ENERGY TRANSFER IN AN ANS–PHYCOCYANIN COMPLEX

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Received 17 April 1972

1. Introduction

Phycobiloproteins occur in a variety of algae [1], and apparently serve as ancillary light absorbers in the photosynthetic apparatus [2]. The light energy "trapped" by the prosthetic groups (substituted linear tetrapyrroles) is presumably transferred to chlorophyll; the phycobiloproteins do not fluoresce *in vivo*, but are highly fluorescent *in vitro*. Further details and additional references to the original work are given in a recent paper by Teale and Dale [3], where the isolation and spectral characterization of several phycobiloproteins are reported.

During the course of a characterization of a new phycocyanin isolated from *Mastigocladus laminosus* [4], we investigated the interaction of the protein with 8-anilinonaphthalene-1-sulfonic acid magnesium salt (ANS), a probe for "hydrophobic" areas on the surface of proteins [5]. ANS was found to bind to the protein, as judged by a characteristic increase in ANS fluorescence but at the same time, the intrinsic fluorescence of the protein was quenched.

2. Materials and methods

C-Phycocyanin was isolated from the thermophilic blue-green alga *Mastigocladus laminosus* as described earlier [4]. ANS analytical grade was used.

Quantitative titrations were made by adding microliter amounts (1–150 μl) of a 0.01 M ANS solution in water to 0.2 ml of phycocyanin solution (0.495 mg/ml 0.05 M phosphate buffer, pH 8.1) in the spectrophotofluorimeter Fluorispec SF-1. The face of the cell was oriented in the Fluorispec such that it made an angle of ~40° with respect to the exciting beam, and the front surface fluorescence spectrum was recorded as a function of ANS concentration.

3. Results and discussion

The increase in ANS fluorescence due to binding (apparent λ'_{max} = 520 nm) was accompanied by a concomitant decrease in the native phycocyanin fluorescence (apparent λ'_{max} = 640 nm), and the existence of a sharply defined isobestic point at 606 nm clearly shows the complementarity of the binding and quenching processes (fig. 1).

The increase in ANS fluorescence due to simple binding is given by

$$\frac{F_{520}}{[P_0]} = \frac{\phi k [X_0]}{1 + k[X_0]}; \qquad \phi = \eta \epsilon a \ln 10 \qquad 1)$$

where η is the quantum yield of the ANS–protein complex, ϵ is the molar (decadic) extinction coefficient of the complex in cm^2/mmole, and a is the depth of the cell. The quantities $[P_0]$ and $[X_0]$ are the total concentrations of protein and ANS, respectively, and $k = [PX]/[P][X]$ is the equilibrium constant for the association reaction in cm^3/mmole. If the decrease in quantum yield (intensity) of the intrinsic protein

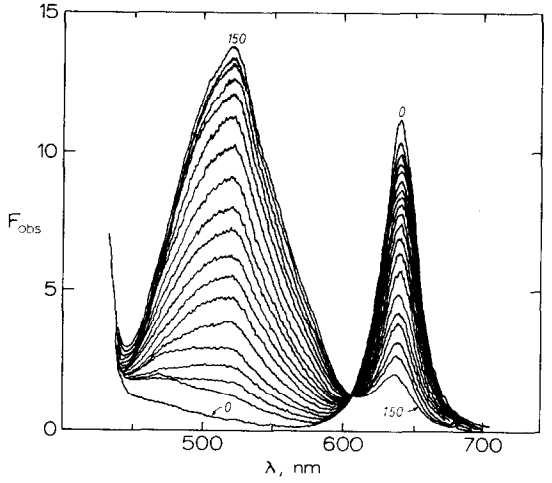

Fig. 1. Titration of C-Phycocyanin with ANS (1–150 μl 0.01 M ANS solution). ANS fluorescence: λ_{max} = 520 nm; intrinsic fluorescence of the protein: λ_{max} = 640 nm.

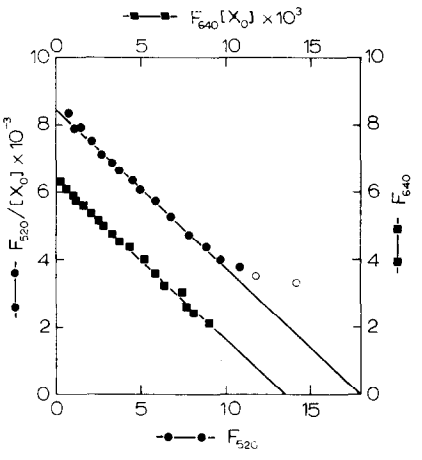

Fig. 2. Scatchard plot: 1) $F_{520}/[X]$ vs. F_{520} (increase in ANS fluorescence). 2) F_{640} vs. $F_{640}[X]$ (decrease of the intrinsic protein fluorescence).

fluorescence is due to the formation of a complex between the quencher (ANS) and the fluorescent moiety of the protein, then the quenching can be described by the Stern–Volmer law:

$$\frac{F^0_{640}}{F_{640}} = 1 + k[X]; \qquad F^0_{640} = \eta^0 \epsilon^0 a[P_0] \ln 10 \quad 2)$$

The superscripts on F_{640}, η, and ϵ designate that these quantities refer to protein in the absence of quencher. Equations 1) and 2) can both the rearranged in linear forms which, when plotted, will contain the entire titration curve within the plot limits (Scatchard-type plots). Thus, plots of $F_{520}/[X]$ vs. F_{640} vs. $F_{640}[X]$ will be linear with slope equal to $-k$, the equilibrium constant for complex formation. The fluorescence data plotted in this fashion are shown in fig. 2, and within the limits of experimental error, the binding constants calculated from the slopes are identical (473 and 467 cm^3/mmole for the increase in ANS fluorescence and the decrease in protein fluorescence, respectively).

The fluorescence spectrum of the protein excited in the short wavelength absorption band ($\lambda_{max} \sim 375$ nm) is identical with that observed from excitation in the long wavelength band ($\lambda_{max} \sim 590$ nm), modified by the ratio of the absorbancies at the two excitation wavelengths. Thus, essentially complete internal energy transfer takes place from the short wavelength oscillator to the long wavelength oscillator responsible for fluorescence, as observed by others [6, 7]. The radiationless process corresponding to this internal transfer must be very fast, with a probability of the order of 10^{11} or higher (reciprocal of the product of the natural lifetime estimated from the short wavelength absorption band, and the quantum yield for short wavelength fluorescence, less than approx. 0.1%). The most likely mechanism for the quenching of the long wavelength fluorescence involves transfer of the excitation energy from the short wavelength oscillator of the tetrapyrrole moiety to ANS. The transfer rate for this process would have to be in excess of 10^{11}, implying a very short interaction distance (of the order of a molecular diameter) between the donor and the acceptor [8, 9]. We interpret the above results to mean that the quenching of protein fluorescence is due to the interaction of ANS and the prosthetic group of the protein, and that the binding site for ANS is probably the tetrapyrrole ring system.

The fluorescence of bound ANS appears with an apparent maximum at 520 nm, which is more characteristic of ANS in a polar surrounding medium [10] than in a "hydrophobic" environment. Barring complexities due to spectral shifts introduced by the front face geometry [11], or inherent in the proposed ANS–tetrapyrrole complex, the latter result may in-

dicate that the tetrapyrrole is in a highly polar environment on the surface of the protein. Dale and Teale [12] found that the experimental values of the emission anisotropies of C-phycocyanin are more consistent with surface locations for the chromophores, than for their distribution throughout the volume. Our results tend to confirm this conclusion, that the prosthetic groups are on the surface, and furthermore indicate that they are highly accessible to external binding probes such as ANS.

Acknowledgements

We wish to thank Miss Hanni Schneider for excellent technical assistance. This work was supported by research grants from the U.S. National Science Foundation (GB18016), and the Schweizerische National-fonds (3.379.70).

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