Hypothesis

AN ALTERNATIVE TO KOK'S MODEL FOR THE OXYGEN-EVOLVING SYSTEM IN PHOTOSYNTHESIS

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The model proposed in 1970 by Kok et al. [1] is presently considered as the best explanation of the damped oscillations of periodicity 4 in oxygen emission induced in dark-adapted chloroplasts or intact cells by a sequence of brief, saturating flashes. This model may be summarized by the following linear, 4-step scheme:

$$S_0 \xrightarrow{h\upsilon} S_1 \xrightarrow{h\upsilon} S_2 \xrightarrow{h\upsilon} S_3 \xrightarrow{h\upsilon} O_2$$

In order to account for the damping, the model assumes that some centres do not react ('misses') and that others react twice ('double hits') during a flash. Misses and double hits are random events. From the typical pattern observed in dark-adapted systems ($Y_1 = 0$, $Y_2 \simeq 0$, Y_3 = maximum, Y_n being the O_2 yield at flash number n), it is assumed that only states S_0 and S_1 are stable in darkness, with the relative distribution: $S_0 \simeq 0.3$, $S_1 \simeq 0.7$; this implies that, upon cessation of light, S_2 and S_3 decay into S_0 and S_1 (deactivation). An essential property of this model is that the System II centres are isolated from each other and do not cooperate in the oxidation of water and emission of oxygen. My purpose is to show that many experimental results do not fit easily in this framework and are better explained if the hypothesis of non-cooperativity is relaxed.

In my laboratory, we have recently developed a numerical procedure (σ analysis [2]) for the objective evaluation of the damping coefficients of O_2 sequences and we have undertaken to study the effect of various substances or treatments on these coefficients. Our results – as well as those of others which we have analyzed – argue against the concept that misses are only of photochemical origin, as proposed in Kok's model. According to the above model, the steady-state O_2 yield Y_{ss} is related to the total miss coefficient σ_1 ($= \Sigma\alpha_i$, α_i being the miss coefficient for the transition $S_i \longrightarrow S_{i+1}$) as shown in fig.1: the

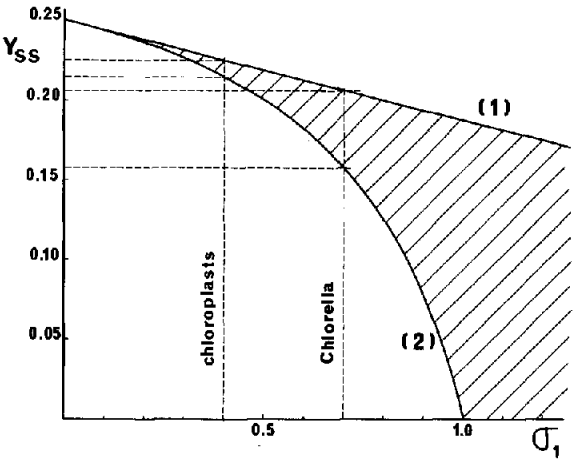

Fig.1. Relationship between the steady-state O_2 yield Y_{ss} and the sum of miss coefficient σ_1 in Kok's model. The exact relationship depends upon the specific distribution of misses among the S states; however the representative points must always be between curve (1) corresponding to homogeneous misses and curve (2) corresponding to a single miss (see text). The tow vertical dashed lines show the range of Y_{ss} corresponding to the average σ_1 found in the literature for chloroplasts and *Chlorella* [2].

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl-urea; CCCP, carbonylcyanide m-chlorophenyl-hydrazone; DNB, *m*-dinitrobenzene; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone.

representative points must lie inside the hatched area bounded by the upper and lower curves corresponding to equal misses on all states ($\alpha_i = \sigma_1/4$) and a single miss on one state ($\alpha_i = \sigma_1$, $\alpha_j = 0$ for $j \neq i$) respectively). Obviously, an inverse relationship is predicted between Y_{ss} and σ_1; if misses increase, the quantum yield must decrease (a direct relationship may only be possible by moving inside the permissible region through a change in α_i distribution among states).

This rule is contradicted by a number of results. In general, *Chlorella* has a higher σ_1 (0.7 ± 0.1) than isolated chloroplasts (0.4 ± 0.1), see ref. [2], and thus following the above rule the latter should have a better photochemical efficiency than the former (see fig.1). This is doubtful considering that chloroplasts, unless prepared and handled with special precautions, are subject to a rapid loss of photochemical activity. In addition, it is seen in fig.1 that Y_{ss} in *Chlorella* would be between 0.6 and 0.8 of the theoretical maximum, a range of values barely consistent with the known overall quantum requirement of 8 photons per evolved O_2 molecule. Several treatments have been found to decrease both Y_{ss} and σ_1, inplying a direct relationship between these quantities: DCMU [3], CCCP [4], DNB [5], *p*-benzoquinone [6], mild heat pretreatment [7]. It was verified that in every case the representative points could not be placed in the permissible region of fig.1. The same is true for NaN_3 [8] although in this case an inverse relationship between Y_{ss} and σ_1 was observed. A most striking case is that of the partial thermal denaturation of the O_2 evolving system in *Chlorella* observed recently in my laboratory [7]: after this treatment, the O_2 sequence is even less damped than that of a typical chloroplast preparation. The only examples being in agreement with the concept of photochemical misses are cases where the acceptor side of System II becomes strongly limiting and Q, the primary acceptor, mostly reduced (chloroplasts without external electron acceptor [9], mutant deficient in System I [10], (HCO_3^- depletion [11], DBMIB [12]). However, these conditions raise a difficult problem. If the photochemical misses are explained by a large proportion of centres being in state Q^- [9], we must admit that this state is very rapidly and randomly distributed among the centres; this contradicts the findings of Bouges-Bocquet [13] and Velthuys and Amesz [14] who showed that no such fast exchange was possible, at least at the level of Q.

From the above evidence, it is clear that the hypotheses at the basis of Kok's model must be re-examined in order to dissociate the quantum yield from the damping. Now, damping can probably be explained by a variety of mechanisms besides photochemical misses. For instance, I have recently proposed [15] that a side carrier could reversibly store a positive charge on the donor side of the System II centres; such a mechanism would give rise to purely non-photochemical 'misses' and 'double hits' (this proposal however may not be acceptable as such because it implies that the extent of misses and double hits are equivalent, which is not supported by experiments). Contrary to the concept inherent in Kok's model, such damping factors could be called conservative.

I propose to introduce another model of the conservative type allowing most naturally a positive correlation between Y_{ss} and σ_1. The structural hypothesis of this model are (see fig.2):

(H1.) The System II centre is dissociated into a charge storage system or 'O_2 enzyme' E and a charge separating system P (which may be conceived as the usual Z P Q complex).

(H2.) As in Kok's model, E has four states: E_0, E_1, E_2, E_3 and O_2 emission occurs during the transition from E_3 to E_0:

$$E_3 + \oplus \longrightarrow E_0 + O_2$$

(H3.) E is contained within the internal space of the thylakoid where it may be either free or bound to the donor side of P; the latter according to current concepts, is integrated in the thylakoid membrane; the transfer of a positive charge from P to E occurs in the bound state:

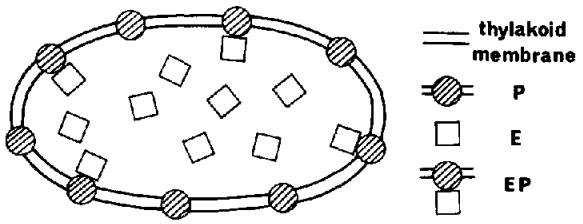

Fig.2. Structural properties of the model. A schematic cross-section of an isolated thylakoid is shown. See text.

$$E_nP^+ \longrightarrow E_{n+1}P$$

(H4.) The numbers n_E of E molecules and n_P of P centres per thylakoid are not necessarily equal.

At least one mechanistic hypothesis is required:

(H5.) A given E has a small probability of visiting more than one P^+ and thus seldom acquires more than one extra ⊕ per flash. A P^+ not visited may back react.

The above set of hypotheses is sufficient to account for a positive correlation between Y_{ss} and σ_1. This is best explained considering the effect of extreme variations in the ratio n_E/n_P. If $n_E > n_P$, the total number of E contribute to O_2 emission, but there is not enough ⊕ production per flash to supply every O_2 enzyme with a ⊕: some of the Es get behind the others in the $E_0 \longrightarrow E_3$ cycle. The yield is maximal ($Y_{ss} = n_P$, expressed per thylakoid) and yet misses are large. They obviously are conservative misses. This may correspond to the normal situation (e.g. *Chlorella*). If on the contrary $n_E < n_P$, almost every E is supplied with a ⊕ at each flash and the small probability of double visits prevents much 'double hitting'. Now, the yield is low ($Y_{ss} \simeq n_E$) and the misses are decreased. Yet there are many photochemical misses (back reaction of excess P^+), but these are not seen kinetically. This situation may result from inactivation of part of the O_2 enzyme (e.g. isolated chloroplasts or heat-pretreated *Chlorella*). Obviously, the binding equilibrium plays also an important role in determining the O_2 yield and the damping. To get some insight into this role, let us consider a state E_i; its behaviour is described by,

$$PE_i \underset{}{\overset{K_i}{\rightleftharpoons}} P + E_i$$

$$\frac{(P)\ (E_i)}{(PE_i)} = K_i$$

K_i being the dissociation constant. The miss factor on this state is simply $\alpha_i = (PE_i)/\ [(PE_i) + (E_i)]$ whence it follows that,

$$\sigma_1 = \Sigma \frac{K_i}{K_i + (P)}$$

It is seen that σ_1 is a direct function of the K_i but that, in addition, variations of n_E/n_P also affect σ_1 (through the term (P)) in exactly the directions explained above. A preliminary simulation of the model suggests that the normal situation (*Chlorella*) is well described assuming that

$$\Sigma K_i \simeq 0.1\text{–}0.2 \text{ and } n_E/n_P \simeq 1.$$

Additional arguments in favour of the new model may be given. The first is not related to O_2 emission kinetics. Bennoun [16] has shown that, in the presence of DCMU (saturating), Q^- photochemically formed from the dark-adapted condition (S_0, S_1) subsequently decayed according to biequimolecular kinetics. This observation is very simply accounted for by the model. For instance, assuming that E_2 (corresponding to S_2) and PQ^- (explicitly) were the dominant species produced by light, they will decay following,

$$E_2 + PQ^- \xrightarrow{(1)} E_2PQ^- \xrightarrow{(2)} E_1PQ$$

If the association (1) is limiting as compared to the recombination (2) and considering that $(E_2) = (PQ^-)$, the decay will be exactly biequimolecular. The hypothesis H 3 is very important in this connection. Moreover, from the observation [17] that the decay of Q^- in chloroplasts is accelerated by acidification, it may be inferred that the binding of E_2 to P is favoured by high proton concentration inside the thylakoid and that, more generally, the pH of the internal thylakoid phase plays an important role in the binding equilibria of the E_is. A second argument is provided by Etienne's experiment [18] on System II in *Chlorella* when DCMU is added after the centres have been placed essentially in state S_3Q (*i.e.* by two flashes pre-illumination of the dark-adapted cells). According to Kok's model, a flash given in this condition should produce oxygen, leaving the centres in state S_0Q^-, that is blocking both the variable fluorescence decay and the luminescence emission. Actually, O_2 emission was observed, but is was concomitant with a normal fluorescence decay and luminescence emission. Contrary to Kok's model, the present model can account very easily for these results (e.g. $E_i + PQ^- \rightarrow E_iPQ^- \rightarrow E_{i-1}PQ$). J. Lavergne (private communication) has independently developed similar ideas based on the above argument.

A strong argument in favour of non-cooperativity

between centres was found in the observations [1] that partial inhibition by DCMU or partial destruction of the centres by u.v. irradiation did not essentially disturb the oscillatory pattern of O_2 emission. Any type of cooperativity – as assumed for instance in the present model – apparently would result in appreciable damping under the above conditions. We recall however that inhibition by acceptor blocking did actually induce damping, a fact that Kok's model could only accommodate by unduly assuming a delocalization of the state Q^-. At any rate, it is clear that the DCMU blocking is very different from the other types of acceptor blocking. Admittedly, additional assumptions are required in the present model: one possibility could be that, due to a change in the equilibrium constants K_i in DCMU bound centres, the increase in n_E/n_p is partially compensated by a decrease in ΣK_i; it is also to be noted that the disapearance of E_2 and E_3 by back reaction in DCMU bound centres somehow alleviates the $n_E > n_P$ condition favorable to damping. The result of u.v. treatment is a much weaker argument since it is known that this treatment inactivates both the acceptor and donor sides of System II [19] and thus is not expected to change n_E/n_P much.

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