ABSORPTION AND FLUORESCENCE SPECTRA OF MONOMERS, DIMERS AND POLYMERS OF CHLOROPHYLL-a IN SOLUTION

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The absorption spectrum of a fresh solution of chlorophyll-a in hexane at 20 °C has peaks at about 430 and 665 nm, and a strong and a very weak shoulder at about 675 and 450 nm. After 4 days of storage at 5 °C the shoulder at 450 nm develops into a strong band and a new band appears at 748 nm, while the two bands and the strong shoulder observed in fresh solutions decrease considerably.

The fluorescence maximum of a fresh solution is at 667 nm when excited by light of 430 nm and at 685 nm when excited by 450 nm. After storage for 4 days, the main fluorescence peak by excitation with 450 nm is at 668 nm with a very weak peak at 755 nm, and by excitation with 430 nm it is at the same position as observed for fresh solution.

The results are explained by ascribing the absorption peaks at 430 and 675 nm to monomers, the peak at 665 and 748 nm to dimers and to polymers, respectively. The peak observed at 450 nm is common for dimers and polymers. The fluorescence maxima of monomers, dimers, and polymers are at 667, 685, and 755 nm, respectively.

Introduction

The study of the absorption and fluorescence spectra of chlorophyll-a in solutions is important for the understanding its *in vivo* spectra. Chlorophyll-a *in vivo* exists in different forms. Both pigment-pigment and pigment-lipoprotein interactions have been suggested to explain the appearence of the different pigment forms.

AMSTER and PORTER [1], TOMITA [2] and SAUER *et al.* [3] studied the aggregation of chlorophylls in hydrocarbon and non-polar solvents. The dimer peak was observed between 650 and 690 nm and the polymer peak at about 745 nm. QUINLAN [4] studied the spectrum of chlorophyll-a in aqueous formamide solution to find the influence of the linkage of amide on the dimerization of chlorophyll-a.

Its seems to be clarified that chlorophyll-a dimers have their red absorption peak between 680 and 690 nm, the polymers (including colloidal and microcrystalline particles) between 740 and 750 nm. The blue peak of the dimers in carbon tetrachloride was reported at 441 nm [5] and in aqueous formamide at 462 [1].

The fluorescence of chlorophyll-a dimers in solution was studied by BROYDE

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and BRODY [6] and AMSTER [5]. Both in [6] and [5] low temperature fluorescence of dimers were reported.

In this paper the absorption and fluorescence spectra of dimers in *n*-hexane at room temperature and the appearence of a fluorescent polymer are reported.

Experimental

Chlorophyll-a was prepared with a method suggested by STRAIN [7]. The purity was checked by comparing the ratios of the heights of the blue maximum to the red one and that of the red maximum to the minimum with the values given in [8]. Freshly distilled n-hexane of analytical grade was used as solvent. The concentration of the solution was 10^{-4}M. The absorption spectra were determined with a grating photoelectric spectrophotometer Optica Milano CF—4 and the fluorescence spectra with an instrument assembled in the laboratory. The fluorescence was measured on the lightexposed side of the cell of 0,1 cm thickness. The fluorescence spectra were corrected for reabsorption of fluorescence and photomultiplier response. The solutions were stored at 5 °C.

Results and discussion

In Figs. 1 and 2 the absorption spectra of a fresh solution (measured in 2—4 hours after preparation) and of solutions stored during two days and four days are shown. On ageing, aggregation of chlorophyll-a occurs. The bands at 430 and 675 nm are monomer bands, but also in fresh solutions there are dimers with absorption band at 665 nm (in the blue band the dimer peak is hidden within the monomer band). In two days both monomer and dimer bands decrease and a polymer band at 748 nm develops. In the absorption spectrum of the 2 days old solution the dimer band (closely together with the polymer band) begins to appear also in the blue band at 447—450 nm. The polymer band at 748 nm becomes very high after 4 days of storage and, simultaneously, a low dimer band is seen at 665 nm, while the monomer band at 675 nm practically disappears. In the blue band the polymer peak develops at 450 nm. After four days no further change was found in the absorption spectrum.

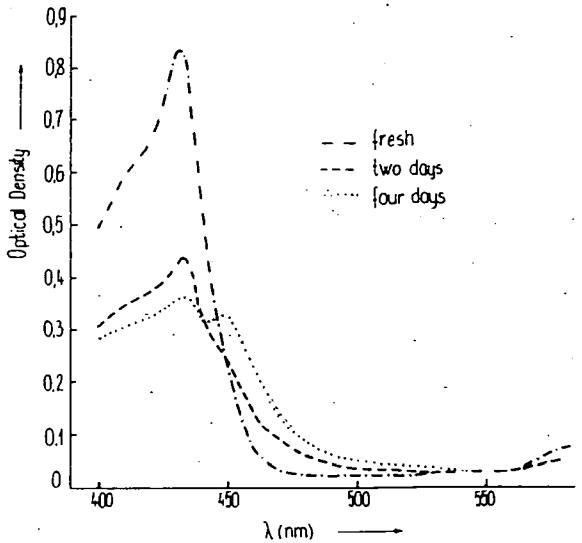

Fig. 1. Blue band of the absorption of chlorophyll-a in *n*-hexane

In Fig. 3 the fluorescence

spectra are shown; the samples were kept under the same conditions as before. The fluorescence spectrum excited with 430 nm wavelength does not change with the time of storage. At 430 nm the peak of the monomer form of chlorophyll-a is found and in this region the aggregated forms developing during storage, obviously, practically do not absorb. The maximum of fluorescence is found at 667 nm. The fluorescence excited with 450 nm, however, shows considerable changes during the time of storage. In fresh solutions this spectrum compared with the former one

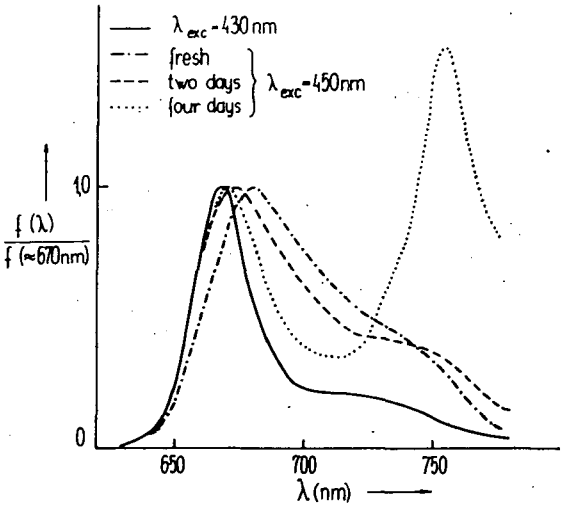

Fig. 2. Red band of the absorption of chlorophyll-a in *n*-hexane

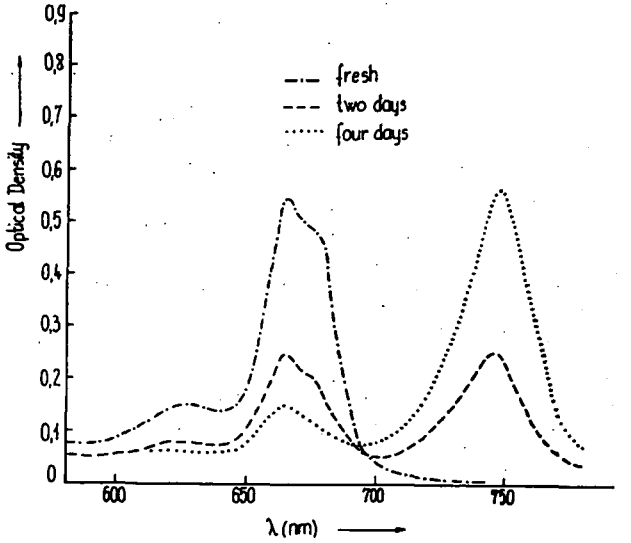

Fig. 3. Fluorescence spectrum of chlorophyll-a in *n*-hexane

shows an enhancement at longer waves, demonstrating the appearence of fluorescent aggregates. This is in accordance with the absorption spectrum which exhibits a dimer and (very close to it) a polymer absorption band. In 4 days old solutions the structure of fluorescence spectrum is completely changed, a new polymer fluorescence band emerges at 755 nm. Simultaneously, the relative intensity of monomer and dimer fluorescence decreases.

The absorption and fluorescence maxima of the different forms of 10^{-4} M chlorophyll-a in n-hexane at 20 °C in nm are the following:

	monomer	dimer	polymer
absorption			
blue	433	447	450
red	675	665	748
fluorescence	667	685	755

These results demonstrate that in a non-polar solvent, *n*-hexane, the different states of aggregation (monomer, dimer, polymer) can be coexisting in a single system. At 20 °C the proportion of these species depends upon the time for which the solutions have been allowed to stand after their preparation. In the absorption the peak positions of the different species could not be precisely determined because of the strong overlap of the components.

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СПЕКТРЫ ПОГЛОЩЕНИЯ И ЛЮМИНЕСЦЕНЦИИ МОНОМЕРОВ, ДИМЕРОВ И ПОЛИМЕРОВ ХЛОРОФИЛЛА-а В РАСТВОРАХ

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Свежеприготовленный раствор хлорофилла-а в гексане при 20 °С имеет максимумы поглощения при 430 и 666 нм, и плечо при 675 и 450 нм. Через 4 дня хранения раствора при 5 °С на месте плеча появляется хорошо выраженная полоса при 450 нм, и новая полоса при 748 нм с одновременным уменьшением основных полос и сильно выраженного плеча, наблюдаемых в свежем растворе.

Максимумы спектра люминесценции свежеприготовленного раствора при λ (возб.) 430 и 450 нм находятся при 667 и 685 нм соответсвено. У четырехдневных растворов при λ (возб.) 450 нм наблюдается максимум при 668 нм и слабая полоса при 755 нм, а при λ (возб.) 430 нм главная полоса люминесценции совпадает с максимумом для свежего раствора.

Полученные экспериментальные данные объясняются тем, что полосы поглощения при 430 и 675 нм принадлежат мономерам, а полосы при 665 и 748 нм характерны для димеров и полимеров хлорофилла. Полоса при 450 нм общая для димеров и полимеров. Полосы люминесценции мономеров, димеров и полимеров находятся при 667, 685 и 755 нм соответственно.