reaction than diradical reduction, it would not be surprising to find that the physiological state of the particular plant sample which determines the relative rate constants of these reactions would also determine the dependence of the 6 T effect on quinone concentration. It is, of course, in principle possible to observe 6 T stimulation if quinone is not rate determining provided that the diradical reduction may be stimulated by an increase in (S•). If the reduction of XH is absolutely limited, however, then the 6 T effect cannot be stimulatory. Therefore, quinone dependence is a sufficient but not necessary condition for stimulation, while light saturation is not a sufficient condition and only a necessary one in the case of a naturally low quantum decay rate.

Summary

1. Conditions have been realized under which the Hill reaction by cellular chloroplasts of Scenedesmus is stimulated by prior incubation with synthetic 6-thioctic acid.
2. The conditions for stimulation are sensitive functions of the oxidant concentration, 6-thioctic acid concentration, light intensity, suspension density, chlorophyll content of the algae, culture conditions, and incubation condition.
3. The three most general experimental conditions for stimulation are: a) dependence of the Hill control rate on oxidant concentration, b) saturation of the control rate with respect to light intensity, and c) incubation of algae with 6-thioctic acid aerobically prior to oxidant addition. The second and third conditions are necessary, the first is sufficient.
4. Under conditions in which quinone is self-inhibitory, the 6-thioctic acid is also inhibitory.
5. The observed stimulatory effects are consistent with the proposal that 6-thioctic acid is the primary quantum converter in photosynthesis.

Appendix

In the steady state solution R, defined as,

$R \equiv$ net steady state rate of Hill reaction

is also equal to the rate of every individual reaction in the sequence. We may therefore express the concentrations of the reactants in terms of R.

$$R = k_4(SH)(Q)$$

$$R = k_3(XH)^2(S\cdot)$$

$$\text{and} \quad R = k'K \equiv \frac{k'k_1(S\text{-}S)}{k_2 + k_1(S\text{-}S)}$$

where $k' \equiv kI(chl)$

Using the additional relation

$$m = (S\text{-}S) + (S\cdot) + (SH)$$

(SH), (S·) and (S–S) may be eliminated and R expressed as a function of m:

$$R = \frac{m + \frac{k_2}{k_1} + \frac{k'}{k_4(Q)} + \frac{k'}{k_3(XH)^2} \pm \sqrt{\left(m + \frac{k_2}{k_1} + \frac{k'}{k_4(Q)} + \frac{k'}{k_3(XH)^2}\right)^2 - \frac{4\,mk'}{k_4(Q)} - \frac{4\,mk'}{k_3(XH)^2}}}{\frac{2}{k_4(Q)} + \frac{2}{k_3(XH)^2}}$$

The physically significant root may be expanded in a power series to yield, for the leading terms,

$$R \cong \frac{mk'}{m + \frac{k_2}{k_1} + \frac{k'}{k_4(Q)} + \frac{k'}{k_3(XH)^2}} + \frac{\left(\frac{1}{k_4(Q)} + \frac{1}{k_3(XH)^2}\right)m^2k'^2}{\left(m + \frac{k_2}{k_1} + \frac{k'}{k_4(Q)} + \frac{k'}{k_3(XH)^2}\right)^3} + \cdots$$

The limiting cases of interest are summarized below:

limit	R
$Q \longrightarrow 0$	$k_4 mQ$
$\begin{cases} Q \longrightarrow \infty \\ (XH)^2 \longrightarrow \infty \end{cases}$	$\dfrac{mk_1k'}{mk_1 + k_2}$
$I \longrightarrow 0$	$\dfrac{mk_1k'}{mk_1 + k_2}$
$I \longrightarrow \infty$	$\dfrac{m}{\dfrac{1}{k_4(Q)} + \dfrac{1}{k_3(XH)^2}}$
$m \longrightarrow 0$	$\dfrac{mk'}{\dfrac{k_2}{k_1} + \dfrac{k'}{k_4(Q)} + \dfrac{k'}{k_3(XH)^2}}$
$m \longrightarrow \infty$	k'

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Footnotes

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(****) The following abbreviations will be used throughout this paper: 6 T, 6,8-dithiooctanoic acid; 6 DT, 6,8-dithioloctanoic acid; 6 MO, 6,8-dithiooctanoic acid monoxide; 6 TR, 6,8-dithiyloctanoic acid; 5 T, 5,8-dithiooctanoic acid; 4 T, 4,8-dithiooctanoic acid; DPN, diphosphopyridine nucleotide; FAD, flavin adenine dinucleotide.

(*****) We are indebted to Dr. T. H. Jukes of Lederle Laboratories for making samples of synthetic 6 T, 6 DT, 6 MO, 5 T, 4 T available to us for this investigation.

Table I

Contribution of 6 T to oxygen evolution

mm^3O_2/mg quinone (controls, 67 exp.)	86.1
mean deviation	3.2
standard error of the mean	0.5
mm^3O_2/mg quinone (+ 6 T, 54 exp.)	87.0
mean deviation	3.2
standard error of the mean	0.6
mean mg 6 T/mg quinone (+ 6 T)	0.36
Stoich. mm^3O_2/mg quinone if 6 T is oxidant	19.6
Observed "	0.9 ± 0.7

Captions to Figures

Fig. 1 - Chemical specificity of 6 T. 40 mm^3 Scenedesmus and 1.5 mg. quinone per vessel. 0.25 mg. 6 T, 6 MO, 6 DT when used. A) Standard dark incubation, B) Light incubation.

Fig. 2 - 6 T effect as a function of 6 T concentration. 30 mm^3 Scenedesmus and 1.4 mg. quinone per vessel. Standard dark incubation.

Fig. 3 - 6 T effect as a function of incubation conditions. 40 mm^3 Scenedesmus and 1.5 mg. quinone per vessel. 0.25 mg. 6 T when used.

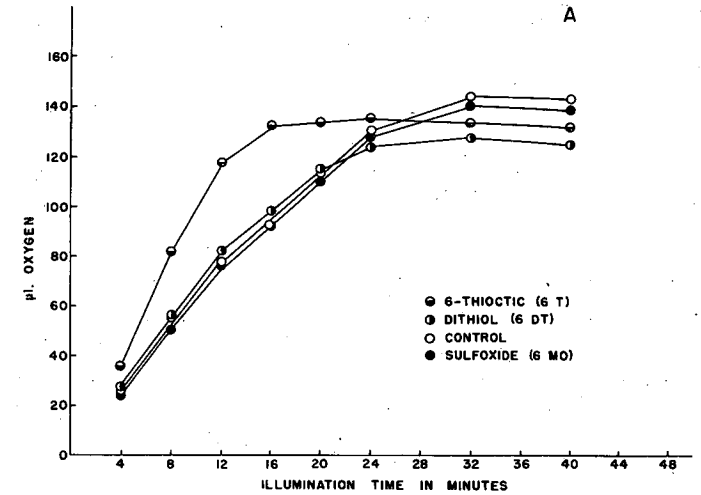
Fig. 4 - 6 T effect as a function of quinone concentration. 40 mm^3 Scenedesmus per vessel. 0.25 mg. 6 T when used. Standard dark incubation.

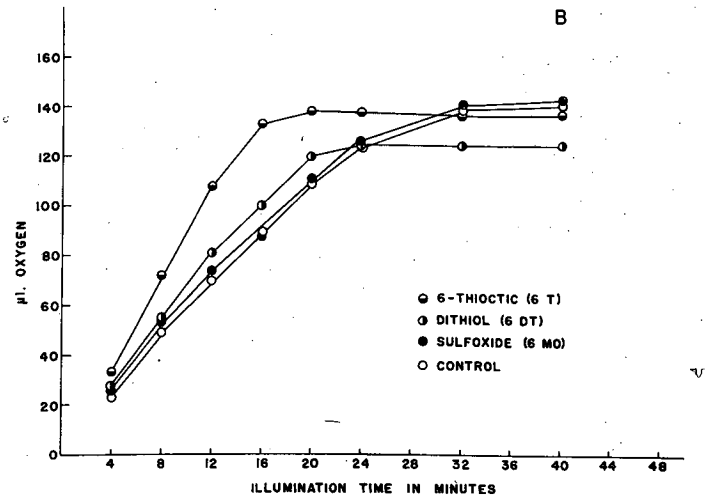
Fig. 5 - 6 T effect as a function of quinone preillumination. 20 mm^3 Scenedesmus and 1.5 mg. quinone per vessel. 0.5 mg. 6 T when used.

Fig. 6 - 6 T effect as a function of light intensity. 40 mm^3 Scenedesmus per vessel. 0.5 mg. 6 T when used. Standard dark incubation.

Fig. 7 - 6 T effect as a function of suspension density. Scenedesmus and 1.5 mg. quinone per vessel. A) 0.25 mg. 6 T when used, B) 0.5 mg. 6 T when used.

Fig. 8 - 6 T effect in Chlorella. 20 mm^3 cells per vessel. 0.5 mg. 6 T when used. Standard dark incubation.

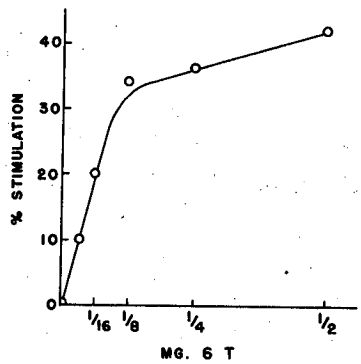




Chemical specificity of 6 T. 40 mm^3 *Scenedesmus* and 1.5 mg. quinone per vessel. 0.25 mg. 6 T, 6 MO, 6 DT when used. A) standard dark incubation. B) light incubation.

MU-5173

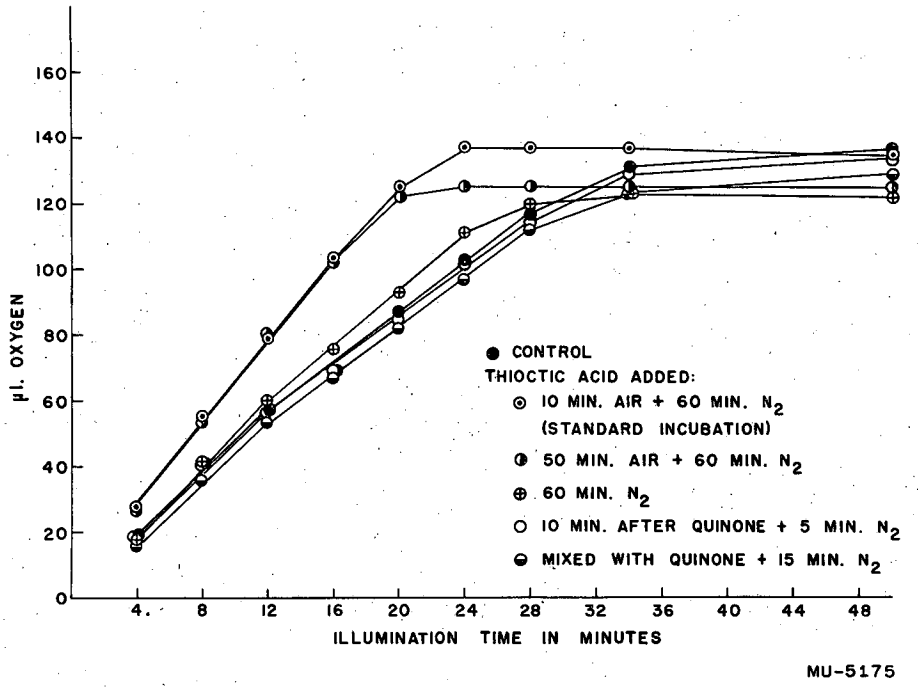
Fig. 1



6 T effect as a function of 6 T concentration. 30 mm^3 *Scenedesmus* and 1.4 mg. quinone per vessel. Standard dark incubation.

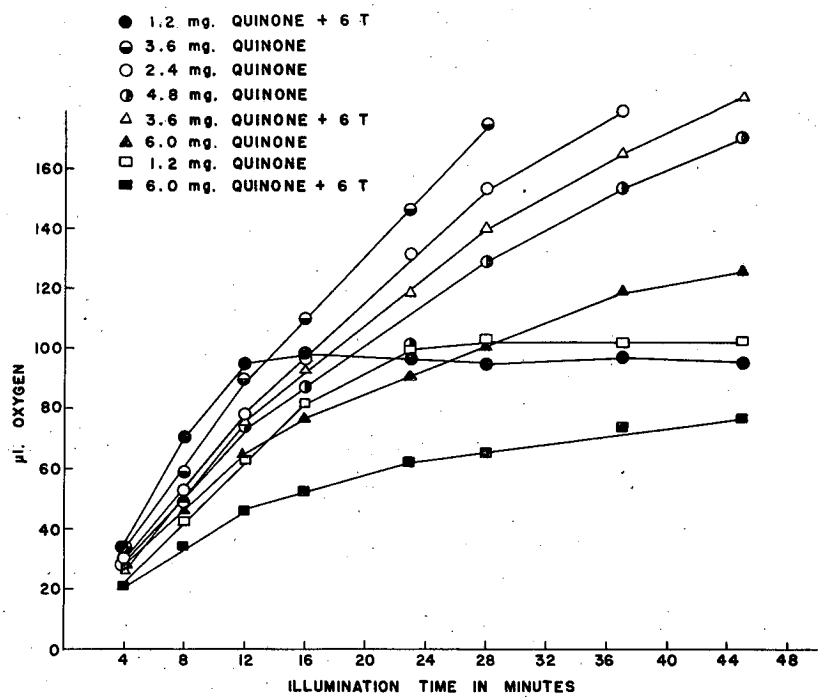
MU-5177

Fig. 2



6 T effect as a function of incubation conditions. 40 mm^3 *Scenedesmus* and 1.5 mg. quinone per vessel. 0.25 mg. 6 T when used.

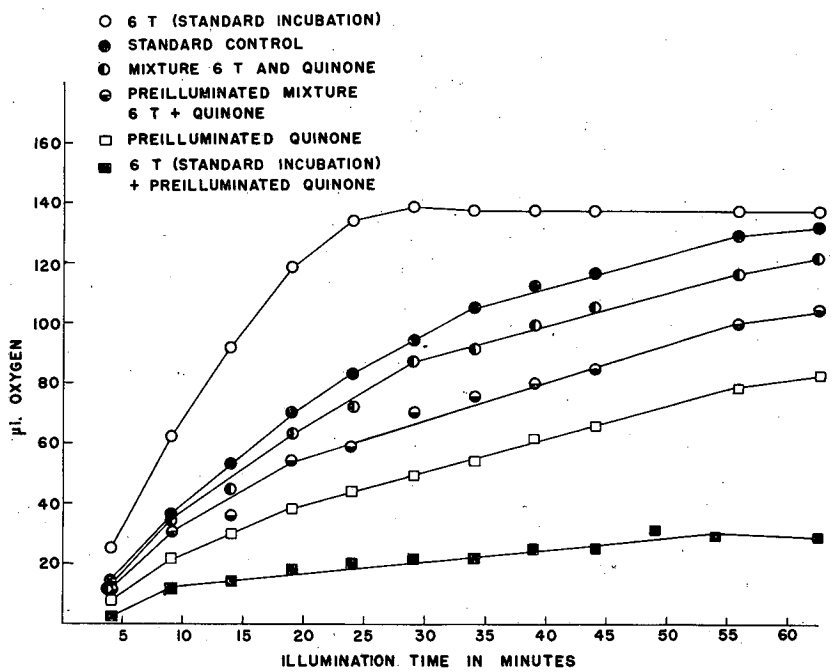
Fig. 3



6 T effect as a function of quinone concentration. 40 mm^3 *Scenedesmus* per vessel. 0.25 mg. 6 T when used. Standard dark incubation.

MU-5176

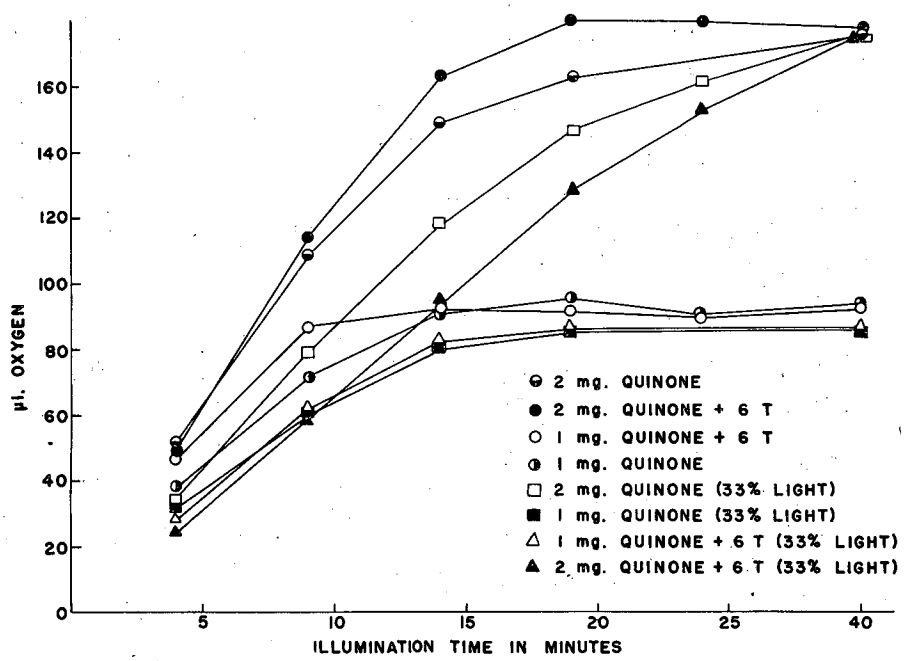
Fig. 4



6 T effect as a function of quinone preillumination. 20 mm^3 *Scenedesmus* and 1.5 mg. quinone per vessel. 0.5 mg. 6 T when used.

MU-5178

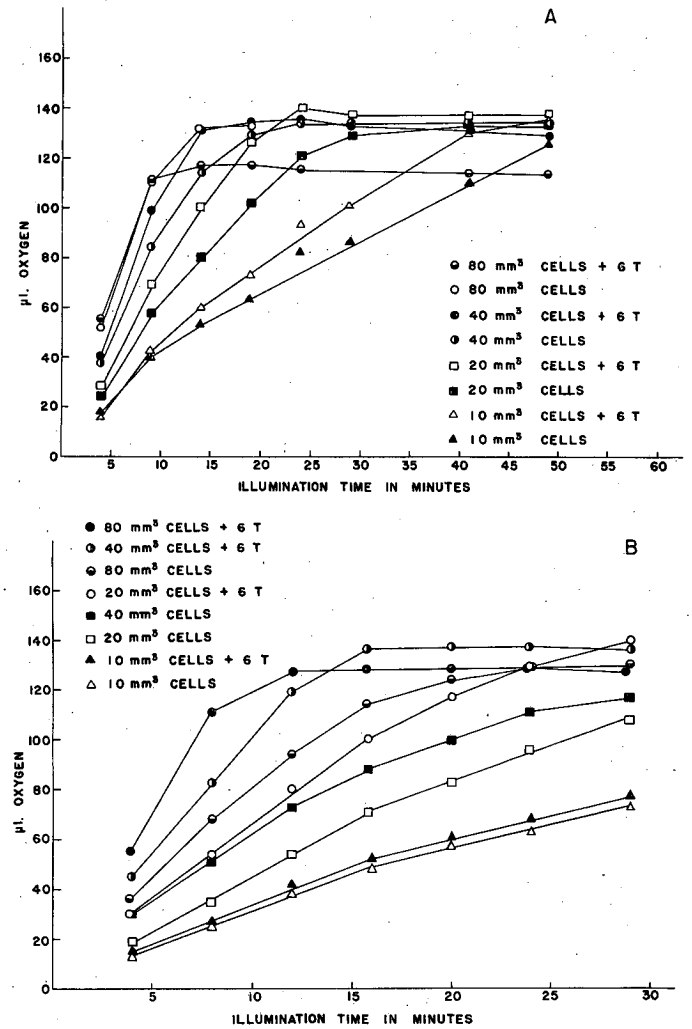
Fig. 5



6 T effect as a function of light intensity. 40 mm^3 *Scenedesmus* per vessel. 0.5 mg. 6 T when used. Standard dark incubation.

MU-5179

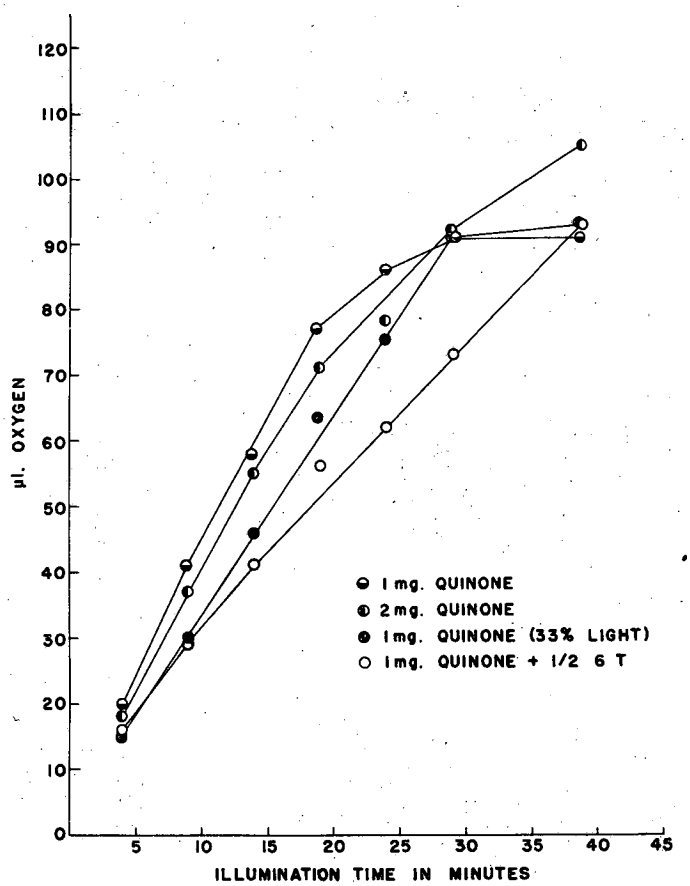
Fig. 6



6 T effect as a function of suspension density. *Scenedesmus* and 1.5 mg. quinone per vessel. A) 0.25 mg. 6 T when used. B) 0.5 mg. 6 T when used.

MU-5174

Fig. 7



6 T effect in *Chlorella*. 20 mm^3 cells per vessel.
0.5 mg. 6 T when used. Standard dark incubation.
MU-5180

Fig. 8