Acta Biologica Szeged. 26 (1—4), pp. 33—39 (1980)

PREPARATION AND SPECTROSCOPIC PROPERTIES OF AN ALBUMIN-CHLOROPHYLL-a COMPLEX

ERZSÉBET TOMBÁCZ and ESZTER VOZÁRY

Department of Biophysics, Attila József University, Szeged

(Received November, 1979)

Abstract

Albumin in phosphate buffer and chlorophyll-a in dioxane were mixed, dialysed and gel-filtered. A uniform, homogeneous system was separated. The absorption, fluorescence and excitation spectra show that a complex was formed: a non-stochiometric aggregate of albumin, chlorophyll-a and dioxan molecules. In this complex chlorophyll-a is attached to albumin in monomeric or dimeric (possibly higher aggregated) forms, with very loose energetic interaction, as revealed by the absence of intermolecular energy transfer from albumin to chlorophyll-a. The system does not seem a very suitable choice as a model for protein-pigment complexes.

Introduction

For a more detailed understanding of the primary processes of photosynthesis, it is necessary to know the environment of the pigment molecules taking part in hem. In recent years methods have been developed by means of which pigment, protein complexes can be isolated from photosynthetising membranes (THORNBER, 1975). Antenna pigment-protein complexes have molecular weights in the range 20.000—30.000 dalton and contain only a few pigment molecules; their protein moieties consist chiefly of non-polar amino acids (KVEY-SVEY KAN and THORNBER, 1976).

It is of interest to examine artifical pigment-protein complexes, for their constituents are well known. Accordingly, from the changes observed as a result of variations in their compositions, conclusions can be drawn regarding their interactions. From a study of the interaction of chlorophyll aggregates and proteins, SEMICHAEVSKI et al. established that human serum albumin takes part in a hydrophobic interaction with chlorophyll and enhances the photochemical activity of aqueous chlorophyll (SEMICHAEVSKI et al., 1971). GILLER et al. (1970, 1972) prepared a water-soluble pigment-protein-lipid complex from milk and a chlorophyll solution in acetone; they examined this with spectroscopic methods and attempted to draw conclusions on the bonding state of the pigment from treatment with organic solvents. The photochemical activities of the chlorophyll in analogous complexes were determined by DIAMANT and AGHION (1973).

The aim of the present work is to prepare a complex containing protein and chlorophyll, and to examine this spectroscopically. Bovine serum albumin was selected as protein, for this is well known both spectroscopically (KONEV, 1967) and as regards the binding sites of the pigment (KARUSH, 1950). The chlorophyll was dis-

persed in aqueous albumin solution with the aid of dioxan, as this is miscible with water and causes only reversible changes in proteins, while at the same time it can be removed by dialysis (SINGER, 1962). A further advantage is that it absorbs only at wavelengths shorter than 270 nm.

Materials and Methods

Albumin (crystallized, product of BDH Chemicals Ltd) was used without preliminary purification. Chlorophyll was prepared from spinach according to STRAIN et al., (1963), and was stored in diethyl-ether at ca. −10 °C. The concentration of both the chlorophyll- a and the protein in the solutions was 5.10^{-6} M. The protein was dissolved in 0.15 M phosphate buffer of pH 7.4. Chlorophyll-a in dioxan was mixed with the protein solution for 15 minutes with a magnetic stirrer on a 40 °C water-bath (ratio of protein solution to chlorophyll-a solution 64:1 by volume). (The necessary amount of chlorophyll-a was obtained by evaporation of a diethyl-ether solution to dryness, and the residue was dissolved in dioxan.) After stirring, the solution was dialysed at 4 °C for 48 hr in the dark against a 20-fold volume of phosphate buffer of pH 7.4 to remove the dioxan from the medium. The external buffer was exchanged at 10-hr intervals. The dialysing membrane was permeable for substances with molecular weights up to 10.000 dalton. Similar methods were used to prepare a buffered solution not containing albumin, and also the same buffered solution not containing chlorophyll-a. The chlorophyll content of dioxan removed by dialysis was 1—5% of chlorophyll content of the solution before dialysis.

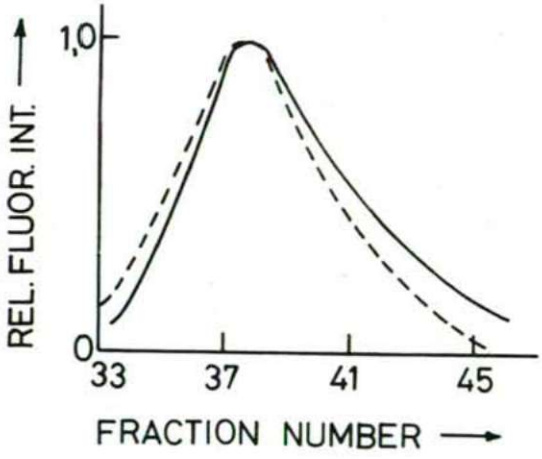

Fig. 1. Relative fluorescence intensities of gel-filtered fractions of dialysed albumin-chlorophyll-a solutions excited at 280 nm, observed at 330 nm (albumin fluorescence, solid line), and excited at 435 nm, observed at 685 nm (chlorophyll-a fluorescence, broken line).

The homogeneity of the solutions was checked by gel-filtration on Sephadex G-100. The solution transferred to the filtration column was washed through with phosphate buffer. One ml fractions of the eluate were taken and the intensities of their fluorescence were measured at 300 nm (albumin fluorescence) and 685 nm (chlorophyll-a fluorescence) with excitation at 280 nm and 435 nm, respectively. The relative fluorescence intensity is shown as a function of the fraction number in Fig. 1. The protein (continuous line) and chlorophyll-a (dashed line) fluorescence curves practically coincide; this indicates that the albumin and the chlorophyll-a pass through the filter together.

The filtered solutions were stored at 4 °C in the dark for about 24 hr. Next the absorption spectra were recorded at room temperature with an SP-300 Unicam spectrophotometer, and the fluorescence and excitation spectra were recorded at room temperature with an MPF 44A Perkin-Elmer spectrofluorimeter.

Results and Discussion

Absorption Spectra

Figure 2 shows the absorption spectra of chlorophyll-a in dioxan, and dioxan dispersion in buffer solution before and after dialysis in terms of $k(\lambda)$, the absorption coefficient $(k(\lambda)=E(\lambda)/l)$; and l are the extinction and layer thickness, respectively. In the aqueous dispersion before dialysis the red absorption band of chlorophyll-a is shifted about 20 nm towards longer wavelength, and the half-bandwidth is increased from 20 to 24 nm, this indicates a solvent effect and the formation of chlorophyll-a aggregates. If this dispersion is dialysed the maximum is at around

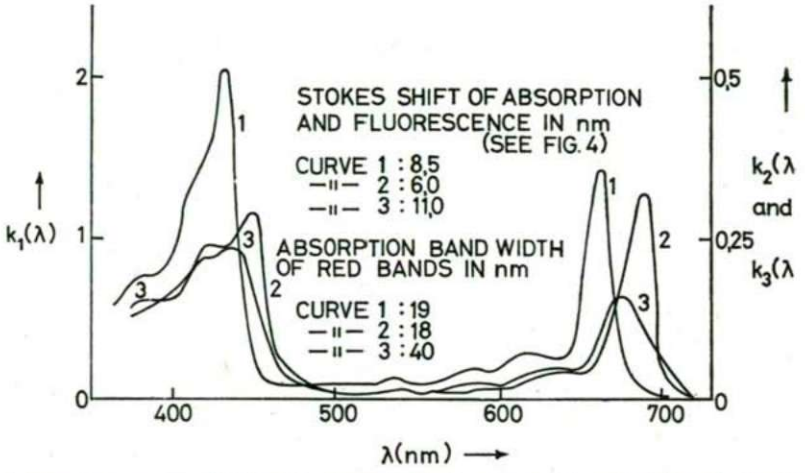

Fig. 2. Absorption spectra of 5.10^{-6}M chlorophyll-a in dioxan (1), $k_1(\lambda)$-left scale, in dioxan and phosphate buffer before (2) and after dialysis (3), $k_2(\lambda)$ and $k_3(\lambda)$-right scale.

675 nm, the half-bandwidth is increased to about 40 nm, and the intensity of absorption is considerably decreased. Since the dialysing membrane is not permeable to species with molecular weights greater than 10,000 dalton, the absorption changes suggest that large chlorophyll-a aggregates are also present in the system. Dialysis removes the dioxan and this gives rise to enhancement of the aggregation. The absorptions of solutions containing both albumin and chlorophyll-a are almost the same before and after dialysis (Fig. 3). The halfwidths of the red band before and after dialysis are 25 and 24 nm, respectively, and the positions of the maxima 682 and 683 nm, respectively. The very small change in the red band can be interpreted in that the presence of the albumin stabilizes monomeric and possibly dimeric chlorophyll-a, but not larger aggregates (SHERMAN, 1963).

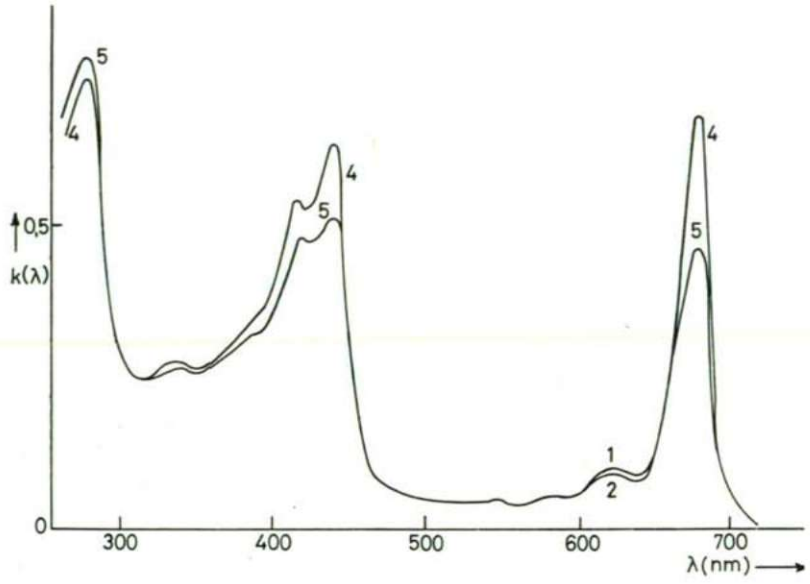

Fig. 3. Absorption spectra of mixture of 5.10^{-6}M chlorophyll in dioxan and 5.10^{-5}M albumin in phosphate buffer before (4) and after dialysis (5).

Fluorescence spectra

For the fluorescence spectra similarly as for the absorption spectra appreciable changes can be observed between the fluorescence spectra of a chlorophyll-a solution in dioxan and buffer before and after dialysis (Fig. 4) excited at 435 nm. The fluorescence spectra of solutions containing albumin and chlorophyll-a together are practically the same before and after dialysis (Fig. 5). In both cases, prior to dialysis the band with maximum at 688 nm displays the highest intensity; indeed, in the system not containing albumin, virtually only this band is present. (Fig. 4, curve 2; Fig. 5, curves 4 and 5). The quantum yield of chlorophyll fluorescence in the solution containing water too, is about 250 times less than that for chlorophyll dissolved in pure dioxan.

The Stokes-shifts of the maxima of the absorption and fluorescence spectra of systems containing protein are not changed after dialysis. This permits the conclusion that the interaction between the chlorophyll molecules is not changed either. Thus, the protein acts as a stabilizer of the structure of the chlorophyll-a aggregates.

The protein fiuorescence spectra similarly support the assumption that dioxan still remains in the system after dialysis, for this is related with the fact that fluorescence spectra of protein solutions containing dioxan are broadened towards shorter waves (Fig. 5).

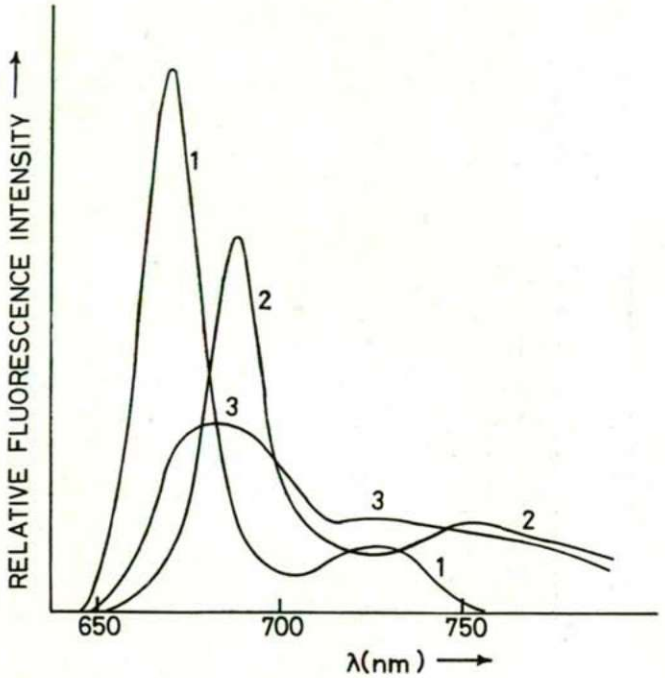

Fig. 4. Fluorescence spectra of 5.10^{-6}M chlorophyll-a solutions in dioxan (1), in dioxan and buffer mixture before (2) and after dialysis (3).

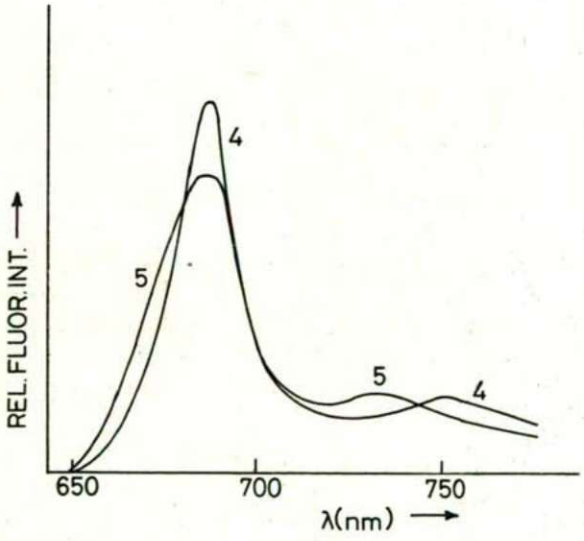

Fig. 5. Fluorescence spectra of a mixture of 5.10^{-6}M albumin buffer solution and chlorophyll-a dioxan solution before (4) and after dialysis (5).

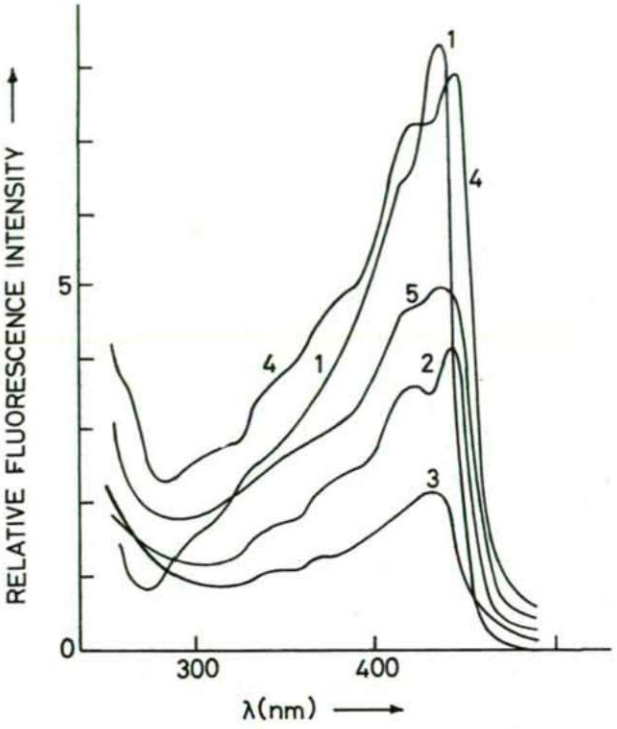

Fig. 6. Fluorescence excitation spectra of 5.10^{-6}M chlorophyll-a in dioxan with 670 nm observation in dioxan and phosphate buffer mixture before (2) and after (3) dialysis with 690 nm observation in mixture of 5.10^{-6}M albumin in phosphate buffer and 5.10^{-6}M chlorophyll-a in dioxan before (4) and after dialysis (5) with 687 nm observation.

Fluorescence excitation spectra

The fluorescence excitation spectra (Fig. 6) coincide with the absorption spectra (Fig. 2—3) with the exception of the ultraviolet region. In solutions containing albumin, the 280 nm band characteristic of the absorption (see Fig. 3) does not appear, which shows, that the electron excitation energy is not transferred from the chlorophyll-a, i.e. these compounds are not in an energy-transfer relation. It may be assumed that the albumin interacts close with the chlorophyll aggregates in dioxan, as if it were their "carrier".

To summerize, it may be stated that with the above method it is possible to prepare an albumin-chlorophyll complex but only in very low yield. The majority of the chlorophyll-a added to the aqueous buffer forms aggregates with the dioxan before it can couple with the albumin; these dioxan-chlorophyll aggregates are washed out of the system during dialysis. It is however not possible to remove the dioxane completely from the chlorophyll-albumin complex. Since the result is low yield of a non-stoichiometric albumin-chlorophyll complex which, in addition to chlorophyll

monomers also contains dimers and slightly larger aggregates, and even dioxane molecules, the complex is not an appropriate choice as a model for photosynthetic pigment-protein complexes.

Acknowledgement. The authors wish to express their thanks to Dr. L. SZALAY, Head of the Department for his interest in this work and for the valuable discussions during the preparation of the manuscript.

References

DINANT, M., AGHION, J. (1973): Aggregation des Chlorophylls *in vitro* II. Photodecoloration de la Chlorophylle a Adsorbee sur des Particules de Lipoproteines Extraites du Lait. — Photochem. and Photobiol. *17,* 25—30.

Гиллер, Х. Е., Красичкоа Г. Б. Сапожиков, Д. И. (1970): Спектральныме свойства сопгстояние фотосинтетических пигментов в искуссентвном водорастворимо игмент-белковолипоидном комплексе. — БИОФИЗИКА *15,* 38—46.

Гиллер, Ю. Е., Юханова, Л. Н., Абдуллаева, С. К. (1972): О природе агрегированных форм хлорофилла, образующихся в искусственном пигмент-белковом комплексе. — ДАН. *207,* 1475—1478.

KARUSH, F. (1950): Heterogenity of the Binding Sites of Bovine Serum Albumin. — Journal of American Chemical Soc. *72,* 2705—2713.

KONEV, S. V. (1967): Fluorescence and Phosphorence of Proteins and Nucleic Acids, ed. S. Udenfried, Plenum Press, New York.

KVEY-SVEY KAN, THORNBER, J. P. (1976): The Light-Harvesting Chlorophyll a/b — Protein Complex of *Chlamydomonas reinhardii.* — Plant Physiol. *57,* 47—52.

SHERMAN, G. M. (1963): Circular Dichroism of Long Wavelength Forms of Chlorophyll a. — Nature *224,* 1108—1110.

SINGER, S. J. (1962): The Properties of Proteins in Nonaqueous Solvents. — Advances in Protein Chemistry *17,* 1—69.

STRAIN, H. H., THOMAS, M. R., KATZ, J. J. (1963): Spectral Absorption Properties of Ordinary and Fully Deuteriated Chlorophyll *a* and *b* — Biochem. et Biophys. Acta *75,* 306—311.

Семичаевский, В. Д., Лось, С. И., Лозовая, Г. И. (1971): О взаимодействии ассоциатов хлорофилла а с белками. — Биофизика *16,* 1117—1120.

Семичаевский, В. Д., Лозовая, Г. И. (1971): Количественные соотношения при взаимодействии ассоциатов хлорофилла а с белками. — ДАН. *199,* 965—967.

THORNBER, J. P. (1975): Chlorophyll Proteins: Light-Harvesting and Reaction Center Components Fully Deuteriated Chlorophyll *a* and *b* — Biochem. et Biophys. Acta *75,* 306—311.
of Plants. — Annual Review of Plant Physiology *26,* 127—158.

Address of the authors:
Dr. ERZSÉBET TOMBÁCZ
Dr. ESZTER VOZÁRY
Department of Biophysics
A. J. University, H-6701 Szeged,
P. O. Box 428. Hungary