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SPECTROSCOPIC PROPERTIES OF PROTOCHLOROPHYLL FORMS IN TRITON X-100 DETERGENT MICELLES

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Protochlorophyll (PChl) forms were performed in Triton X-100 detergent micelles. The concentration of Triton X-100 was $7 \cdot 10^{-4}$ M (above the critical micellar concentration); the concentration of PChl varied between $1.6 \cdot 10^{-5}$ and $1.8 \cdot 10^{-4}$ M. Absorption, fluorescence and circular dichroism (CD) spectra were registered. The absorption spectra were resolved into Gaussian components by computer analysis. PChl forms with absorption bands at 632–634, 638, 652–654, 663–664, 668 and 676 nm and with fluorescence emission bands at 634–636, 640–644, 652–655, 677–678, 686 and 694–696 nm were observed in micellar solutions of different PChl concentrations. The CD spectra showed a strong dependence on the concentration of PChl: positive CD signals or positive Cotton effects were observed in the vicinity of 650 nm. The intensity of these signals increased in parallel with increasing concentration of PChl. No CD signals were found in the region of the longer wavelength absorption bands. These data show that the PChl exists in many different forms in this system, and the spectroscopic properties of these forms are determined by different molecular interactions viz., interactions of PChl with Triton X-100 or water molecules and/or by the aggregation of PChl.

Introduction

It is well established that protochlorophyll/ide (PChl/ide) in vivo has different spectroscopic forms which are characterized by their absorption and/or fluorescence maxima [1,2]. Model systems of different nature are often used to investigate the structural origin of the differences in spectroscopic characteristics of these pigment forms. The results of these experiments with pigment solutions in the case of polar and nonpolar [3–10] and mixed water/dioxane [11] solvents, with solid films [12–14] and mono or multilayers [15–18] of pigments, made us draw the conclusions that the spectroscopic properties of the chlorophyll pigment forms are determined by the effect of interactions of the pigment molecules with solvent molecules or other nonchomophore ones and/or by interactions of the pigment molecules with each other. On the basis of infrared [4,9], CD [6,8,14] and NMR [10] spectroscopy data, the molecular structure or arrangement of several aggregates of PChl was discussed.

Detergent micellar solutions of photosynthetic pigments constitute a remarkable class of model systems. The interaction of chlorophyll *a* (Chl *a*) molecules with detergent molecules was observed at high concentrations (well above the critical micellar concentrations) of different detergents (Triton X-100, Brij 35, Brij 58, cetylpyridinium chloride, SDS, etc.) which resulted in the appearance of a simple absorption maximum of Chl *a* at 672 nm [19] and the shapes of the absorption [19] and fluorescence [20] spectra were found to be

very similar to those of Chl *a* solutions in acetone [19]. Detergents in a high concentration (also well above the critical micellar concentration) were observed to solubilize crystals of Chl *a* dihydrates with an absorption maximum at 745 nm, which caused a shift of this maximum to 672 nm [19,20]. When Chl *a* was dissolved in detergent solutions and the concentration of detergent was around its critical micellar concentration, the chlorophyll pigments showed complex spectroscopic properties because of splitting of the absorption and fluorescence maxima [21]. The detergent micelles can concentrate the pigment molecules in themselves; the local concentration of pigment in the micelles may be higher by 1–3 orders of magnitude than the overall concentration of pigment in the solution. In this way the local concentration of chlorophyll can reach the in vivo concentration of 0.1 M [22]. The structure and size of the Triton X-100 micelles – aggregation number, 134–140; diameter, 48 Å; for pure micelles [23–25] – make possible at least two ways of localization for chlorophyll pigments: molecules can associate with the hydrophilic region, or they can build into the hydrophobic microenvironment of the micelles [26,27]. The study of energy migration, concentration-dependent fluorescence depolarization and concentration-dependent fluorescence quenching showed that the chlorophyll can exist in the micelles in monomeric and in aggregated forms [26,28]. Micellar solutions of Triton X-100 proved to be suitable for preparing different Chl *a* and Chl *b* forms [21], and for studying the photochemical properties of chlorophyll pigments [29–31].

In this work PChl forms were prepared in micellar solutions of Triton X-100, and their spectroscopic properties were examined by absorption, fluorescence and CD spectroscopy.

Materials and Methods

PChl was extracted from pumpkin seed coat and then purified by column chromatography [4]. To study the effect of the nonionic detergent Triton X-100 (Serva) on the spectroscopic properties of PChl, PChl was dissolved in pure Triton X-100 and in Triton X-100 solutions of 10^{-2} and $7 \cdot 10^{-6}$ M (well above and below, respectively, the critical micellar concentration of $3 \cdot 10^{-4}$ M [21]). For the preparation of micellar solutions Triton X-100 was used in a concentration of $7 \cdot 10^{-4}$ M. Triton X-100 was diluted in every case with phosphate buffer (0.05 M, pH 7.2). The concentration of PChl was $1.6 \cdot 10^{-5}$ M in pure Triton X-100 and in Triton X-100 solutions of 10^{-2} and $7 \cdot 10^{-6}$ M. The final concentration of PChl ranged from $1.6 \cdot 10^{-5}$ M to $1.8 \cdot 10^{-4}$ M in the micellar solutions. The preparation of micellar solutions was carried out as follows: to a given amount of Triton X-100 solution at the above-mentioned concentration of $7 \cdot 10^{-4}$ M, different volumes of PChl solution (diethyl ether as solvent) were added gradually with continuous stirring. This solution was then heated to 40°C under low vacuum to remove the diethyl ether. The absorption spectra were registered with an SF 18 recording spectrophotometer with integrating sphere. These spectra were then analyzed by a computer program used also in other works [21,32]. The fluorescence spectra were measured with a Perkin-Elmer MPF 44B type spectrofluorometer using a high-sensitivity cell-holder accessory. The CD spectra were registered with a CNRS-Roussel-Jouan-Dichrograph III type spectropolarimeter. Cells of 1 mm thickness were used for CD measurements and CD spectra were taken with the sample directly in front of the photomultiplier.

Results

The absorption and fluorescence emission spectra of PChl dissolved in pure Triton X-100 and in Triton X-100 solution of $2 \cdot 10^{-2}$ M were very similar to those of PChl dissolved in acetone or in diethyl ether [5,6] (similar to Chl *a* [19]), but the maxima were shifted: the Q_x band appeared at about 610 nm and the Q_y band was observed at 632 nm in the absorption spectrum. The fluorescence emission spectrum showed a simple, sharp band at 634 nm and a broad shoulder around 685 nm with low intensity. When PChl was dissolved in Triton X-100 solution of $7 \cdot 10^{-6}$ M, the solution had a large extent of light scattering and the absorption and fluorescence spectra showed broad maxima between 635 and 640 nm with low intensity. The latter results are very similar to those for PChl dissolved in water or in phosphate buffer [33,34].

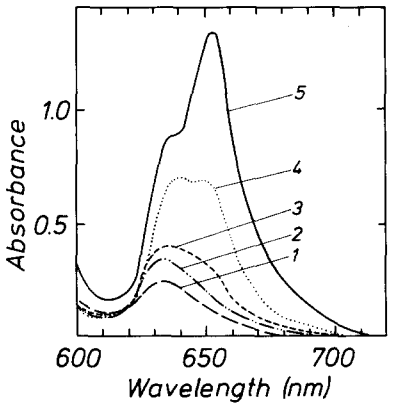

Fig. 1. Absorption spectra of Triton X-100 micellar solutions (Triton X-100 concentration: $7 \cdot 10^{-4}$ M) with different concentrations of PChl: (curve 1) $1.6 \cdot 10^{-5}$ M, (curve 2) $3.6 \cdot 10^{-5}$ M, (curve 3) $6.3 \cdot 10^{-5}$ M, (curve 4) 10^{-4} M, (curve 5) $1.8 \cdot 10^{-4}$ M.

The presence of Triton X-100 micelles had a remarkable effect on the spectroscopic properties of PChl dissolved in micellar solutions: the absorption and fluorescence maxima became complex. For the characterization of PChl forms in this system, the red region of the absorption spectra was studied. At low concentrations of PChl ($1.6 \cdot 10^{-5}$ M) the main absorption maximum appeared at 632 nm and a broad shoulder was observed in the vicinity of 680 nm. At higher concentrations of PChl the main absorption maximum shifted to 652 nm (Fig. 1). The fluorescence emission spectra showed bands at 634–636, 640, 677–678 and 686 nm and a shoulder at about 690 nm when the excitation was carried out with 450 nm light (Fig. 2A). When the excitation wavelength was shifted to 480 nm the fluorescence bands appeared at longer wavelengths, viz., 644, 652–655, 678, 686 and 694–696 nm (Fig. 2B). This dependence of the fluorescence emission spectra on excitation wavelength is due to the complexity of the Soret region of the fluorescence excitation spectra which showed two groups of bands: when the excitation spectra were measured at 640 nm the main Soret bands were found at 440, 452–455 and 462–467 nm while in the excitation spectra measured at 700 nm the main bands appeared at 480 and 492 nm. Excitation between 440 and 467 nm resulted in the appearance of shorter wavelength bands at 643–636 and 640 nm with higher intensity and excitation between 480 and 492 nm caused the appearance or increase in bands at 652–655, 677–678 and 694–696 nm. In parallel with the increasing concentration of PChl the intensity of the latter group of bands became higher. Also, the CD spectra of the solutions showed a

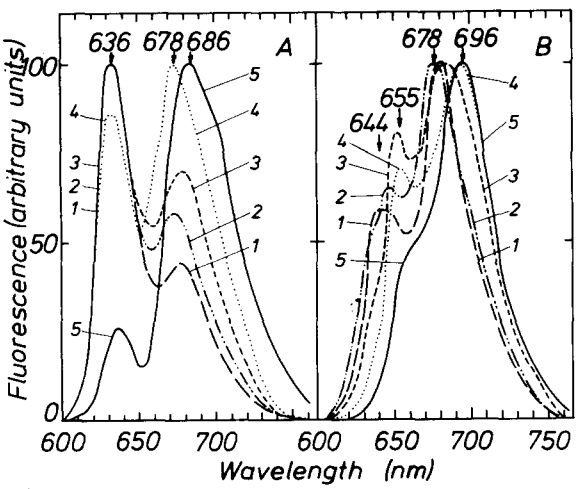

Fig. 2. Fluorescence emission spectra of Triton X-100 micellar solutions (Triton X-100 concentration: $7 \cdot 10^{-4}$ M) with different concentrations of PChl (curves denoted as in Fig. 1). (A) Wavelength of excitation, 450 nm; (B) Wavelength of excitation, 480 nm.

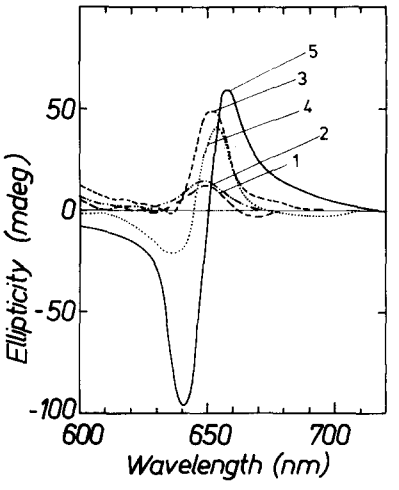

Fig. 3. CD spectra of Triton X-100 micellar solutions (Triton X-100 concentration: $7 \cdot 10^{-4}$ M) with different concentrations of PChl (curves denoted as in Fig. 1). The spectra are normalized with respect to the concentration of PChl of the solution depicted by curve 5 ($1.8 \cdot 10^{-4}$ M).

strong dependence on the concentration of PChl. In the CD spectra – normalized with respect to the concentration of PChl (Fig. 3) – the intensity and also the shape of the signals changed. In the case of $1.6 \cdot 10^{-5}$ and $3.6 \cdot 10^{-5}$ M PChl concentrations, positive signals of low intensity appeared in the vicinity of 650 nm. The CD signal of the solution of $6.3 \cdot 10^{-5}$ M PChl concentration increased significantly, which may be connected with the great relative increase in absorbance of this solution at 650 nm (see below). When the concentration of PChl was higher – 10^{-4} and $1.8 \cdot 10^{-4}$ M –, positive Cotton effects appeared in the CD spectra. Computer analysis of the red region of the absorption spectra showed that for every solution the component at about 650 nm was present. The ratio of the heights of this Gaussian component at 650 nm to the component at 634 nm gradually increased (0.24, 0.27, 0.64, 0.70 and 0.81, respectively, for the five examined solutions) in parallel with increasing PChl concentration. The computer analysis showed also the presence of long-wavelength bands at 663–664, 668 and 676 nm. For the sake of illustration the analysed absorption spectra of the solutions with the lowest – $1.6 \cdot 10^{-5}$ M (Fig. 4), and highest – $1.8 \cdot 10^{-4}$ M (Fig. 5) – PChl concentrations are presented.

In order to study the effect of the micellar structure of the solutions on the appearance of different PChl forms, the above-studied solutions were diluted, different volumes of phosphate buffer were added to them, and the absorption, fluorescence emission and CD spectra were measured. When the PChl and Triton X-100 concentrations were reduced in this way 2-, 5- and 10-fold, the intensity of the absorption bands decreased to different extents: the decrease in the bands at 634 and 650 nm was smaller, therefore, the ratio of these bands increased in the spectra, and the bands at longer wavelengths showed a relatively greater decrease. In the case of the 100-fold diluted solutions these bands at 663–664, 668 and 676 nm disappeared and the ratio of the bands at 634 and 650 nm showed a further increase. In the fluores-

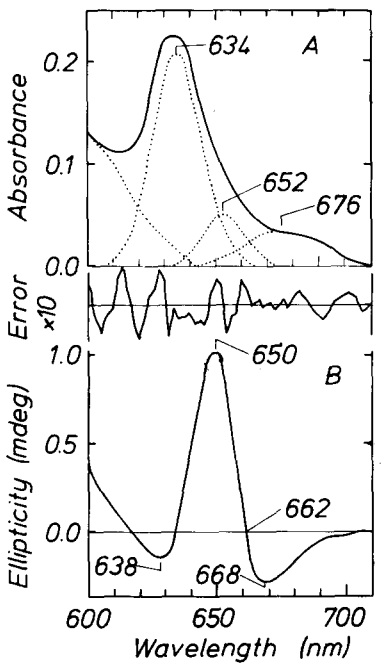

Fig. 4. Absorption spectrum, computer analysis of the absorption spectrum with the error of the analysis (A) and CD spectrum (B) of a micellar solution with a Triton X-100 concentration of $7 \cdot 10^{-4}$ M and with a PChl concentration of $1.6 \cdot 10^{-5}$ M.

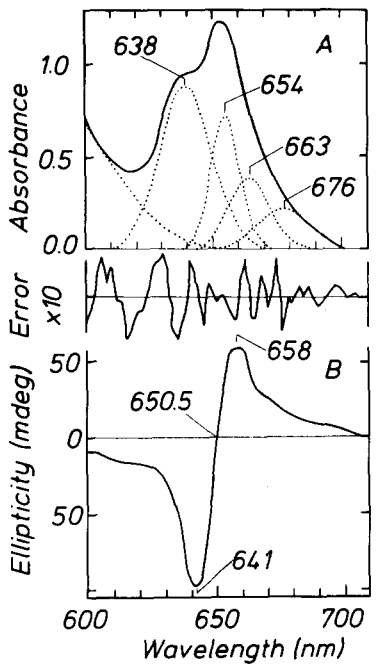

Fig. 5. Absorption spectrum, computer analysis of the absorption spectrum with the error of the analysis (A) and CD spectrum (B) of a micellar solution with a Triton X-100 concentration of $7 \cdot 10^{-4}$ M and with a PChl concentration of $1.8 \cdot 10^{-4}$ M.

cence emission spectra the ratio of the long-wavelength maxima at 677–678, 686 and 694–696 nm decreased significantly. Dilution had an effect only on the intensity of the CD signals; neither the position nor the shape (the ratio of the positive signals to the negative ones) changed compared with the spectra in Fig. 3.

Discussion

The results presented in the literature and also the data of this work indicate that micellar solutions of Triton X-100 are suitable models for preparation of different chlorophyll pigment forms and for the examination of their spectroscopic properties. In the solutions studied in this work, PChl forms with similar absorption and fluorescence properties to those in vivo could be observed. The ratio of these forms depended on the concentration of PChl and in the case of the highest concentration studied in this paper, this ratio was very similar to that of PChl/ide forms in etiolated leaves [1,2] or in pumpkin seed coat [35,36]: their absorption spectra showed great similarities; in all three cases the main absorption band could be found at about 650 nm and a shoulder appeared around 635 nm. Also, the fluorescence emission spectra exhibited bands in positions similar to those in vivo [2,36], but the ratios of the intensities of bands (for example, I_{678}/I_{636} or I_{678}/I_{650}, etc.) showed considerable differences compared with those in vivo: these ratios were significantly higher in the micelles than in vivo. These results, compared with the data of absorption spectroscopy, can be explaned by the great efficiency of energy migration in Triton X-100 micelles [26,27] which takes place in our systems from the short-wavelength PChl forms with absorption maxima at 632–634, 638 and 652–654 nm to the longer wavelength ones. In this way the long-wavelength bands dominate in the fluorescence emission spectra of micellar solutions with high PChl concentrations.

PChl/ide forms with spectroscopic properties similar to those of forms observed in Triton X-100 micellar solutions were also found in other in vitro systems: in nonpolar solvents [3–10] and in solid films [12–14,37]. There were similarities in the CD properties of these forms: the forms with a 635 nm absorption maximum and no CD signal in solid films [14] and also the CD spectra of the micellar solutions showed no CD signals in this region (Fig. 3, curves 1–3); the form with a 650 nm absorption maximum had a positive Cotton effect around 650 nm in the case of solid films treated with solvent vapor [14,37] and of pumpkin seed coat [36], similar to PChl forms studied in this work. On the other hand, the PChlide form with a 650 nm absorption maximum had a negative CD signal around 650 nm in liquid paraffin oil [8], and homogenates of etiolated leaves had broad negative CD signals in the red region of the CD spectrum [38,39]. These differences may refer to the differences in the structures – molecular arrangements – between PChl and PChlide forms [14]. Fewer data are available about the PChl forms with long-wavelength maxima. The etiolated leaves and their homogenates had several absorption and fluorescence emission maxima in the 660–710 nm region of the spectra [40,41] but it has not been exactly verified if these bands were due only to certain forms of PChl/ide. However, the PChl-containing *Cyclanthera* seed coat was found to have mainly these long-wavelength forms which were attributed to 'watered' or 'hydrated' crystals of PChl [42,43], and these forms were found also in several in vitro systems [7,12] – similar to the micellar solutions described here. Despite the similarities in the absorption and fluorescence properties of these forms, there were differences in their CD spectra: the forms of *Cyclanthera* seed coat had very intense CD signals [42] but these forms observed in the micellar solutions had only very weak or no CD signals at all (the disappearance of these long-wavelenght forms in the case of the 100-fold diluted solutions had no effect on the shape of the CD signals around 650 nm).

The spectroscopic properties of the PChl forms described in this paper are determined by different molecular interactions of PChl. Taking into consideration that the main absorption and fluorescence bands of PChl dissolved in pure Triton X-100 or in its solution of 10^{-2} M concentration were found at 632 and 634 nm, respectively, and that these bands appeared also in the spectra of the micellar solutions, it can be concluded that PChl molecules can interact with Triton X-100 molecules also in micellar solutions. The splitting

of the red absorption and fluorescence maxima, the large red shift of several bands and the dependence of these bands on the concentration of PChl, and the presence of the bands at 480 and 492 nm in the Soret region on the fluorescence excitation spectra (these bands are well known in the absorption spectra of solid films containing aggregates [13] and in the fluorescence excitation spectra of *Cyclanthera* seed coats containing PChl crystals [42]) are good evidence for the aggregation of PChl in Triton X-100 micelles. Further evidence for the aggregation of PChl is given by CD spectroscopy: the increase in intensity of the CD signals in the spectra normalized with respect to the concentration of PChl and the changes of the shapes of these signals are arguments for aggregation [44]. Aggregation was notable in the case of a PChl concentration of $6.3 \cdot 10^{-5}$ M and at higher concentrations: the absorbance at 650 nm and the intensity of the CD signals increased significantly. The average maximal number of PChl molecules contained in one micelle is 12.6 in the solution of $6.3 \cdot 10^{-5}$ M PChl concentration (these values are 3.2, 7.2, 12.6 20 and 36, respectively, for the examined five solutions). This value seems to be necessary for the accumulation of aggregates. Because all the CD signals were found in the vicinity of 650 nm, the forms with bands at 652–654 nm may be due to aggregates of PChl exhibiting exciton-type interactions. These aggregates must have a stable structure: after the 100-fold dilution of the micellar solutions, the absorption of fluorescence bands around 650 nm could be observed (in Triton X-100 solution of the same, $7 \cdot 10^{-6}$ M Triton X-100 concentration and with a similar PChl concentration or in phosphate buffer this form cannot be prepared) and this dilution reduced the intensity of the CD signals but had no effect on their shape. This dilution caused great changes in the long-wavelength region (above 660 nm) of the absorption and fluorescence spectra: the long wavelength band gradually decreased. It shows that their appearance is connected with the micellar structure. On the basis of the position of these bands it can be concluded that the forms corresponding to these bands are aggregates of PChl with a high degree of aggregation. Their structure, however, differs from that of the aggregates of crystals of PChl found in *Cyclanthera* seed coat with similar absorption and fluorescence properties, which probably causes the differences in their CD properties. The absence of CD signals corresponding to the long-wavelength absorption and fluorescence bands of micellar solutions can be explained by a random geometry (arrangement) of the aggregate. Because of the limited volume of the micelles such 'stacks' of PChl molecules can probably appear via overlap of the π-electron systems of their porphyrin rings. This overlap can cause large red shifts in the absorption and fluorescence spectra [45]. As in this case the interactions are realised not via the interactions between magnesium atoms and keto C = O groups [46] of PChl molecules (interactions which have specific steric requirements), the molecular arrangement corresponding to magnesium-keto C = O interactions is not assured and consequently, the PChl molecules can interact via π-π interactions in any positions. A CD signal belonging to a π-π interaction or to an aggregate with a given geometry (a geometry which determines the shape of CD signals of Chl aggregates [47]) can be cancelled by another (opposite) CD signal belonging to another aggregate with different geometry. The spectroscopic properties of all PChl forms described in this work are probably also influenced by interactions with water molecules.

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References

1 Shibata, K. (1957) J. Biochem. (Jap.) 44, 147–173
2 Virgin, H.I. (1981) Annu. Rev. Plant. Physiol. 32, 451–463
3 Seliskar, C.J. and Ke, B. (1968) Biochim. Biophys. Acta 153, 685–691
4 Houssier, C. and Sauer, K. (1969) Biochim. Biophys. Acta 172, 476–491
5 Houssier, C. and Sauer, K. (1969) Biochem. Biophys. Acta 172, 492–502
6 Houssier, C. and Sauer, K. (1970) J. Am. Chem. Soc. 92, 779–791
7 Brouers, M. (1972) Photosynthetica 6, 415–423
8 Brouers, M. (1975) Photosynthetica 9, 304–310

9 Brouers, M. (1979) Photosynthetica 13, 9–14
10 Rasquain, A., Houssier, C. and Sironval, C. (1977) Biochim. Biophys. Acta 462, 622–641
11 Zenkevitch, E.I. and Losev, A.P. (1976) Mol. Biol. 10, 294–303 (in Russian)
12 Krasnovsky, A.A., Bystrova, M.I. and Láng, F. (1971) Dokl. Akad. Nauk. S.S.S.R. 201, 1485–1488 (in Russian)
13 Bystrova, M.I., Láng, F. and Krasnovsky, A.A. (1972) Mol. Biol. 6, 77–86 (in Russian)
14 Böddi, B., Soós, J. and Láng, F. (1980) Biochim. Biophys. Acta 593, 158–165
15 Ke, B. and Sperling, W. (1966) Brookhaven Symp. Biol. 19, 319–327
16 Aoshima, R., Iriyama, K. and Asai, H. (1975) Biochim. Biophys. Acta 406, 362–369
17 Hirsch, R.E. and Brody, S.S. (1979) Photochem. Photobiol. 29, 589–596
18 Rich, M. and Brody, S.S. (1981) Photochem. Photobiol. 33, 271–274
19 Mukherjee, T., Sapre, A.V. and Mittal, J.P. (1978) Photochem. Photobiol.28, 95–96
20 Brown, R.G. and Evans, E.H. (1980) Photochem. Photobiol. 32, 103–105
21 Lehoczki, E. (1975) Biochim. Biophys. Acta 408, 223–227
22 Csatorday, K., Lehoczki, E. and Szalay, L. (1975) Biochim. Biophys. Acta 376, 268–273
23 Yedgar, S., Barenholz, Y. and Cooper, V.G. (1974) Biochim. Biophys. Acta 363, 98–111
24 Helenius, A. and Simons, K. (1975) Biochim. Biophys. Acta 415, 29–79
25 Kalyanasundaram, K. and Thomas, J.K. (1977) in Micellization, Solubilization and Microemulsions (Mittal, K.L., ed.), pp. 307–326, Plenum Press, New York
26 Zenkevitch, E.I., Losev, A.P. and Gurinovich, G.P. (1972) Mol. Biol. 6, 824–832 (in Russian)
27 Lehoczki, E., Csatorday, K., Szalay, L. and Szabad, J. (1975) Biofizika 20, 44–50 (in Russian)
28 Lehoczki, E. and Csatorday, K. (1975) Biochim. Biophys. Acta 396, 86–92
29 Massini, P. and Voorn, G. (1968) Biochim. Biophys. Acta 153, 589–601
30 Shaposnikova, M.G., Drozdova, N.H. and Krasnovsky, A.A. (1971) Biokhimiya 36, 704–710 (in Russian)
31 Van Ginkel, G. (1980) Photochem. Photobiol. 30, 397–404
32 Demeter, S., Horváth, G., Joó, F., Halász, N. and Faludi-Dániel, Á. (1974) Physiol. Plant. 32, 222–227
33 Krasnovsky, A.A., Kosobutskaya, L.M. and Voynovskaya, K.K. (1953) Dokl. Akad. Nauk. S.S.S.R. 92, 1201–1204 (in Russian)
34 Krasnovsky, A.A. and Bystrova, M.I. (1962) Biokhimiya 27, 958–966 (in Russian)
35 Inada, Y. and Shibata, K. (1960) Plant Cell Physiol. 1, 311–316
36 Böddi, B., Soós, J. and Láng, F. (1979) Plant Sci. Lett. 16, 75–79
37 Bystrova, M.I., Safronova, I.A. and Krasnovsky, A.A. (1982) Mol. Biol. 16, 291–301 (in Russian)
38 Mathis, P. and Sauer, K. (1972) Biochim. Biophys. Acta 267, 498–511
39 Schultz, A. and Sauer, K. (1972) Biochim. Biophys. Acta 267, 320–340
40 Láng, F., Vorobyeva, L.M. and Krasnovsky, A.A. (1972) in Photosynthesis, Two Centuries after its Discovery by Joseph Priestley (Forti, G., Avron, M. and Melandri, A. eds.), Vol. 3, pp. 2309–2317, Junk, The Hague
41 Litvin, F.F. and Stadnitsuk, I.N. (1980) Fiziol. Rast. 27, 1024–1030 (in Russian)
42 Sundqvist, C., Ryberg, H., Böddi, B. and Láng, F. (1980) Physiol. Plant 48, 297–301
43 Ryberg, H., Liljenberg, H. and Sundqvist, C. (1980) Physiol. Plant. 50, 333–339
44 Imae, T. and Ikeda, S. (1976) Biopolymers 15, 1655–1667
45 Krasnovsky, A.A., Bystrova, M.I. and Pakshina, E.V. (1966) Dokl. Akad. Nauk S.S.S.R. 167, 691–694 (in Russian)
46 Ballschmiter, K. and Katz, J.J. (1972) Biochim. Biophys. Acta 256, 307–327
47 Dratz, E.A. (1966) Ph. D. Thesis, University of California, Berkeley