Biochimica et Biophysica Acta, 460 (1977) 230–238

BBA 47291

ENERGY TRANSFER AND THE DISTRIBUTION OF EXCITATION ENERGY IN THE PHOTOSYNTHETIC APPARATUS OF SPINACH CHLOROPLASTS

R. J. STRASSER and W. L. BUTLER

Department of Biology, University of California, San Diego, La Jolla, Calif. 92093 (U.S.A.)

(Received October 19th, 1976)

SUMMARY

Equations are derived from our model of the photochemical apparatus of photosynthesis to show that the yield of energy transfer from Photosystem II to Photosystem I, $\varphi_{T(II\to I)}$, can be obtained from measurements on an individual sample of chloroplasts frozen to −196 °C by comparing the sum of two specifically defined fluorescence excitation spectra with the absorption spectrum of the sample. Then, given that value of $\varphi_{T(II\to I)}$, the fraction of the quanta absorbed by the photochemical apparatus which is distributed initially to Photosystem I, α, can be determined as a function of the wavelength of excitation from the same fluorescence excitation spectra. The results obtained in this study of individual samples of chloroplasts frozen to −196 °C in the absence of divalent cations, namely, that $\varphi_{T(II\to I)}$ varies from a minimum value of 0.10 when the Photosystem II reaction centers are all open to a maximum value of 0.25 when the centers are all closed and that α has a value of about 0.30 which is almost independent of wavelength for wavelengths shorter than 675 nm (α increases rapidly toward unity at wavelengths longer than 675 nm), agrees quite well with results obtained previously from comparative measurements of chloroplasts frozen to −196 °C in the presence and absence of divalent cations.

INTRODUCTION

A model for the photochemical apparatus of photosynthesis was developed by Butler and Kitajima [1] in which the distribution of excitation energy between Photosystem I and Photosystem II was specified by two types of parameters. The initial distribution of the energy absorbed by the photochemical apparatus was specified in terms of α, the fraction of the quanta delivered initially to Photosystem I, and β, the fraction delivered to Photosystem II. It was also assumed that, following the initial distribution of energy, some of the quanta in the β fraction going to Photosystem II might be transferred to Photosystem I and a photochemical rate constant term, $k_{T(II\to I)}$, was introduced into the equations to account for such an energy

transfer process. It was recognized in the initial presentation of the model [1] that specific values for α, β and the yield of energy transfer from Photosystem II to Photosystem I, $\varphi_{T(II\to I)}$, should be obtainable from measurements of fluorescence at low temperature.

The model was first tested in a study of the fluorescence of spinach chloroplasts frozen to −196 °C in the presence and absence of Mg^{2+} to determine if the expected Mg^{2+} regulation of energy distribution would be predicted from the fluorescence measurements [2]. The results of that investigation indicated that Mg^{2+} controlled the distribution of energy by regulating both the initial distribution and the subsequent transfer of energy from Photosystem II to Photosystem I; α was 20 % greater and $k_{T(II\to I)}$ was 90 % greater when the chloroplasts were suspended in the absence of Mg^{2+}. Furthermore, there was sufficient information from the measurements made on chloroplasts in the presence and absence of Mg^{2+} to calculate the absolute values of α, β and $\varphi_{T(II\to I)}$. While the results obtained in that study of chloroplasts in the presence and absence of Mg^{2+} were very useful for showing how the energy distribution could be regulated, the method for determining the values of the energy distribution parameters required a comparative study in which conditions could be varied so that both α and $k_{T(II\to I)}$ could be altered in a controlled manner. The purpose of the present paper is to develop a method whereby the distribution of excitation energy can be determined from measurements on a single sample and to compare the results obtained from this method with those obtained earlier.

THEORETICAL CONSIDERATIONS

According to the model [1, 2] the equations for fluorescence from Photosystem II and Photosystem I can be written as:

$$F_{II} = \beta I_a \varphi_{FII}\left[A + \frac{1-A}{1-\varphi_{TII}\varphi_{tII}}\right] \tag{1}$$

$$F_I = I_a \varphi_{FI}\left[\alpha + \beta\varphi_{T(II\to I)}\right] \tag{2}$$

where

$$\varphi_{T(II\to I)} = \varphi_{T(II\to I)\,(0)}\left[A + \frac{1-A}{1-\varphi_{TII}\varphi_{tII}}\right] \tag{3}$$

F_{II} and F_I were measured at −196 °C at 694 and 730 nm, respectively. I_a is the quantum flux absorbed by the photochemical apparatus, φ_{FII} and φ_{FI} are the probabilities that an exciton in the antenna chlorophyll of Photosystem II or Photosystem I will be emitted as fluorescence, φ_{TII} is the probability that an exciton in Photosystem II will be trapped by a Photosystem II reaction center, φ_{tII} is the probability that an exciton trapped by a closed Photosystem II reaction center will be transferred back to the antenna chlorophyll of Photosystem II and A is the fraction of the Photosystem II reaction centers that are open. It is apparent from Eqns. 1 and 3 that F_{II} and $\varphi_{T(II\to I)}$ increase to the same relative extent as the Photosystem II reaction centers are transformed from the open ($A = 1$) state to the closed ($A = 0$) state. F_I also increases from a minimum, $F_{I(0)}$, level to a maximum, $F_{I(M)}$, level as the Photosystem II reaction centers close but all of this fluorescence of variable yield from Photo-

system I, $F_{I(V)}$, is due to the variation in the yield of energy transfer from Photosystem II to Photosystem I. In higher plants, no fluorescence yield changes result from the primary photochemical activity of Photosystem I [1,3]. (In red and blue-green algae, however, photooxidation of P_{700} at −196 °C does cause a small increase in the yield of Photosystem I fluorescence [4].)

It is apparent from Eqn. 2 that F_I can be considered as a sum of two parts, $F_{I(\alpha)}$, due to the direct excitation of Photosystem I, plus $F_{I(\beta)}$, due to excitation of Photosystem II with subsequent energy transfer to Photosystem I. $F_{I(\alpha)}$ can be determined readily from measurements of F_M and F_V of Photosystem I and Photosystem II.

$$F_{I(\alpha)} = F_{I(M)} - \frac{F_{II(M)}}{F_{II(V)}} F_{I(V)} \tag{4}$$

Eqn. 4 represents for any given wavelength of excitation the extrapolation of the straight-line plot of F_I as a function of F_{II} (see ref. 5) back to the F_I axis to determine that part of F_I which is due solely to α.

It will be convenient for purposes of calculation to introduce the terms α_N and β_N which represent the fractions of $F_{I(M)}$ which are due to $F_{I(\alpha)}$ and $F_{I(\beta)}$. (Previously [4, 5] we normalized the value of $F_{I(\alpha)}$ on $F_{I(0)}$. In the present work the normalization will be on the basis of $F_{I(M)}$ since excitation spectra can be measured more readily at the F_M level. Thus, the term $F_{I(\beta)}$ will be understood to represent energy transfer at the maximum $\varphi_{T(II\to I)\,(M)}$ level.)

$$\alpha_N = \frac{F_{I(\alpha)}}{F_{I(M)}} = \frac{\alpha}{\alpha+\beta\varphi_{T(II\to I)\,(M)}} \tag{5}$$

$$\beta_N = \frac{F_{I(\beta)}}{F_{I(M)}} = \frac{\beta\varphi_{T(II\to I)\,(M)}}{\alpha+\beta\varphi_{T(II\to I)\,(M)}} \tag{6}$$

It is apparent that:

$$F_{I(M)} = F_{I(\alpha)} + F_{I(\beta)} = (\alpha_N+\beta_N)F_{I(M)} \tag{7}$$

The purpose of this theoretical treatment is to show that $\varphi_{T(II\to I)}$ and α can be deduced from measurements on a single sample. The excitation spectrum for $F_{I(\alpha)}$ can be obtained from Eqn. 4 with the F_I and F_{II} terms being the appropriate excitation spectra. Given the excitation spectrum for $F_{I(\alpha)}$, the excitation spectrum of $F_{I(\beta)}$ can be obtained as the difference spectrum $F_{I(M)} - F_{I(\alpha)}$. Given these two excitation spectra consider the sum:

$$F_{I(\alpha)} + \frac{F_{I(\beta)}}{\varphi_{T(II\to I)}} = \frac{\alpha+\beta}{\alpha+\beta\varphi_{T(II\to I)\,(M)}} F_{I(M)}$$

Substituting Eqn. 2 for $F_{I(M)}$ and recalling that $\alpha+\beta = 1$, we find:

$$F_{I(\alpha)} + \frac{F_{I(\beta)}}{\varphi_{T(II\to I)\,(M)}} = I_a\varphi_{FI} \tag{8}$$

Thus, we shall try to fit the absorption spectrum I_a (measured on a linear photometric scale) with a weighted sum of the excitation spectra, $F_{I(\alpha)} + KF_{I(\beta)}$, in order

to find the value of K which gives the best fit. This value of K will be taken as the reciprocal of $\varphi_{T(II\rightarrow I)\,(M)}$. Given the value of $\varphi_{T(II\rightarrow I)\,(M)}$, we should also be able to obtain the wavelength dependence of α since:

$$\frac{F_{I(\alpha)}}{F_{I(\alpha)}+F_{I(\beta)}/\varphi_{T(II\rightarrow I)\,(M)}} = \frac{\alpha}{\alpha+\beta} = \alpha \tag{9}$$

The fitting of the weighted sum of the excitation spectra to the absorption spectrum will be attempted only at wavelengths longer than 570 nm where we assume that all of the absorbed light energy is absorbed by the photochemical apparatus. At shorter wavelengths any light energy absorbed by carotenoid pigments which is not transferred to chlorophyll is not included in I_a, i.e., pigments which do not transfer their excitation energy to chlorophyll are not considered to be a part of the photochemical apparatus. Thus, in the blue region of the spectrum the absorption of light by the chloroplasts may be greater than I_a.

MATERIALS AND METHODS

Chloroplasts were prepared by methods described previously [6] and were suspended in a reaction medium consisting of 0.4 M sucrose, 0.01 M NaCl, 0.05 M Tris · HCl, pH 7.8. All measurements were carried out on 0.3 ml aliquots of chloroplasts (10 μg chlorophyll/ml) frozen to liquid nitrogen temperature which gave samples approximately 2-mm thick in our vertical cuvette and Dewar system [7].

Spectral measurements were made with our computer-linked, single-beam spectrophotometer [7]. For fluorescence excitation spectra, the monochromatic exciting light was transmitted from the Cary Model 14 monochromator (0.4 mm slits) to the top of the frozen sample with a fiber optic light pipe assembly [3] which also transmitted the fluorescence from the top of the sample back to the phototube of the spectrophotometer. Fluorescence was measured either at 694 nm (defined by a filter system consisting of a Baird Atomic 694 nm interference filter with Corning 9830 and 2030 glass filters) or at 730 nm (Baird Atomic 730 nm interference filter with Corning 2600 and 5031 filters). The passband of the monochromatic exciting light was approximately 1.5 nm and the intensity of excitation was so low that the measurement of the excitation spectra at the minimum, F_0, level did not alter the fluorescence yield of the frozen sample. Fluorescence excitation spectra were measured at the F_0 level and the F_M level (obtained after a saturating irradiation with blue light). The fluorescence excitation spectra are corrected for equal quantum flux.

The absorption spectrum of the frozen sample was measured on a linear photometric scale. The transmittance of the sample, T, was measured as the ratio of photocurrent obtained with the frozen sample to that obtained with a frozen buffer blank and the absorption spectrum, I_a, was plotted as $(1-T)$.

The computer was used to calculate difference spectra or ratio spectra between various pairs of spectra and to multiply a spectrum by a constant and to take the first derivative of a spectrum. The spectra presented in the figures of this paper were all plotted out directly from the computer from data measured on-line with the spectrophotometer.

RESULTS

Excitation spectra for Photosystem II fluorescence (measured at 694 nm) and for Photosystem I fluorescence (measured at 730 nm) at the F_0 and F_M levels of fluorescence at −196 °C are presented in Fig. 1. Excitation spectra for the fluorescence of variable yield are also presented as the difference spectra, $F_V = F_M - F_0$. The long wavelength limit of these excitation spectra is determined by the wavelengths at which the 694 and 730 nm filters begin to transmit.

We assume that all of the fluorescence measured at 694 nm is due to Photosystem II so that all of the excitation spectra measured at 694 nm should have the same shape. That assumption was examined in Fig. 2 by having the computer divide two excitation spectra, F_V and F_M, and plot the ratio $F_{694(V)}/F_{694(M)}$ as a function of wavelength. The results show that that ratio is constant independent of the excitation wavelength to within limits of about ±1 %. (The same results were also obtained when the excitation spectrum for F_0 was used in the ratio.) Thus, the ratio of $F_{II(M)}/F_{II(V)}$ in Eqn. 4 can be taken as a constant equal to 1.7 from the data in Fig. 2 and $F_{I(\alpha)}$ can be obtained from the excitation spectra measured at 730 nm. The model also predicts that F_V measured at 730 nm and at 694 nm should both be due to Photosystem II excitation. The wavelength independence of the ratio $F_{730(V)}/F_{694(V)}$ shown in Fig. 2 is consistant with this prediction. Thus, all of the factors in the second term of Eqn. 4 are due solely to Photosystem II excitation. The excitation spectrum of $F_{I(\alpha)}$ presented in Fig. 3 was obtained from the computer by the operation stated in Eqn. 4, i.e., the excitation spectrum of $F_{I(V)}$ (see Fig. 1B) was multiplied by 1.7 and subtracted from the excitation spectrum of $F_{I(M)}$. The excitation spectrum of $F_{I(\beta)}$, also shown in Fig. 3, was obtained as the difference spectrum $F_{I(M)} - F_{I(\alpha)}$.

It was decided that the most sensitive criterion by which to fit the curve of $F_{I(\alpha)} + KF_{I(\beta)}$ to the absorption spectrum would be to match the curves for the wavelength position of their maxima (the wavelength maximum for I_a lies between the

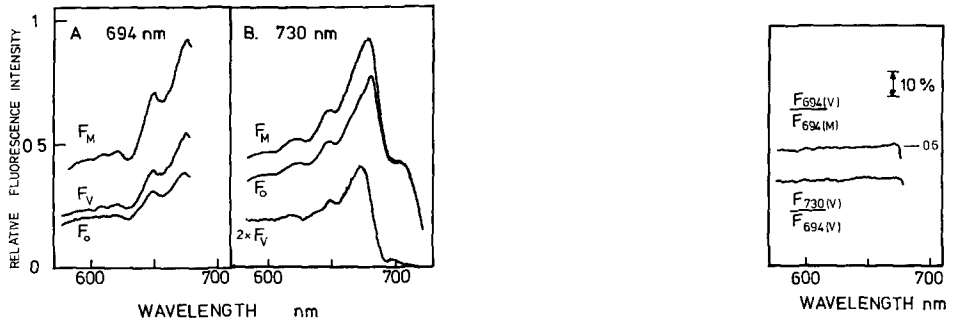
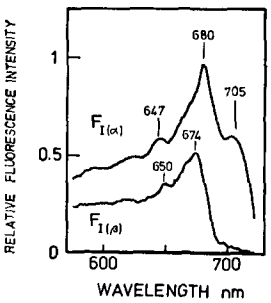


Fig. 1. Excitation spectra of fluorescence from spinach chloroplasts at −196 °C measured at the F_0 and F_M levels. The excitation spectra for F_V were calculated from the difference spectra $F_M - F_0$. A. Excitation spectra for fluorescence measured at 694 nm. B. Excitation spectra for fluorescence measured at 730 nm.

Fig. 2. Ratios of the fluorescence excitation spectra from Fig. 1. The ratio $F_{694(V)}/F_{694(M)}$ has a well-defined value equal to about 0.6. The ratio $F_{730(V)}/F_{694(V)}$ is plotted in a relative scale since the absolute value of the ratio will depend on spectral sensitivity of the phototube. The two ratios, however, are plotted on the same relative sensitivity scale.



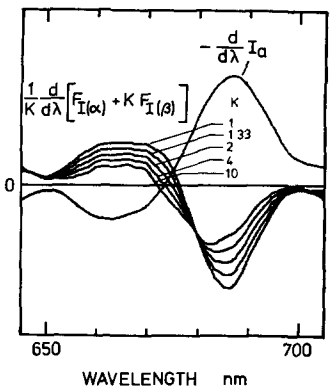
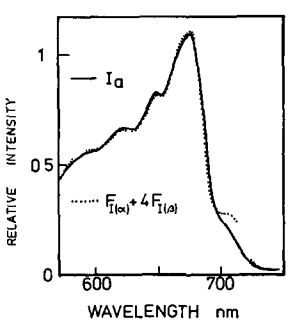


Fig. 3. Excitation spectrum of $F_{I(\alpha)}$ calculated by the computer by the operation stated in Eqn. 4. Excitation spectrum of $F_{I(\beta)}$ calculated as the difference spectrum $F_{I(M)} - F_{I(\alpha)}$.

Fig. 4. A comparison of the negative of the first derivative of the absorption spectrum, I_a, with the first derivative spectra of $F_{I(\alpha)}+KF_{I(\beta)}$. Different values of K are examined to find the value ($K = 4$) such that derivative of the sum of excitation spectra crosses the zero line at the same wavelength as the derivative of the absorption spectrum. The absorption spectrum, I_a, was measured on a linear photometric scale.

maxima of $F_{I(\alpha)}$ and $F_{I(\beta)}$). Thus, the negative of the first derivative of I_a was plotted (see Fig. 4) and different values of K were tried to find a value such that the first derivative of $F_{I(\alpha)}+KF_{I(\beta)}$ crossed the zero line at the same wavelength as the first derivative of the absorption spectrum. The family of curves for the first derivative of $F_{I(\alpha)}+KF_{I(\beta)}$ tended to be somewhat confusing because of overlaps near the zero line so that, for presentation in Fig. 4, each curve was divided by K. This division process changes the magnitudes of the derivative curves but not the positions where they cross the zero line. The family of curves shows that our curve matching criterion is satisfied most closely when $K = 4$. These same measurements and analysis procedures were repeated on several different chloroplast samples prepared on different days and in each case a value of K of 5 was detectably too large



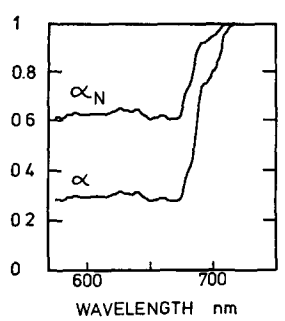


Fig. 5. A comparison of the absorption spectrum, I_a, (solid line) with the weighted sum of excitation spectra, $F_{I(\alpha)}+4F_{I(\beta)}$ (dotted line).

Fig. 6. Spectral dependence of α_N calculation as the ratio $F_{I(\alpha)}/F_{I(M)}$. Spectral dependence of α calculated by Eqn. 9 using a value of 0.25 for $\varphi_{T(II\to I)\,(M)}$.

and a value of 3 was detectably too small. Thus, this criterion of matching the wavelength maxima indicates a value of $\varphi_{T(II\rightarrow I)\,(M)}$ of 0.25 ± 0.05. We also estimate from the ratio that $F_{II(M)}/F_{II(0)}$ is 2.4 (since $F_{II(M)}/F_{II(V)} = 1.7$) that $\varphi_{T(II\rightarrow I)\,(0)}$ is 0.10. A comparison of the absorption spectrum of I_a and the sum of the excitation spectra $F_{I(\alpha)}+F_{I(\beta)}/\varphi_{T(II\rightarrow I)\,(M)}$ using a value of 0.25 for $\varphi_{T(II\rightarrow I)\,(M)}$ is shown in Fig. 5 as the solid and dotted curves, respectively.

The values and wavelength dependencies of α_N and α are shown in Fig. 6. The spectral distribution of α_N was plotted as the ratio of $F_{I(\alpha)}/F_{I(M)}$ according to Eqn. 5. The spectral distribution of α was calculated and plotted (by the computer) according to the operations indicated in Eqn. 9 using a value of 0.25 for $\varphi_{T(II\rightarrow I)\,(M)}$. α can also be calculated from α_N according to Eqn. 5

$$\alpha = \frac{\varphi_{T(II\rightarrow I)\,(M)}\alpha_N}{1-(1-\varphi_{T(II\rightarrow I)\,(M)})\alpha_N}$$

At wavelengths shorter than 675 nm α_N is approximately 0.63 and α is approximately 0.3 and both are practically independent of wavelength. At wavelengths beyong 675 nm α rises steeply as Photosystem I becomes the dominant absorber in the photosynthetic apparatus.

DISCUSSION

The values of $\varphi_{T(II\rightarrow I)}$ and α reported here from measurements on individual samples of chloroplasts frozen to -196 °C in the absence of divalent cations are in close agreement with the results obtained earlier from comparative studies of chloroplasts frozen to -196 °C in the presence and absence of Mg^{2+}. The earlier studies [2] indicated that $\varphi_{T(II\rightarrow I)}$ for chloroplasts in the absence of Mg^{2+} varied from a minimum value of 0.12 when the Photosystem II reaction centers were all open to a maximum value of 0.28 when the centers were all closed and that α was 0.32. The comparable values from the present study are 0.10 for $\varphi_{T(II\rightarrow I)\,(0)}$, 0.25 for $\varphi_{T(II\rightarrow I)\,(M)}$ and 0.30 for α.

The two studies used different methods to estimate $\varphi_{T(II\rightarrow I)}$. The earlier work required comparative measurements of fluorescence made in the presence and absence of Mg^{2+} which altered both α and the rate constant for energy transfer, $k_{T(II\rightarrow I)}$. The present method derives the necessary information from the absorption and fluorescence properties of a single sample of chloroplasts at -196 °C. The close agreement between these two methods indicates the general validity of both methods and further substantiates the model of the photochemical apparatus from which the two methods were derived. The close agreement, however, does not rule out the possibility that systematic errors in the measurement may affect the results of both methods. It was pointed out previously [5] that an overlap of fluorescence emission bands in the 692 nm region (where Photosystem II emission was measured) would tend to result in values of α which were too low. The same uncertainties apply to the results reported here so we assume that the method reports a minimum value of α.

The comparative method based on a divalent cation induced change of $k_{T(II\rightarrow I)}$ probably gives a more sensitive and reliable determination of $\varphi_{T(II\rightarrow I)}$ than the method based on the curve fitting of absorption and fluorescence excitationspectra, at least for spinach chloroplasts. It is apparent that the curve fitting procedures will

be effective only in spectral regions where the excitation spectra of $F_{I(\alpha)}$ and $F_{I(\beta)}$ are appreciably different. Since $F_{I(\alpha)}$ and $F_{I(\beta)}$ are proportional to αI_a and βI_a, respectively, these procedures will be applicable for spinach chloroplasts only in the long wavelength region where the wavelength dependencies of α and β are appreciably different. In the work reported here we chose as our criterion for curve fitting the position of the long wavelength maxima and we used the first derivatives of the curves to achieve a higher degree of sensitivity. However, the curve fitting procedures would be more sensitive to the value of $\varphi_{T(II\to I)}$ with samples which had a stronger wavelength dependence of α and β across the spectrum. A special case of the general procedure was used previously [8] to determine $\varphi_{T(II\to I)}$ for individual samples of the red alga, *Porphyridium cruentum*, in which α shows a marked wavelength dependence across the visible spectrum [4]. We also look forward to using these procedures to examine the distribution of excitation energy in dark grown bean leaves which have been partially greened by a series of brief flashes. We expect that these leaves will show a greater variation of α and β with wavelength than mature chloroplasts because they lack the light-harvesting chlorophyll *a/b* protein which appears to buffer out the changes of α which would otherwise arise from differences in the absorption spectra of Photosystem I and Photosystem II units [5].

The curve fitting procedures are not applicable in the extreme long wavelength region beyond 700 nm because of the presence of an excitation band at about 705 nm for the 730 nm fluorescence. This excitation band is due to a species of chlorophyll denoted previously as C-705 (9) which absorbs in the 705 nm region (note the shoulder between 700 and 720 nm in the absorption spectrum in Fig. 6) and emits at 730 nm at −196 °C. C-705 appears to act as a trap for the excitation energy in the antenna chlorophyll of Photosystem I. It is apparent from our attempt to match the absorption spectrum of I_a with the weighted sum of the excitation spectra in Fig. 6 that the yield of 730 nm fluorescence is greater when C-705 is excited directly than when the antenna chlorophyll of Photosystem I is excited. However, the amount of C-705 is quite low relative to the main bulk of the chlorophyll so that the effects of the direct excitation of C-705 are apparent only at wavelengths longer than 700 nm. We conclude that our model for the photosynthetic apparatus and the equations which are the essence of that model are valid for excitation wavelengths up to 700 nm. We could modify the model to accommodate the special role of C-705 in Photosystem I fluorescence but such modifications do not appear to be essential at this time.

ACKNOWLEDGEMENTS

This work was supported by a grant from the National Science Foundation, BMS73-06884. Dr. Strasser is the recipient of a Fellowship from The Swiss National Science Foundation NR. 831.190.73.

REFERENCES

1 Butler, W. L. and Kitajima, M. (1975) Proceedings of the Third International Congress on Photosynthesis (Avron, M., ed.), pp. 13–24, Elsevier, Amsterdam
2 Butler, W. L. and Kitajima, M. (1975) Biochim. Biophys. Acta 396, 72–85

3 Strasser, R. J. and Butler, W. L. (1976) Biochim. Biophys. Acta, 449, 412–419
4 Ley, A. C. and Butler, W. L. (1977) Plant Cell Physiol., in the press
5 Kitajima, M. and Butler, W. L. (1975) Biochim. Biophys. Acta 408, 297–305
6 Yamashita, T. and Butler, W. L. (1968) Plant Physiol. 43, 1978–1986
7 Butler, W. L. (1972) Methods in Enzymology (San Pietro, A., ed.), Vol. 24, Part B, pp. 3–25, Academic Press, New York
8 Ley, A. C. and Butler, W. L. (1976) Proc. Natl. Acad. Sci. U.S., 73, 3957–3960
9 Butler, W. L. (1961) Arch. Biochem. Biophys. 93, 413–422