STUDIES ON THE KINETICS OF CATION-ASSOCIATED FLUORESCENCE CHANGES IN CHLOROPLAST MEMBRANES

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1. Introduction

Since cation effects on chlorophyll fluorescence were first discovered [1,2] this phenomenon has excited great interest. Murata's initial suggestion that cations control energy distribution between the two photosystems has found widespread support though there is still no consensus concerning details of the mechanism. Generically cations are considered to interrupt energy flow from PSII to PSI (the so called 'spillover') and three main ideas have been invoked to explain this:

(1) Butler and Strasser [3] and Butler [4] consider this to be a consequence of a more efficient energy coupling between PSII units in the presence of cations;

(2) Leto and Arntzen [5], on the other hand, find that cations can influence energy flow from LHCP to PSI in a mutant which lacks a functional PSII, suggesting a direct influence at the level of energy transfer between LHCP and PSI;

(3) The assumption that cations directly affect energy flow from PSII to PSI is also often made (e.g., [6,7]) and such an idea gains support from the model proposed by Andersson and Anderson [8] for the distribution of the photosystems along the thylakoids (but see [9]).

Andersson and Anderson [8] envisage that in the partition zones of the grana PSII and LHCP are concentrated to the almost total exclusion of PSI, which is distributed on all the stroma exposed membranes. As cations induce grana stacking at approximately the same concentrations as those which regulate energy distribution the idea is that the physical separation of PSII from PSI which accompanies grana formation mechanistically explains the cation effect on energy distribution. Hypothesis (1) differs from both (2) and (3) in that the primary effect of cations is at the level of PSII and does not invoke changes in the steric relationship between PSII (+LHCP) and PSI.

Here we present an analysis of the kinetics of cation effects on chlorophyll fluorescence yield in an attempt to distinguish between these various possibilities. The main finding we report is that the fluorescence decline which occurs on EDTA addition to the chloroplast membranes incubated in the presence of Mg^{2+} can be explained adequately by second-order reaction kinetics involving two different substrates in an environment in which one of the substrates is present in two kinetically distinguishable 'subpopulations'. This finding is in agreement with either of hypothesis (2) or (3) but not with hypothesis (1).

Abbreviations: DCMU, 3-(3,4 dichlorophenyl)-1,1 dimethylurea; LHCP, light harvesting chlorophyll-protein complex; PS, photosystem

2. Materials and methods

Chloroplasts were prepared as in [10] in the presence of $MgCl_2$ (2.5 mM). Chlorophyll fluorescence measurements were performed in a Perkin-Elmer MPF-3 spectrofluorometer. The excitation wavelength was 470 nm and the emission wavelength 681 nm. A Balzers B-40 interference filter (681 nm) was placed in front of the photomultiplier. All reactions were performed with continual agitation in 3 ml Tricine–Na 30 mM (pH 8) containing DCMU, 10 μM and $MgCl_2$ (see figure legends). The chlorophyll concentration was 4 μg/ml. A preincubation period of 10 min in the desired reaction conditions preceded the addition of

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3.5 mM EDTA. All reactions were followed until the maximal fluorescence change was achieved, which usually required 20–30 min.

3. Results and discussion

When chloroplasts were prepared and incubated in the presence of 2.5 mM $MgCl_2$ it was found that >2.5 mM EDTA induced a fluorescence decline which was zero-order with respect to the EDTA. Thus in these conditions the velocity of the fluorescence changes is a measure of the rate-limiting step at the level of the membrane-associated reactions.

Fig.1 represents the kinetics of the EDTA-induced fluorescence decline plotted as a second order reaction in which the two substrates are assumed to be present in equal concentrations [11]. The principal assumption involved here is that the fluorescence parameters are directly proportional to the concentrations of the hypothetical reactants. A reasonably close fit is achieved between the data and the assumptions made

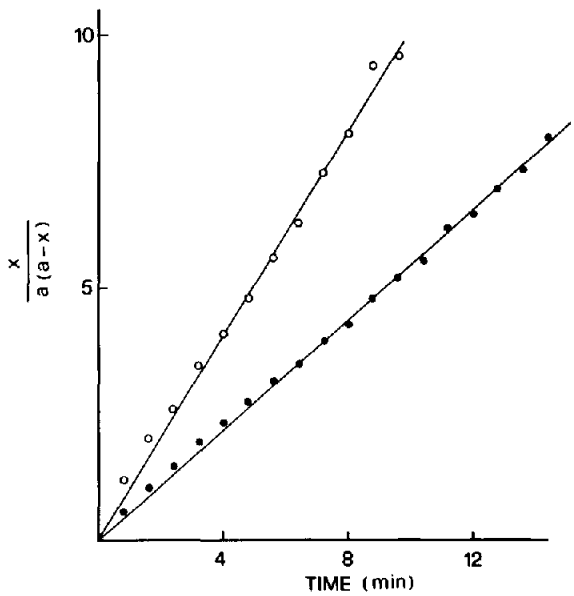

Fig.1. Kinetics of the fluorescence decline in chloroplasts induced by addition of EDTA. The chloroplasts were incubated with 2.5 mM $MgCl_2$ and 3.5 mM EDTA. The plot is according to the theory of second-order reaction kinetics in which the two substrates involved are present in equal concentrations: (a) maximal fluorescence decrease; (x) the fluorescence decline at any time; (•) experiment conducted at 9°C; (○) experiment conducted at 18°C. Each data point is the average of 3 separate determinations.

for ≥90% of the total change. Experimental errors are large above this value due to uncertainties in attaining measurements of the necessary precision. When these data were plotted according to first-order reaction kinetics, or second-order kinetics assuming different concentrations for the two substrates, the discrepancy between data and theory was great.

Thus to a first approximation the reaction seems to be one involving two components in the rate-limiting step. This observation is not easy to reconcile with hypothesis (1) above on cation effects. In this case a complex involving PSII and PSI (together with LHCP) should be present at the beginning of the reaction prior to EDTA addition. Upon EDTA addition decreased PSII–PSI energy coupling (possibly via a looser LHCP–PSII coupling) would occur resulting in more energy transfer to PSI (with the associated fluorescence quenching). Such a reaction should display the parameters of a first-order reaction. Second-order kinetics imply that at least two reactants are involved, one of which is presumably the fluorescence quencher (probably PSI). Since it is mainly PSII fluorescence which one measures at the temperatures used here the other component is probably PSII (+LHCP). Experiments with antibodies are underway to study this aspect.

The rate-limiting step could in theory be either due to the potential energy barrier associated with the formation of the transition state complex between the two substrates, or that associated with the diffusion process. Diffusion itself obeys first order kinetics but this is expected to display the characteristics of a second-order reaction when two diffusing reactants must collide with one another. Diffusion is most probably the rate-limiting step here due to the relatively low fluidity of biological membranes and the large size of the molecules or complexes suspected to be involved here. Experimental evidence supporting this view appears in [12,13]. This assumption was also made in [7].

During the initial stages of the reaction there is a small positive deviation of the data from the theory (fig.1). This can be accounted for by assuming a heterogeneity in the system. The possible biochemical nature of such a heterogeneity will be discussed below. The rate equation for a homogeneous second-order reaction is:

$$\frac{dx}{dt} = k[A] \cdot [B] \qquad (1)$$

where all the symbols have their usual meaning and [A] = [B] in the present case. However one can envisage the situation in which one of the substrates consists of more than one 'subpopulation'. Thus we assume that the molecular species A consists of two subpopulations A_1 and A_2. The rate equation for such a situation is:

$$\frac{dx}{dt} = k_1[A_1] \cdot [B] + k_2[A_2] \cdot [B] \qquad (2)$$

where $[A_1] + [A_2] = [A] = [B]$.

The reaction product is assumed to be similar in both cases. Clearly if $k_1 = k_2$ then the equation becomes the familiar one cited above (eq.1). However, the principal aspect of the present hypothesis is that they are not equal. Assigning arbitrary values to $[A_1]$, $[A_2]$, k_1 and k_2 in eq.(2) permits the calculation of $x/[a(a-x)]$ at different times (fig.2a): If a small fraction of one of the substrates interacts relatively rapidly with the other, there is an initial positive deviation from linearity. The size of this deviation depends clearly on the proportion of fast and slow reactions. We note that with the assumed k_1- and k_2-values of 6 and 1, respectively, the relative concentrations of A_1 and A_2 which yield a result comparable with the experimental data in fig.1 is 0.15 and 0.85. When the relative concentration differences become large, e.g., 0.30 and 0.70 (fig.2a) with the same k-values, the points can no longer be reasonably approximated by a straight line passing through the origin.

A possible biochemical basis for the postulated heterogeneity relates to PSII, demonstrated to consist of two different kinds of unit in [14]. The β-units in [14] probably have a smaller optical cross-section than the α-units [15], as the β-units have little or no LHCP attached, unlike the α-units [16]. Thus, the β-units would be smaller than the α-units and would have a correspondingly larger diffusion coefficient than the α-units. The β-units are responsible for only ~15% of the total PSII fluorescence yield [17]. Thus we tentatively suggest that in our analysis A_1 is equivalent to the β-PSII units and A_2 is equivalent to the α PSII units. PSI is not known to display any such heterogeneity and would thus be represented by B.

The proportion of α- and β-units is sensitive to $[Mg^{2+}]$ [14]. Thus on passing from 3 mM to zero $MgCl_2$ the α-units decreased from 62% of the total to 36% [14]. We have qualitatively confirmed this observation [10]. According to the above hypothesis, an increase in the proportion of β-units on lowering the $[Mg^{2+}]$ should lead to an increase in the initial deviation of the second-order plot (see fig.2a). This is best seen in the ratio plot of fig.2b where the initially high ratio

$$\left[\frac{x}{a(a-x)}\right]A_1 = 0.30 \Big/ \left[\frac{x}{a(a-x)}\right]A_2 = 0.15$$

decreases towards unity.

The experimental data presented in fig.3 in which the ratio is plotted for the situation

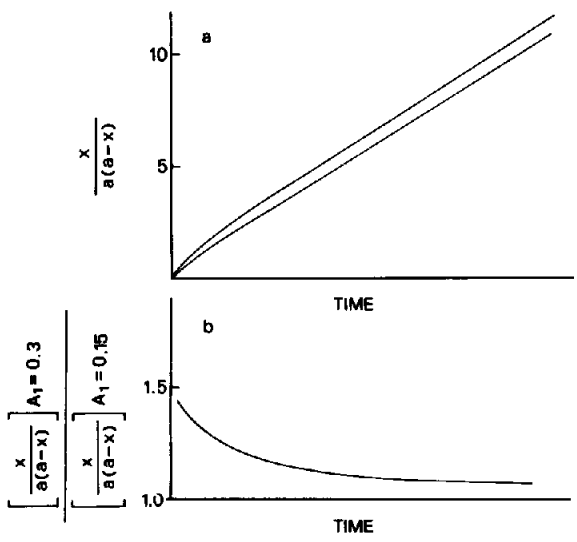

Fig.2. (a) Theoretical plots of the kinetics of a second-order reaction of the type described by eq.(2). $k_1 = 6, k_2 = 1, B = 1$. In the lower curve $A_1 = 0.15, A_2 = 0.85$. In the upper curve $A_1 = 0.3, A_2 = 0.7$. (b) The ratio of the two curves presented in fig.2a.

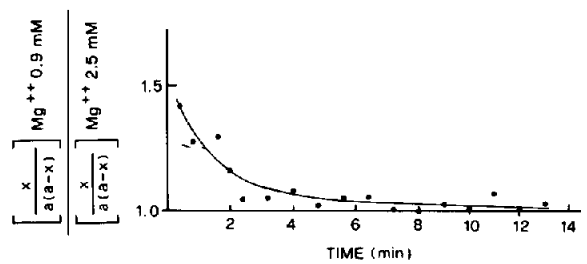

Fig.3. The ratio of the function $x/a(a-x)$ calculated for the fluorescence decline induced by EDTA (3.5 mM) added to chloroplasts prepared and incubated with 0.9 mM and 2.5 mM $MgCl_2$. The experiment was performed at 18°C.

$$\left[\frac{x}{a(a-x)}\right][Mg^{2+}]\ 0.9\ mM/\left[\frac{x}{a(a-x)}\right][Mg^{2+}]\ 2.5\ mM$$

resemble reasonably closely the theoretical situation in fig.2b.

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