

## A KINETIC VERSION OF THE MODEL OF KOK

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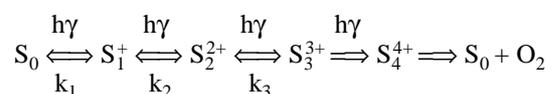
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**Summary.** A kinetic model was developed on the basis of the model of Kok et al. (1970), which makes possible the estimation of back deactivation reactions of higher oxidized  $S_2$  and  $S_3$  states as well as the so-called Emerson and Arnold period, a time needed for forward reactions of oxygen evolving centres.

**Key words:** photosynthesis, oxygen evolution,  $S_i$  states, Kok's model

**Abbreviations:**  $S_i$  states – oxidized states of the photosynthetic oxygen evolving enzyme system;  $R_i$  states –  $S_i$  states after capturing of a substrate molecule in the darkness

The extremely important discovery of Joliot and co-workers (1969) showed that after several minutes dark incubation of unicellular algae suspensions or of isolated chloroplasts, the first flash does not lead to oxygen evolution, and the yields from the succeeding flashes show damping oscillations with a maximum after the third flash and a periodicity of four flashes. These results were used by Kok and co-workers (1970) in the elaboration of the so-called four-quantum four-step linear model on the functioning of oxygen-evolving reaction centres.



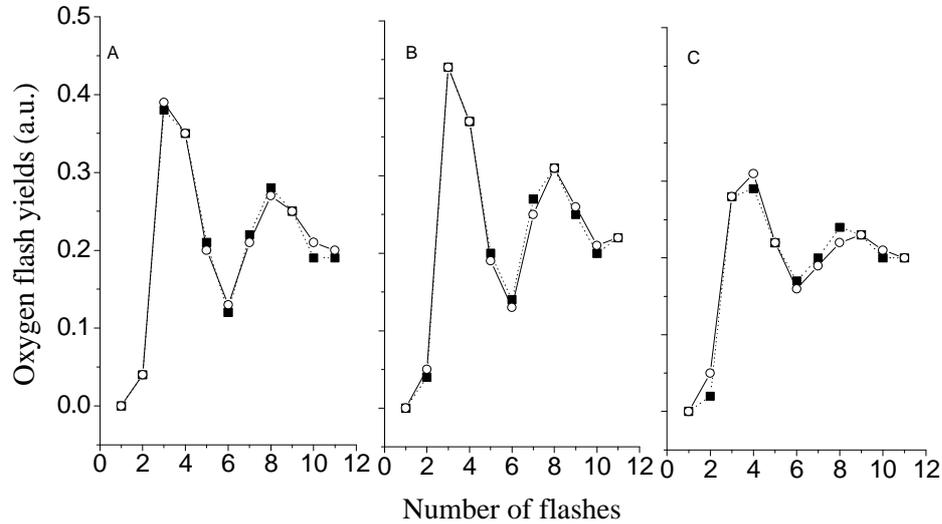
According to Kok's scheme the photochemical reactions in water splitting system of photosynthesis follow the absorption of photons ( $h\nu$ ) and are connected with the successive accumulation of positive charges in the respective  $S_i$  states of each individual oxygen evolving centre. After reaching the  $S_4^{4+}$  state one oxygen molecule is evolved and the centre returns to the initial  $S_0$  state. The damping of oxygen yield

oscillations is explained by the assumptions that there exists a definite percentage of probabilities of every centre after the absorption of one photon to pass to the next state (single hits – “ $\gamma$ ”), to pass two steps forward (double hits – “ $\beta$ ”) or to rest in the same state (misses – “ $\alpha$ ”). The maximum oxygen yield at the third flash, reflecting the initial concentration of the centres in  $S_1$  state, is explained by the assumption that in the dark the higher oxidised  $S_2$  and  $S_3$  states deactivate to  $S_1$  state through back reactions represented in the scheme by the rate constants  $k_2$  and  $k_3$  and consequently after several (5–10) minutes in darkness 75% of the centres will be in  $S_1$  and 25% in  $S_0$  state.

During the last 25 years the linear four-step model of Kok (1970) was widely applied for investigations of photosynthetic oxygen evolving mechanisms and contributed to a great extent to our contemporary understanding of this process.

There are some problems concerning the model, which are not satisfactorily explained so far. One of them is the nature of the “misses” ( $\alpha$ ) and “double hits” ( $\beta$ ) parameters. Several models about the cause of misses exist. Fourbush and co-workers (1970) have accepted that the misses are identical for each  $S_i$  state. This statement was questioned by Delrieu (1983), who showed that a non-homogenous distribution of misses fits better the experimental data. Moreover, Meunier and Popovich (1990) have shown that misses become lower with the flash number in a sequence of  $O_2$  evolution. As it has been pointed out by Lavorel (1980) the  $O_2$  yields is expected to decrease in experimental conditions where damping due to photochemical misses increases. However, this assumption was disproved in a number of cases (e.g. the photochemical misses in *Chlorella* and *Scenedesmus* are higher than in isolated chloroplast suspensions). On the other hand, according to Sun and Sauer (1972), the quantum yield of photosystem II reaction could reach  $1.00 \pm 0.05$  which is in contradiction with the estimated value of the miss parameters (0.2–0.3) in *Chlorella* and *Scenedesmus* cells. The existence of a stable  $S_1$  state in the dark is also a problem.

On Fig. 1 are presented experimentally obtained oxygen flash yields (squares) using pea chloroplasts under 0.1s (A), 0.3s (B) and 1s (C) dark intervals between the flashes. Theoretically calculated flash yields using the original Kok’s model and a programme for minimization the mean quadratic deviation between experimentally registered and the predicted yields are presented with the circles. On Table 1 are presented the calculated values of the initial  $S_i$  states distribution as well as the values of the misses ( $\alpha$ ) and double hits ( $\beta$ ). The most serious disadvantage of Kok’s model is the absence of time as a free parameter. Data on Table 1 show that not only the values of misses and double hits are depending on the dark spacing between the flashes (which is not easily understandable), but also the values of the  $S_0$  and  $S_1$ . If we are interested to know the dark distribution of these two states stable in the dark, our results show that this distribution is depending on the dark intervals between the flashes. Obviously, the oxygen-evolving centres cannot “know” in advance how high the flash frequency is.

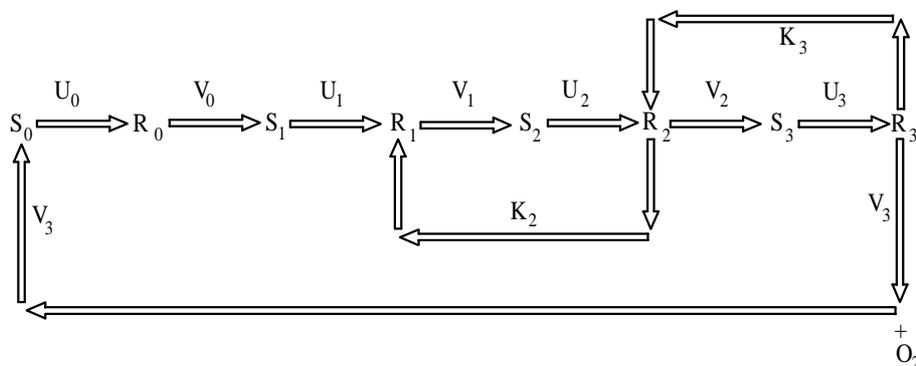


**Fig. 1.** Experimentally obtained oxygen flash yields in spinach chloroplasts (squares) and the yields, calculated according to the model of Kok (circles). The values of the different parameters used are presented in Table 1. Spacing between flashes: A–0.1s; B–0.3s; C–1.0s.  $SD = 1.153 \cdot 10^{-4}$

**Table 1.** Dark (initial) distribution of  $S_i$  states and values of misses in dependence on the spacing between flashes according to the model of Kok

Intervals between the flashes	$S_0$		$S_1$		Misses		Double hits	
	a.u.	%	a.u.	%	$\alpha$	%	$\beta$	%
100 ms	0.30	100	0.81	100	0.221	100	0.029	100
300 ms	0.30	100	0.87	107	0.200	90	0.035	120
1000 ms	0.42	140	0.62	77	0.257	116	0.055	190

On Fig. 2 is presented an extended version of the model of Kok et al. (1970). In this scheme it is assumed that every oxygen evolving centre exists in two different states –  $S_i$  and  $R_i$ . The centres from  $S_i$  states could pass to the next  $R_i$  states after capturing in the dark a special substrate partner. It could be a  $CO_2$  molecule or more probable  $HCO_3^-$  ion as it is assumed by Warburg (1964) and Metzner (1978). The steps ( $S_i \rightarrow R_i$ ) are depending on the concentration of substrate molecules and this is expressed by the rate constant  $k_i$ , so that the rates of the transients ( $U_i$ ) are equal to the  $k_i \cdot S_i$ . The steps  $R_i \rightarrow S_{i+1}$  are photochemical reactions, i.e. these steps should be expressed as  $R_i + h\nu \rightarrow R_i^* \rightarrow S_{i+1}$ . However, having in view that the first step is very fast it could be neglected. It is assumed that the last transient  $R_3 \rightarrow S_0$  which is also a photochemical step is leading to the production of one molecule oxygen. In the scheme it is also assumed that the centres in  $R_3$  and  $R_2$  states could deactivate with the rate constants  $k_3$  and  $k_2$  to the  $R_2$  and  $R_1$  states respectively.



**Fig. 2.** Scheme of the kinetic model. The transients  $U_0$ – $U_3$  are dark reactions, most probably connected with the participation of an unknown substrate. The steps designated by  $V_i$  are photochemical reactions and reactions  $k_3$  and  $k_2$  are back deactivating processes.

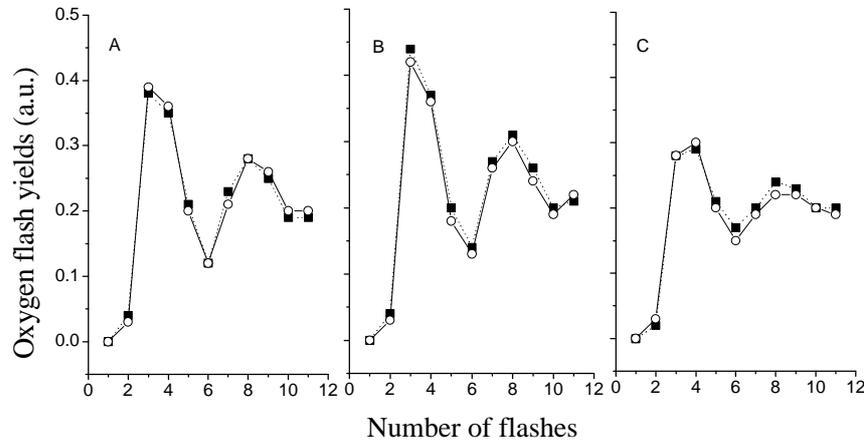
On the bases of the presented scheme the following equations could be derived:

$$\begin{aligned}
 dS_0/dt &= k \cdot R_3 - k_t \cdot S_0 \\
 dS_1/dt &= k \cdot R_0 - k_t \cdot S_1 \\
 dS_2/dt &= k \cdot R_1 - k_t \cdot S_2 \\
 dS_3/dt &= k \cdot R_2 - k_t \cdot S_3 \\
 dR_0/dt &= k_t \cdot S_0 - k \cdot R_0 \\
 dR_1/dt &= k_t \cdot S_1 - k \cdot R_1 + k_2 \cdot R_2 \\
 dR_2/dt &= k_t \cdot S_2 - k \cdot R_2 - k_2 \cdot R_1 + k_3 \cdot R_3 \\
 dR_3/dt &= k_t \cdot S_3 - k \cdot R_3 - k_3 \cdot R_2.
 \end{aligned} \tag{1}$$

In flash experiments the rate constant ( $k$ ) is zero and only after each flash the  $R_i$  states pass to the next  $S_{(i+1)}$  states with the assumed probabilities of misses and double hits.

On Fig. 3 are presented the same experimentally obtained oxygen flash yields (squares) as in Fig. 1 and the theoretically calculated flash yields using the presented model. Despite the fact that the data on Fig. 3 are fitted for the different spacing of the flashes, with the invariable values of the used parameters ( $R_1$ ,  $R_2$ ,  $k_t$ ,  $k_2$ ,  $k_3$ ,  $\alpha$  (misses) and  $\beta$  (double hits)) the deviations between the theoretically calculated yields and the experimentally obtained oxygen flash yields are not higher comparing the results presented on the Fig. 1 where the values of  $S_0$ ,  $S_1$ ,  $\alpha$  and  $\beta$  are calculated separately for the different three cases of the dark spacing between the flashes.

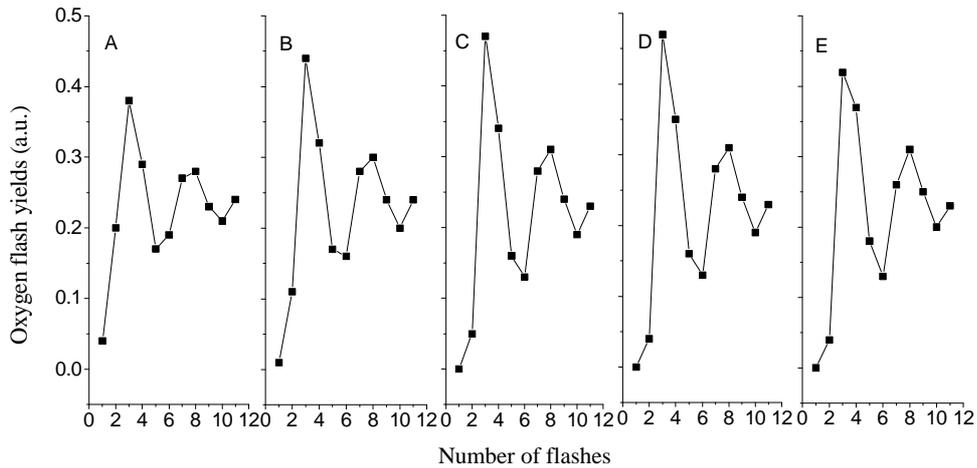
In Fig. 4 is illustrated the behaviour of the considered model in consecutive flash train experiments depending on the dark intervals between the flash trains (A – 5 s, B – 10 s, C – 20 s, D – 30 s, and E – 1 min) and at 300 ms dark spacing between flashes in the trains. The results show that the yields after 1 min dark time between flash trains are similar to the initial distribution of the flash yields shown on Figs. 1 and 3 at



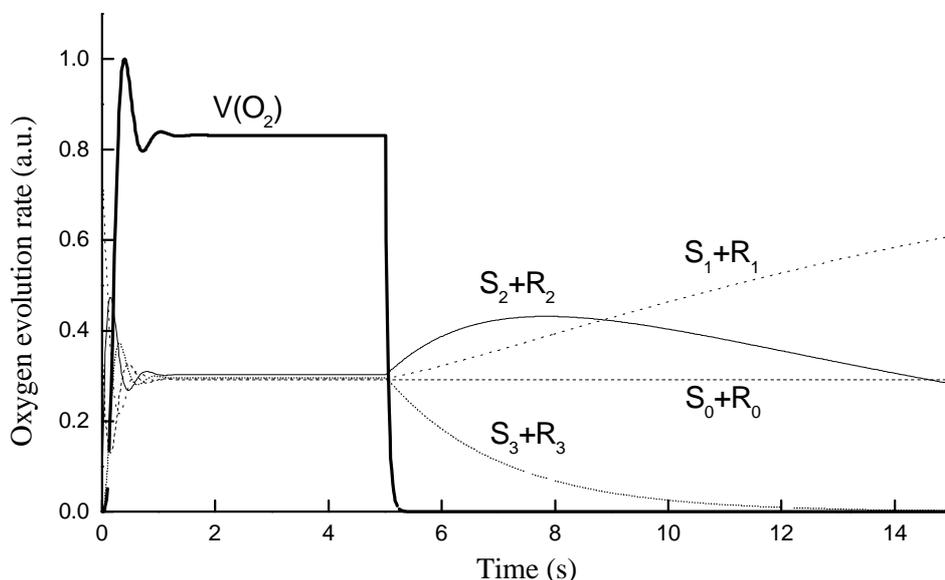
**Fig. 3.** Experimentally obtained oxygen flash yields (squares) as in Fig. 1 and the yields (circles) calculated according to the model presented on Fig. 2. The values of the different parameters used in the model are as follows:  $R_0 = 0.398$ ;  $R_1 = 0.787$ ;  $k_1 = 23.73$ ;  $k_2 = 0.084$ ;  $k_3 = 0.493$ ;  $\alpha = 0.138$ ;  $\beta = 0.057$ . All other parameters –  $R_2$ ,  $R_3$ ,  $S_0$ ,  $S_1$ ,  $S_2$ , and  $S_3$  are equal to zero. Spacing between flashes: A – 0.1 s, B – 0.3 s, C – 1.0 s.  $SD = 1.09 \cdot 10^{-4}$ .

300 ms dark spacing between flashes and 5 min dark time adaptation of the used chloroplast suspensions.

Changing the value of  $k$  from 0 to 10 and 0 a “dark–light–dark” transition experiment is presented on Fig. 5. The model shows an initial oxygen burst which is observed in chloroplast and algae (*Chlorella* and *Scenedesmus*) suspensions.

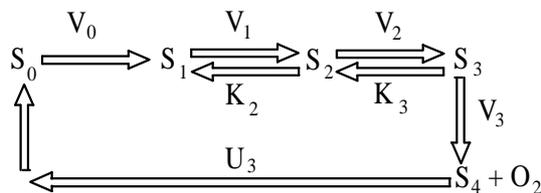


**Fig. 4.** Theoretically estimated oxygen flash yields according to the model presented on Fig. 2 with the same values of the parameters as in Fig. 3 showing the dependence of the yields on the dark intervals between the flash trains: A – 5 min; B – 10 s; C – 20 s, D – 30 s and E – 60 s.

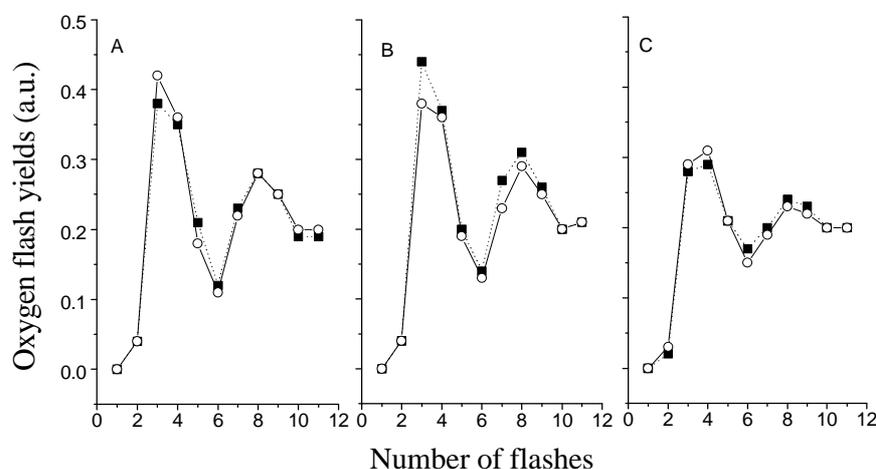


**Fig. 5.** “Dark-Light-Dark” transients or oxygen induction curve as well as the distribution of the different  $S_i$  and  $R_i$  states according to the model presented on Fig. 2.

In the original Kok’s scheme it is assumed that every centre after the absorption of the light quanta passes to the excited states  $S_i + h\nu \rightarrow S_i^*$  after which the centres could pass to the next higher oxidized  $S_{i+1}$  state. According to the authors of the model (Forbush et al., 1970; Kok et al., 1970) the turnover times of the centres ( $S_i^* \rightarrow S_{i+1}$ ) are in the order of 200–400  $\mu\text{s}$  and only the last transient  $S_4 \rightarrow S_0$  has a half time around 2 ms. Neglecting the first three relatively fast transients and considering only the last slow reaction connected with the formation of one oxygen molecule the scheme of Kok could be presented as in Fig. 6. Using this scheme and the same procedure for the fitting of the flash yields the results shown on Fig. 7 are obtained. These data indicate significant deviations between the experimentally obtained and calculated flash yields in comparison to the results presented on Figs. 1 and 3. Especially the deviations of the third flash at 100 ms and 300 ms spacing between flashes are apparent. Obviously, the question about the transient rates of the different  $S_i$  states re-



**Fig. 6.** The kinetic diagram of the original Kok’s model.



**Fig. 7.** Experimentally obtained oxygen flash yields (squares) as in Fig. 1 and the yields (circles) calculated according to the diagram presented on Fig. 6. The values of the different parameters obtained after the fitting are as follows:  $S_0 = 0.418$ ;  $S_1 = 0.694$ ;  $k_1 = 14.45$ ,  $k_2 = 8.2 \cdot 10^{-6}$ ;  $k_3 = 0.433$ ;  $\alpha = 0.156$ ;  $\beta = 0.088$ .  $S_2$ , and  $S_3$  are accepted 0. Spacing between flashes: A – 0.1 s, B – 0.3 s, C – 1.0 s.  $SD = 2.65 \cdot 10^{-4}$

mains open. It could be assumed that the transients  $S_i \rightarrow R_i$  presented on Fig. 2 need really about 20 ms (the so-called Emerson & Arnold time) but after the following transients  $R_i + h\nu \rightarrow R_i^* \rightarrow S_{i+1}$  which are very fast the centres could accept second quanta, i.e. could proceed the next reaction step  $S_{i+1} + h\nu \rightarrow S_{i+1}^* \rightarrow R_{i+1}$ . So, if we investigate the effect of two successive flashes depending on the dark spacing between them we will estimate only the transient rate of the reaction  $R_i^* \rightarrow S_{i+1}$ . No need of saying that this hypothesis should be investigated experimentally.

Trying to exclude the existence of the stable in the dark oxidized  $S_1$  state as well of the misses and double hits we have proposed (Zeinalov and Maslenkova, 1996) a model describing also a significant number of experimental results and leading to linear dependence of the photosynthetic light curves under low light intensities. However we think that the model given on Fig. 2 is more appropriate for the investigation of the experimentally obtained results.

In conclusion, we should point out that the model presented here is only a tentative one. The situation in the oxygen-evolving system is actually more complicated than that described by the system of differential equations (1). The existence of membrane compartments with strong surface and border effects as well as the conjugation of individual electron carriers do not allow us to define correctly the kinetics of the individual  $S_i$  states.

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