Biochimica et Biophysica Acta, 314 (1973) 372–381

BBA 46604

BIPYRIDYLIUM QUATERNARY SALTS AND RELATED COMPOUNDS. V. PULSE RADIOLYSIS STUDIES OF THE REACTION OF PARAQUAT RADICAL WITH OXYGEN. IMPLICATIONS FOR THE MODE OF ACTION OF BIPYRIDYL HERBICIDES*

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(Received March 14th, 1973)
(Revised manuscript received June 27th, 1973)

SUMMARY

1. Rate constants for reduction of paraquat ion (1,1′-dimethyl-4,4′-bipyridylium, PQ^{2+}) to paraquat radical ($PQ^{+\cdot}$) by e^-_{aq} and $CO_2^{-\cdot}$ have been measured by pulse radiolysis. Reduction by e^-_{aq} is diffusion controlled ($k = 8.4 \cdot 10^{10}$ $M^{-1} \cdot s^{-1}$) and reduction by $CO_2^{-\cdot}$ is also very fast ($k = 1.5 \cdot 10^{10}$ $M^{-1} \cdot s^{-1}$).

2. The reaction of paraquat radical with oxygen has been analysed to give rate constants of $7.7 \cdot 10^8$ $M^{-1} \cdot s^{-1}$ and $6.5 \cdot 10^8$ $M^{-1} \cdot s^{-1}$ for the reactions of paraquat radical with O_2 and $O_2^{-\cdot}$, respectively. The similarity in these rate constants is in marked contrast to the difference in redox potentials of O_2 and $O_2^{-\cdot}$ (— 0.59 V and + 1.12 V, respectively).

3. These rate constants, together with that for the self-reaction of $O_2^{-\cdot}$, have been used to calculate the steady-state concentration of $O_2^{-\cdot}$ under conditions thought to apply at the site of reduction of paraquat in the plant cell. On the basis of these calculations the decay of $O_2^{-\cdot}$ appears to be governed almost entirely by its self-reaction, and the concentration 5 μm away from the thylakoid is still 90% of that at the thylakoid itself. Thus, $O_2^{-\cdot}$ persists long enough to diffuse as far as the chloroplast envelope and tonoplast, which are the first structures to be damaged by paraquat treatment. $O_2^{-\cdot}$ is therefore sufficiently long-lived to be a candidate for the phytotoxic product formed by paraquat in plants.

INTRODUCTION

The reaction between oxygen and paraquat radical [$PQ^{+\cdot}$, the stable 1-electron reduction product of the herbicide paraquat**, 1,1′-dimethyl-4,4′-bipyridylium, PQ^{2+}] is of interest in that it is a product of this reaction which is thought to be the true phytotoxic species which gives paraquat dichloride and other bipyridylium salts their biological activity[1]. Conventional techniques have shown[1] that hydrogen perox-

* Part IV: *J. Chem. Soc. Perkin Trans.* 1 (1972) 138–142.

** B.S.I. registered name.

ide is a stable intermediate in the reduction of oxygen to water by paraquat radical, and no intermediate radical, *e.g.* $O_2^{\cdot-}$, between oxygen and hydrogen peroxide has been detected. Such a radical is, however, a possibility for the phytotoxic species, provided it can survive long enough to diffuse to the cell membranes, which is where damage is first observed[2] in plants treated with paraquat. The lifetime of this radical will be determined by its rate of formation from paraquat radical and oxygen relative to its rate of destruction by reaction with further paraquat radical, with cell constituents, or with itself. Pulse radiolysis appeared to offer a method for measuring these rate constants, from which the status of the intermediate radical as the phytotoxic species could be assessed.

METHODS AND RESULTS

The pulse radiolysis equipment and its method of use were essentially as indicated by Land and Swallow[3]. Revised parameters used in calculating radiation doses were, for the hydrated electron, $G = 2{\cdot}80$ (ref. 4), ε (700 nm) = 17400 (ref. 5).

Irradiation of 10^{-4} M aqueous paraquat dichloride with a shorter than 0.1-μs, 600-rad pulse gave almost pure paraquat radical, as judged by comparison of the spectrum of the product formed 10 μs after pulsing with that of authentic paraquat radical solution produced by chemical reduction with zinc. The slight discrepancy between the spectra was probably due to the presence of a product formed from HO· and paraquat (see below). After irradiating a 10^{-5} M solution of paraquat dichloride, the decay curve for e^-_{aq} at 700 nm and the appearance curve for paraquat radical at 395 nm were both first order, with half-reaction times of 1.19 ± 0.01 μs and 1.22 ± 0.02 μs, respectively, giving a second-order rate constant for reaction of e^-_{aq} with paraquat of $8.4 \cdot 10^{10}$ $M^{-1} \cdot s^{-1}$.

After saturating the 10^{-4} M aqueous paraquat dichloride with nitrous oxide, irradiation gave a product with a considerably broader spectrum than paraquat radical. The absorbance at 395 nm was less than 8% of the absorbance in the absence of N_2O, and the rate of formation of the product was much slower ($t_{\frac{1}{2}} = 10$ μs). In the presence of N_2O, the hydrated electron is rapidly converted to HO· (ref. 6) which therefore becomes the major reactive species. This evidently reacts with paraquat, but not to give paraquat radical.

In order to remove this complicating side reaction, all subsequent irradiations were carried out in 0.1 M sodium formate solution. Formate reacts rapidly with HO· and H· to give $CO_2^{\cdot-}$ and either H_2O or H_2 (ref. 7). Hence, both the radicals produced in formate, *viz.* e^-_{aq} and CO_2^-, are reducing species. When 10^{-4} M paraquat dichloride in 0.1 M sodium formate solution was irradiated (200-rad pulses), the spectrum of the product was virtually identical with that of authentic paraquat radical (Fig. 1), and the yield of radical was approximately double that obtained in water alone. Based on an extinction coefficient of $1.33 \cdot 10^4$ at 605 nm for paraquat radical, determined by reducing aqueous paraquat dichloride with zinc in a stepwise manner until maximum absorption was obtained, the G value for paraquat radical formation in aqueous formate was 5.9 (*cf.* $G(e^-_{aq}) + G(HO\cdot) + G(H\cdot)^4 = 5.8 \pm 0.4$).

Irradiation of $2.5 \cdot 10^{-5}$ M paraquat dichloride in formate with pulses of increasing size (up to 9100 rad) showed that paraquat radical was not stable to large pulses. A plot of the yield of paraquat radical against pulse size gave a curve in which the initial gradient decreased with increasing dose and eventually became negative, im-

plying over-reduction of radical to dihydrobipyridyl. This reaction is known to occur with powerful chemical reducing agents, *e.g.* zinc or sodium dithionite[8]. Because of this instability of paraquat radical to large pulses it was not possible to convert, by irradiation, a solution containing paraquat and oxygen to one containing paraquat radical, $O_2^{\cdot-}$ and no oxygen, to allow the reaction of paraquat radical with $O_2^{\cdot-}$ to be followed. Instead, an indirect method had to be used (see below).

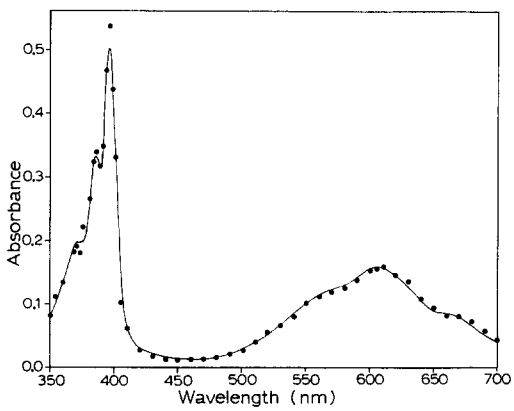


Fig. 1. Optical spectrum of paraquat radical produced by pulse radiolysis of 10^{-4} M paraquat dichloride in 10^{-1} M aqueous sodium formate. Experimental points denoted by ●. The limiting absorbance at each wavelength (10 μs after pulsing) was measured after irradiating with a 0.1-μs pulse and normalised to a constant pulse size. The continuous curve is the spectrum of paraquat radical produced by chemical reduction of paraquat dichloride in water with zinc.

Irradiation of paraquat dichloride in the presence of oxygen

10^{-3} M paraquat dichloride in 0.1 M sodium formate saturated with oxygen was irradiated with a small pulse. The decay of paraquat radical was first order. The products of the reaction were assumed to be paraquat and $O_2^{\cdot-}$ (see Discussion). The second-order rate constant for reaction of paraquat radical with O_2 was calculated (see Table I) assuming the concentration of oxygen had not been altered by the pulse. A closely similar rate constant was obtained when the experiment was repeated after saturating with air rather than oxygen.

In order to find the rate constant for reaction paraquat radical with $O_2^{\cdot-}$, a 10^{-3} M solution of paraquat dichloride in 0.1 M sodium formate saturated with 0.23% oxygen in argon was irradiated with a pulse just sufficient to leave a small residual concentration of paraquat radical after completion of its reaction with O_2 and $O_2^{\cdot-}$. The decay curve for paraquat radical departed significantly from first-order kinetics. It was possible to reproduce it very closely, however, by simulating, on an analogue computer, the differential equations corresponding to the reactions

$$PQ^{\cdot+} + O_2 \rightarrow O_2^{\cdot-} \qquad \text{Rate constant } k_1$$

$$O_2^{\cdot-} + PQ^{\cdot+} \rightarrow \text{products} \qquad \text{Rate constant } k_2$$

Values of k_1 and k_2 and initial concentrations of $PQ^{\cdot+}$ and O_2 were adjusted to produce the best visual fit between the observed and simulated decay curves. The

TABLE I

RATE CONSTANTS FOR REACTION OF PARAQUAT RADICAL WITH OXYGEN (k_1) AND SUPEROXIDE (k_2)

Paraquat radical was generated in the presence of oxygen (see text). Its decay in oxygen- or air-saturated solution was first order and gave k_1 directly. k_1 and k_2 were calculated from the shape of the decay curve in the 0.23% oxygen/argon-saturated solution by the procedure described in the text.

Saturating gas	*pH*	*Wave-length (mn)*	*Pulse (rad)*	*No. of runs*	$t_{\frac{1}{2}}$ (μs)	$k_1(\times 10^8)$ ($M^{-1}\cdot s^{-1}$)	$k_2(\times 10^8)$ ($M^{-1}\cdot s^{-1}$)
Oxygen*	Unbuffered	395	90–2000	5	0.96	5.7	—
Air**	Unbuffered	Various	500	14	4.6	5.9	—
0.23% O_2	6.17	605	1100	1	—	7.5	6.8
0.23% O_2	7.07	605	1100	1	—	7.5	6.9
0.23% O_2	8.09	605	1100	1	—	8.0	6.9
0.23% O_2	Unbuffered	395	430	1	—	7.5	5.8
0.23% O_2	Unbuffered	395	465	1	—	7.5	5.8
0.23% O_2	Unbuffered	395	500	1	—	7.6	6.0
				Preferred values		7.7	6.5

* The concentration of oxygen was assumed to be $1.26\cdot 10^{-3}$ M.
** The concentration of oxygen was assumed to be $2.52\cdot 10^{-4}$ M.

simulations showed that the best conditions for estimating k_2 were when the ratio of initial concentrations of paraquat radical and O_2 was 2:1. They also suggested that an error of 25% in either k_1 or k_2, even when its effect was minimised by optimisation of the other variables, could easily be detected. The preferred values in Table I are probably within 10% of the true values.

DISCUSSION

Excess paraquat radical is known[1] to reduce oxygen to water *via* hydrogen peroxide. We have assumed that this is due to electron transfer and proton uptake, resulting in the successive formation of $O_2^{\cdot-}$ (superoxide ion), hydrogen peroxide and hydroxy radical, *viz.*

$$PQ^{+\cdot} + O_2 \rightarrow PQ^{2+} + O_2^{\cdot-} \qquad \text{Rate constant } k_1 \qquad (1)$$

then

$$O_2^{\cdot-} + PQ^{+\cdot} \rightarrow PQ^{2+} + O_2^{2-} \qquad \text{Rate constant } k_2 \qquad (2)$$

and

$$2O_2^{\cdot-} \rightarrow O_2 + O_2^{2-} \qquad \text{Rate constant } k_3 \qquad (3)$$

followed by

$$2H^+ + O_2^{2-} \rightarrow H_2O_2$$

then

$$H_2O_2 + PQ^{+\cdot} \rightarrow PQ^{2+} + HO\cdot + OH^- \qquad \text{Rate constant } k_4 \qquad (4)$$

and

$$HO\cdot + PQ^{+\cdot} \rightarrow PQ^{2+} + OH^- \qquad \text{Rate constant } k_5 \qquad (5)$$

Both superoxide ion and its protonated form $HO_2^{\cdot}$ (hydroperoxy radical) should react with paraquat radical, but it was expected that the rate constant for reaction of paraquat radical with $O_2^{\cdot-}$ would be greater than that with $HO_2^{\cdot}$ because of ionic interaction. Also, at the pH values used in this investigation (6–8), the equilibrium concentration of $HO_2^{\cdot}$ (pK 4.8)[9] should be less than one-tenth that of $O_2^{\cdot-}$. We therefore ignored any reaction of paraquat radical with hydroperoxy radical. This treatment was justified by the constancy of the calculated k_2 as pH was changed.

Rate constants

The rate constant found for reduction of paraquat by e^-_{aq}, $8.4 \cdot 10^{10}$ $M^{-1} \cdot s^{-1}$, is extremely large. It is exceeded only twice in the list of rate constants for hydrated electron reactions with over 250 organic compounds compiled by Anbar and Neta[6], and the two compounds giving greater rates, catalase and serum albumin, are both molecules having very large areas for electron capture. The theoretical diffusion-controlled rate constant for reaction of the hydrated electron with a doubly charged spherical molecule of the same volume as paraquat is $6.7 \cdot 10^{10}$ $M^{-1} \cdot s^{-1}$ assuming the effective radii, and diffusion coefficients, of $PQ^{\cdot+}$ and e^-_{aq}[10] are 3.5 and 2.5 Å, and $0.9 \cdot 10^{-5}$ (ref. 12) and $4.7 \cdot 10^{-5}$ $cm^2 \cdot s^{-1}$. This value must be very approximate, but it does suggest that the observed rate is faster than that expected for classical diffusion control. It has been suggested[10] that electron tunnelling is the main mechanism for reduction by the hydrated electron, and that reaction at rates faster than those predicted from diffusion control should be possible provided ΔG for formation of the excited product is large. Paraquat is known[11] to have a high electron affinity, consistent with this explanation. Effectively, electron transfer appears to be taking place while the centres of the electron and paraquat are still over 10 Å apart.

The rate constants found for reaction of paraquat radical with oxygen and superoxide ion are $7.7 \cdot 10^8$ and $6.5 \cdot 10^8$ $M^{-1} \cdot s^{-1}$. These are self-consistent values from simulations and only the time scale of the decay curve and the extinction coefficient of $PQ^{\cdot+}$ are required in their calculation. The value of k_1 found directly from the decay of $PQ^{\cdot+}$ in excess oxygen requires the concentration of oxygen in solution to be known and this was not measured directly in these experiments. These rate constants are some 10 times smaller than are required for diffusion control, assuming r and D (ref. 12) for oxygen are 1.2 Å and $1.8 \cdot 10^{-5}$ $cm^2 \cdot s^{-1}$, but even so, k_1 is amongst the highest values so far reported for the reaction of oxygen with other radicals [*cf.* hydroxy cyclohexadienyl radical ($5.0 \cdot 10^8$ $M^{-1} \cdot s^{-1}$)[13] and sodium 2-anthrasemiquinone sulphonate ($4.6 \cdot 10^8$ $M^{-1} \cdot s^{-1}$)[14]].

Comparison between rate constants and redox potentials

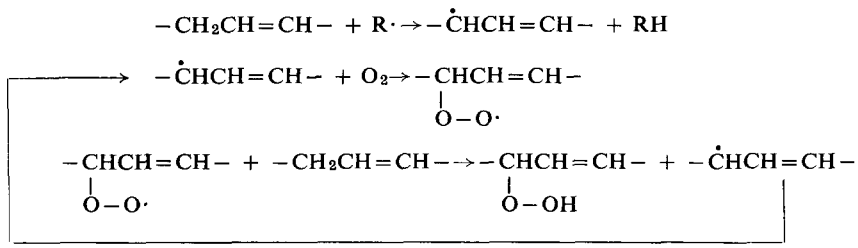
The high values found for the rate constants for reaction of paraquat radical with oxygen and superoxide ion imply that little activation energy is needed for either reaction. This is in line with expectation, since both can be considered as reactions involving only the coupling of unpaired electrons. However, the similarity in rate constants is in marked contrast to the disparity in redox potentials of the two species being reduced. The calculated redox potential of oxygen (at pH 7) is —0.59 V[15], although consideration of biochemical systems in which it is reduced suggests a value of about −0.25 V[16]. On the other hand, the $O_2^{\cdot-}/H_2O_2$ system has a redox potential of +1.12 V[17]. These figures imply that reduction of $O_2^{\cdot-}$ is more favourable

than reduction of O_2 by at least 1.37 eV or 32 kcal/mole. It might have been argued therefore that if paraquat radical reduced oxygen rapidly it would reduce $O_2^{\cdot-}$ even more rapidly. This is not so. Evidently, consideration of redox potential data is not relevant to the discussion of these reaction rates.

Formation of superoxide, hydrogen peroxide and hydroxy radical in the plant

The first observable structural changes which occur in plants treated with paraquat are at membranes[2]. After 6 h, the double membrane surrounding the chloroplast appears to have become leaky, allowing distension and finally bursting of the chloroplast. At about this time the membrane surrounding the vacuole breaks, permitting the cytoplasm and vacuolar contents to mix. The plasmalemma then bursts, allowing the cell contents to escape through the cell wall, and the final result is complete desiccation of the leaf.

Damage to membranes is consistent with a mechanism involving reactive free radicals, which could destroy membrane structure by initiating polymerisation of the unsaturated lipid constituents. Alternatively, a reactive radical could initiate the peroxidation of unsaturated lipids by molecular oxygen



The peroxidation of lipids results in the formation of a small but relatively constant yield of malondialdehyde[18]. This can be detected in paraquat-treated leaves 6 h after treatment, and it continues to increase for a further 48 h[19]. The effects produced by paraquat could, therefore, all be explained chemically on the basis of formation of superoxide ion or hydroxy radical. Equally, they could be explained by hydrogen peroxide which, because of its extreme nucleophilicity or because of its ability to generate $O_2^{\cdot-}$ and $HO\cdot$ in the presence of suitable metals or enzyme systems, could cause oxidation of unstaurated lipids to malondialdehyde.

In order to distinguish between the alternative possibilities of hydrogen peroxide on the one hand and $O_2^{\cdot-}$ or $HO\cdot$ on the other, we have explored the consequences of the latter being extremely short lived and therefore unable to diffuse far from where they are formed. In this connection, Kosower and co-workers[20] have shown that when phenyl and hydroperoxy radicals are generated from oxygen and methyl phenylazoformate within a normal erythrocyte cell, no detectable damage is caused to the membrane. The phenyl and hydroperoxy radicals decay within the cell, without surviving long enough to reach the membrane. However, if they are generated close to it, by allowing azoformate inside the cell and oxygen outside to diffuse together, the membrane is ruptured.

In plants, paraquat radical is formed within the thylakoids of the chloroplast, and then diffuses out into the stroma, which initially is saturated with oxygen. This site of reduction is proposed because herbicidally active bipyridylium salts are known

with redox potentials considerably more negative than $NADP^+$ or even ferredoxin. The data of Black[21], and of Kok *et al.* [22] on the extent of reduction, at equilibrium, of a series of these compounds by illuminated chloroplasts agree well with the idea that they are interacting with an electron carrier with a reducing potential of -550 mV. Such a reductant must be close to, if not identical with, the primary electron donor in Photosystem I, and therefore located within the thylakoid.

Concurrently with diffusion into the stroma of paraquat radical, there will be a parallel diffusion from the thylakoid of oxygen from Photosystem II, which is not inhibited by paraquat[2]. Because they are liberated side by side, paraquat radical and oxygen will react together rapidly. However, 4 moles of paraquat radical are formed for every mole of oxygen, if paraquat completely replaces $NADP^+$ as the terminal electron acceptor in Photosystem I. Only two moles are used up in the fast reactions 1 and 2 with oxygen. Reaction 3 is not significant over short diffusion distances since its rate constant[9] is several orders of magnitude smaller than k_1 and k_2. There will therefore be a residual flux of paraquat radical diffusing out into the stroma, where it meets oxygen diffusing in from the air surrounding the cytoplasm of the cell. As O_2 and paraquat radical react, the concentration of paraquat radical will fall, but, under constant illumination, a steady state should develop, when the net flux of paraquat radical into a given volume just equals its rate of disappearance by chemical reaction. The same applies to oxygen and superoxide ion, but in the case of $O_2^{\cdot-}$, its self-decomposition must also be considered. The steady-state distribution equations are therefore (*cf.* ref. 23)

$$D_A \frac{d^2A}{dx^2} = k_1AB + k_2AC$$

$$D_B \frac{d^2B}{dx^2} = k_1AB - k_3C^2$$

and

$$D_C \frac{d^2C}{dx^2} = -k_1AB + k_2AC + 2k_3C^2$$

where A, B and C are the molar concentrations of paraquat radical, O_2 and $O_2^{\cdot-}$, D_A. D_B and D_C are their diffusion coefficients, x is distance, and k_1, k_2 and k_3 are the rate constants.

These equations are for reaction accompanied by one-dimensional diffusion such as would occur if paraquat radical and oxygen were liberated uniformly and continuously over the surface of an imaginary plane in the stroma. We assumed that the stroma lamellae and grana end walls would approximate to such planes.

Six boundary conditions are needed to solve these equations. By Fick's law, the concentration gradient at any point is $-1/D$ (outward flux). If the molar outward flux of paraquat radical from the thylakoid surface is F, that of oxygen will be $\frac{1}{4}F$ and that of $O_2^{\cdot-}$ will be zero, assuming none is generated inside. The inward flux of

oxygen from outside the cell needed to react with the residual paraquat radical is also $\frac{1}{4}F$. The six boundary conditions are therefore (*cf* ref. 23)

$$\left(\frac{dA}{dx}\right)_0 = -\frac{F}{D_A}, \quad \left(\frac{dB}{dx}\right)_0 = -\frac{F}{4D_B}, \quad \left(\frac{dC}{dx}\right)_0 = 0, \quad A_\infty = 0,$$

$$\left(\frac{dB}{dx}\right)_\infty = \frac{F}{4D_B} \quad \text{and} \quad C_\infty = 0.$$

An order-of-magnitude estimate of F was made as follows.

The maximum rate of photosynthesis by spinach leaves[24] is 0.24 mole of CO_2, or 1.5 gequiv of reducing species per gram of chlorophyll per hour. The chlorophyll content of spinach leaves is about 5%[24], *i.e.* about 1 g in 20 ml of chloroplast. The maximum rate of reduction of paraquat is therefore 1.5 moles per 20 ml of chloroplast per hour. Sections of chloroplasts taken at right angles to the lamellae[26] typically show a total length of thylakoid (double) membrane, either in the form of stroma lamellae or grana, of between 15 and 25$\mu m \cdot \mu m^{-3}$. This is equivalent to a total area of thylakoid of about 20 $\mu m^2 \cdot \mu m^{-3}$ If, as an approximation, paraquat is assumed to be reduced uniformly over this area, the outward flux from a stroma lamella, in $\mu moles \cdot \mu m^{-2} \cdot s^{-1}$ is $(1.5 \cdot 10^6/20) \times (1/20 \cdot 10^{12}) \times (1/3600)$ or about 10^{-12} $\mu moles \cdot \mu m^{-2} \cdot s^{-1}$. From each end of a granum composed of eight lamellae it would be 4.10^{-12} $\mu moles \cdot \mu m^{-2} \cdot s^{-1}$.

The differential equations were set up on an analogue computer using the following parameter values: $k_1 = 7.7 \cdot 10^8$ $M^{-1} \cdot s^{-1}$, $k_2 = 6.5 \cdot 10^8$ $M^{-1} \cdot s^{-1}$, $k_3 = 1.0 \cdot 10^6$ $M^{-1} \cdot s^{-1}$ (appropriate to pH 6.8[9]), $D_A = 0.9 \cdot 10^{-9}$ $m^{-2} \cdot s^{-1}$, $D_B{}^{12} = 1.8 \cdot 10^{-9}$ $m^{-2} \cdot sec^{-1}$, $D_C = 1.8 \cdot 10^{-9}$ $m^{-2} \cdot s^{-1}$, and $F = 4 \cdot 10^{-12}$ $\mu moles \cdot \mu m^{-2} \cdot s^{-1}$. The values of the *k's* and *D's* are those appropriate to water rather than to the stroma of the chloroplast, but since both are likely to be inversely proportional to viscosity, the effect of the medium should cancel out. Sets of initial values of A, B and C were tried until the simulated decay curves for A, B and C satisfied as nearly as possible the boundary conditions for $x = \infty$. These curves are shown in Fig. 2.

CONCLUSIONS FROM THE SIMULATION

The simulation shows that decay of paraquat radical is very rapid, being essentially complete in 3 μm. From this point onward, the decay of superoxide ion is entirely governed by its self-reaction and reaction with cell constituents. Decay by self-reaction is slow at the concentration found, and 90% is still left after 5 μm. Since the chloroplast envelope and tonoplast are in places less than 0.5 μm away from the grana surface, there is no objection, on the basis of rapid decay, to superoxide ion causing damage to these membranes. This is true even if the flux of paraquat radical is very much less than in the case considered, since the rate of decay of superoxide ion with distance is largely independent of the presence of paraquat radical. The concentration of superoxide could be made to fall more rapidly if it encountered a high concentration of a substrate (*e.g.* glutathione[27]) with which it reacted very rapidly, or if k_3 were increased either because of low pH (k_3 at pH 4.88, $22 \cdot 10^6$ $M^{-1} \cdot s^{-1}$)[9] or by the presence of a high concentration of superoxide dismutase[28]. Only if these conditions were found to be operative could superoxide ion be eliminated on the grounds of too

rapid decay. The concentration of superoxide ion formed in the steady state, over 10^{-6} M at the grana surface on the basis of the figures used, is well within the range of concentrations at which biologically active molecules are effective.

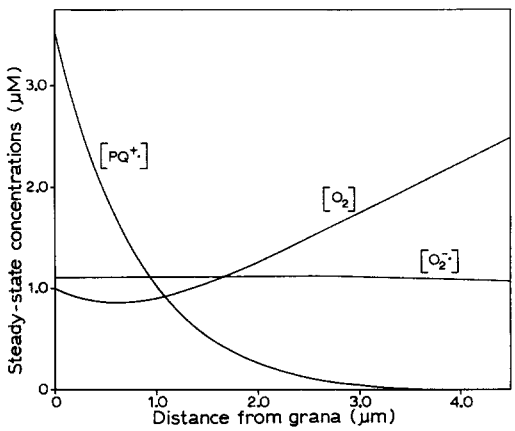


Fig. 2. Calculated steady-state concentrations of paraquat radical, oxygen and superoxide ion close to the surface of a grana stack in an illuminated chloroplast treated with paraquat, assuming (a) movement is entirely due to diffusion, (b) paraquat radical is formed within the grana, (c) the stroma is saturated with oxygen everywhere except close to where paraquat radical is being liberated, (d) paraquat radical decays only by reaction with oxygen and superoxide ion, (e) oxygen reacts only with paraquat radical, and (f) superoxide ion reacts both with paraquat radical and with itself.

This investigation gives no grounds for dismissing superoxide as the phytotoxic product formed by paraquat. On the other hand, hydrogen peroxide also remains as an equally plausible candidate. Both are compounds which may have very different life times and reactivities in the living system compared with those measured *in vitro*, due to the possible availability *in vivo* of substrates and enzymes (superoxide dismutase, and catalase and peroxidase, respectively) which could decompose them. It would appear that the physicochemical approach used in this paper has therefore gone as far as is justifiable, and further progress will require more observations to be made *in vivo*, possibly using added catalase and superoxide dismutase as blocking agents for the superoxidic and peroxidic effects of paraquat.

ACKNOWLEDGEMENTS

The invaluable assistance of R. C. Bridges in all the computational aspects of this work is gratefully acknowledged.

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