TRANSFER OF ELECTRONIC EXCITATION ENERGY IN PROTEIN-DETERGENT SOLUTIONS

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The electronic absorption spectra of mixtures of lysozyme and triton *X*—100 are additive, those of albumin and triton are non-additive in the concentration range of $2.10^{-6}-8.10^{-4}$ *M* protein and $2.10^{-5}-8.10^{-3}$ *M* detergent. In both cases there is an overlap of absorption and fluorescence spectra which leads to transfer of electronic excitation from the detergent to the protein. The dependence of transfer frequency on the distance (*R*) follows an R^{-6}-law for the transfer from triton to lysozyme and an R^{-5}-law for the other case. The activity of lysozyme increases by about 60% in the presence of triton.

Introduction

Photochemical processes of photosynthesis are often investigated on chloroplast fractions obtained by treating chloroplast suspensions with detergents [1—3]. The sizes and properties of the particles forming the fractions depend highly on the characteristics of the detergent and on its concentration. The absorption of light at 280 nm in these systems is often measured in order to determine their protein contents [3]. However, this method can only be applied if the detergents used for the treatment have no absorption at 280 nm. In general, this is not the case, *e.g.* triton *X*—100 has two bands, at 275 nm and 285 nm [4].

The detergents may interact with the particle fractions and may even be incorporated [3], leading to changes in their properties. To permit conclusions concerning the true properties of the particles, the interactions between detergents and proteins and those between detergents and pigments have to be known.

Interactions between pigments and detergents have been investigated by several authors [5—7], and the results provide a broad outline of these interactions. Interactions between proteins and detergents have also been investigated for a long time, but a comprehensive picture of these interactions has still not been obtained. The effects caused by detergents have been studied by measuring the absorption [8, 9] and fluorescence [9, 10], if the detergent has no absorption and fluorescence in the wavelength range of the proteins. However, if protein and detergent absorb in the same wavelength range, but the maximum of the detergent fluorescence lies at a shorter wave than that of the protein excitation, energy transfer from the detergent to the protein becomes possible in mixed solutions. The transfer efficiency depends on the distance (concentration of the components). This dependence may render it possible to characterize the interaction between the proteins and detergents. The aim of this paper is to study the action of triton *X*—100 on lysozyme and albumin.

Materials and methods

The hen ovalbumine lysozyme was a lyophilized, three times crystallized commercial product (Nutritional Biochemical Corporation, Cleveland). Its activity was 6000—10 000 unit/mg. The human serum albumin (Reanal) and Micrococcus lysodeikticus (Worthington Biochemical Corporation, Freehold, New Jersey) used as substrate for activity measurements were also commercial products. Triton *X*—100 (Rohm and Haas, Co.) is a non-ionic detergent; its micelles are formed by about 100 molecules [11], and its critical micelle concentration (c.m.c.) is $3 \cdot 10^{-4} M$ [4]. The protein and detergent were dissolved in phosphate buffer of pH = 7. The concentrations of albumin, lysozyme and triton *X*—100 solutions were varied from $2 \cdot 10^{-6} M$ to $8 \cdot 10^{-4} M$, from $2 \cdot 10^{-6} M$ to $6 \cdot 10^{-4} M$ and from $2 \cdot 10^{-5} M$ to $8 \cdot 10^{-3} M$, respectively. In all mixed solutions the concentration of triton *X*—100 was 10 times greater than that of the proteins.

The absorption and fluorescence spectra were measured with an Optica Milano CF4DR recording spectrophotometer and a Perkin—Elmer MPF—3, spectrofluorimeter, respectively. The fluorescence spectra were corrected for the reabsorption of fluorescence and for the spectral sensitivity of the detector [12]. The layer thickness was selected after BUDÓ and KETSKEMÉTY [13] so that the secondary fluorescence was negligible.

The activity of lysozyme was measured spectrophotometrically with a method by SHUGAR [14].

The absolute quantum yields were determined after DOMBI [15], allowance being made, however, for the different geometry.

Results and discussion

Absorption spectra. Fig. 1 shows that in a mixture of lysozyme and triton the absorption of the mixed solution is the sum of the absorptions of the components, while in a mixture of albumin and triton the additivity does not hold in the short-wave region of absorption. This suggests the existence of some interaction between albumin and triton. This behavior was independent of the concentration in the range studied.

Fluorescence spectra. The relative absorption and fluorescence spectra of lysozyme and triton are presented in Fig. 2. There is a comparatively strong overlap (shaded area) between the fluorescence spectrum of triton and the absorption spectrum of lysozyme. A similar overlap occurs in the case of albumin and triton. This overlap permits the transfer of excitation energy from triton to lysozyme or albumin.

Fig. 3 shows the spectral distribution of the fluorescence of a mixture of lysozyme and triton and that of the components in arbitrary units. It can be seen that the intensity of the fluorescence of triton at 305 nm is decreased in the mixture; in other words, the fluorescence spectra are not additive. The same applies in the case of albumin and triton. This indicates that the excitation energy is partly transferred to the proteins (acceptors) from the detergent (donor). In order to determine the transfer efficiency, the expected contribution of the fluorescence of the components to the total fluorescence must be considered.

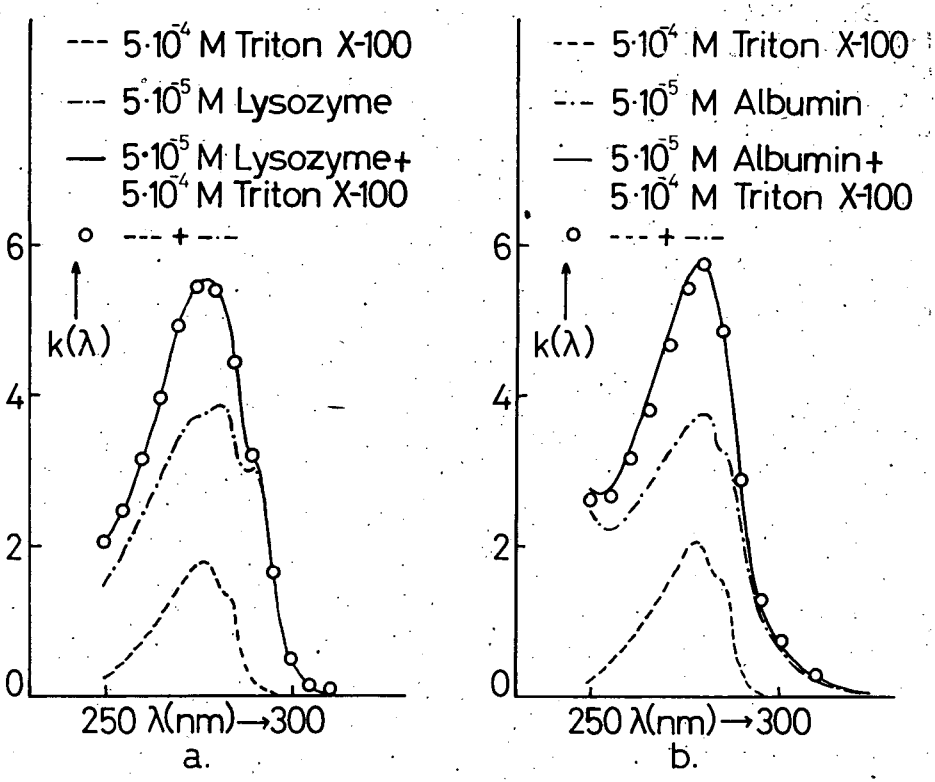
--- 5·10⁻⁴ M Triton X-100
-·- 5·10⁻⁵ M Lysozyme
— 5·10⁻⁵ M Lysozyme+ 5·10⁻⁴ M Triton X-100
k(λ)
250 λ(nm)→300
a.
--- 5·10⁻⁴ M Triton X-100
-·- 5·10⁻⁵ M Albumin
— 5·10⁻⁵ M Albumin+ 5·10⁻⁴ M Triton X-100
k(λ)
250 λ(nm)→300
b.

Fig. 1

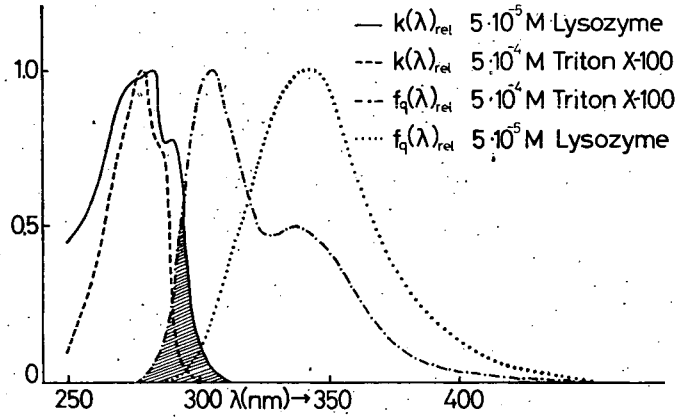
— k(λ)rel 5·10⁻⁵ M Lysozyme
--- k(λ)rel 5·10⁻⁴ M Triton X-100
-·- fq(λ)rel 5·10⁻⁴ M Triton X-100
···· fq(λ)rel 5·10⁻⁵ M Lysozyme
300 λ(nm)→350

Fig. 2

Determination of the transfer efficiency. A relation between the quantum distribution function $f(\lambda')$ of the fluorescence of a mixed solution consisting of two components and the quantum distribution spectra of the fluorescence of the components, $f_1(\lambda')$ and $f_2(\lambda')$, is known for the case when there is no energy transfer between the components:

$$f(\lambda) = \frac{f_1(\lambda)k_1(\lambda)\mu_1(\lambda) + f_2(\lambda)k_2(\lambda)\eta_2(\lambda)}{k_1(\lambda)\eta_1(\lambda) + k_2(\lambda)\eta_2(\lambda)}, \tag{1}$$

where $k_1(\lambda)$ and $\eta_1(\lambda)$, and $k_2(\lambda)$ and $\eta_2(\lambda)$ are the absorption coefficients and the absolute quantum yields of the first and the second component, respectively, at the exciting wavelength λ [15].

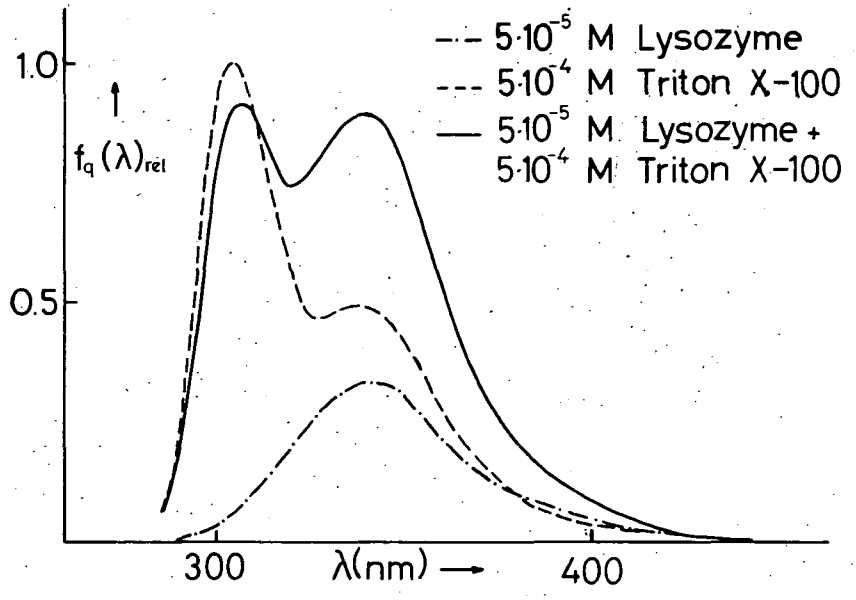

Fig. 3

In Fig. 4 the calculated and measured functions $f(\lambda')$ are plotted for mixed solutions containing albumin-triton (Fig. 4*b*) and lysozyme-triton (Fig. 4*a*). The difference between the calculated and measured spectra is related to the energy transfer.

The efficiency of transfer is given by the general relation [16]

$$f = \frac{(R_0/R)^j}{(R_0/R)^j + 1}, \tag{2}$$

where j is the exponent of the distance-dependence (if $j=6$, the interaction is of inductive resonance type), R is the distance between the molecules, and R_0 is the critical distance, at which the probability of transfer is equal to that of radiative deactivation of the primarily excited molecule.

From data obtained experimentally:

$$f = \frac{I_{mix}(\lambda) - I_A(\lambda) - I_D(\lambda)}{\frac{k_D(\lambda_{exc})}{k_A(\lambda_{exc})} I_A(\lambda) - I_D(\lambda)}, \tag{3}$$

where $I_{mix}(\lambda)$, $I_D(\lambda)$ and $I_A(\lambda)$ are the fluorescence intensities (corrected for reabsorption) of the mixture and solutions containing only the donor molecule and acceptor

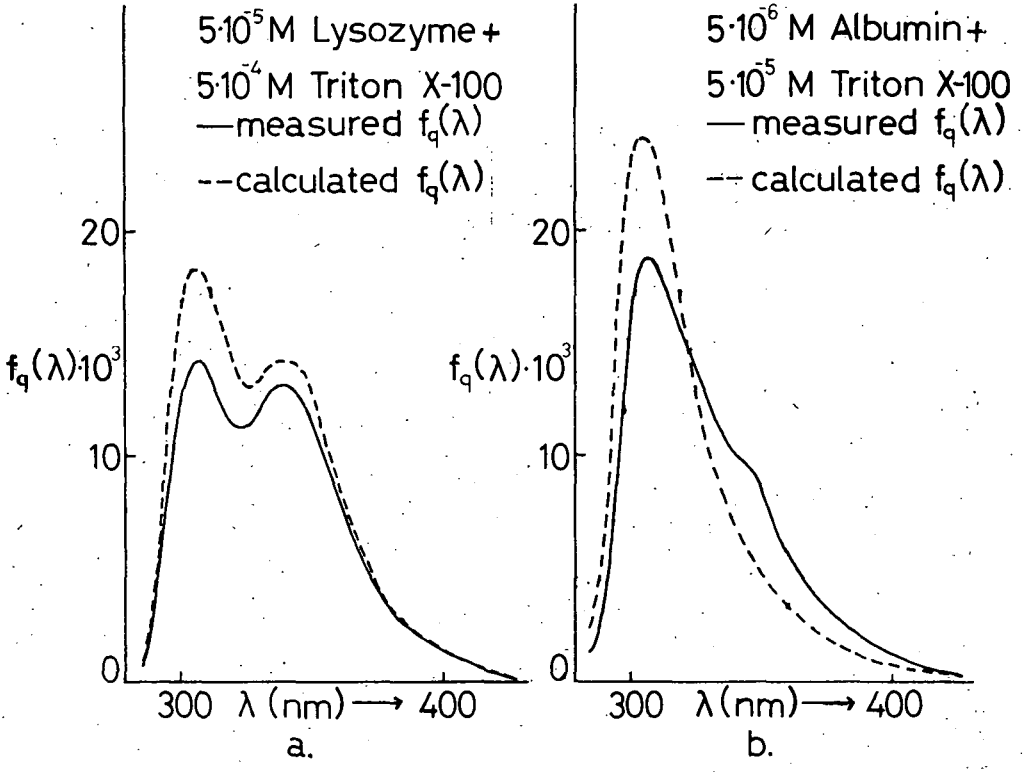

Fig. 4

molecule, respectively; λ denotes the wavelength at the maximum of the fluorescence spectrum of the donor; $k_D(\lambda_{exc})$ and $k_A(\lambda_{exc})$ are the absorption coefficients of solutions containing only the donor and the acceptor molecules respectively; and λ_{exc} is the wavelength of excitation [17].

With values of f calculated with (3) for mixtures of different concentrations, the function $\lg(f^{-1}-1)$ was determined and plotted as a function of $\lg R$ for lysozyme-triton (Fig. 5*a*) and albumin-triton (Fig. 5*b*) systems.

The calculation of the average distance R between donor and acceptor molecules can be omitted, because the ratio of these distances in solutions with different

concentrations is equal to the ratio of the distances between donor molecules in these solutions. Therefore, in Fig. 5 R is the distance between the donor molecules in mixed solutions. R was calculated from the relationship $R^3=3000/4\pi Nc$, where c is the molar concentration of the pigment, and N is Avogadro's number [12].

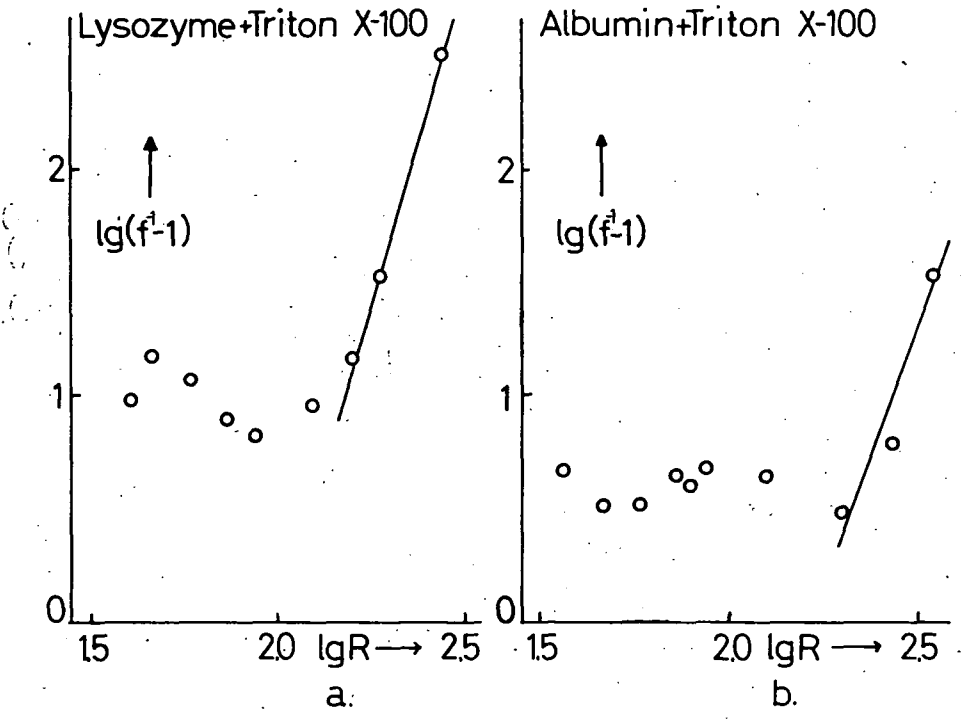

Fig. 5

At low concentrations the function $\lg(f^{-1}-1)$ is linear, the slope being $j\approx 5$ in the albumin-triton system (Fig. 5*b*). This points to the existence of a stronger interaction than that of inductive resonance. Since albumin molecules have about 5 places to bind triton molecules at a molar ratio triton:albumin=10 [8], the bondings here may give the possibility for this phenomenon. In lysozyme-triton systems (Fig. 5*a*) $j\approx 6$, i.e. the energy transfer takes place by inductive resonance.

At higher concentrations, above the critical micelle concentration, the number of free detergent molecules is constant. The efficiency of energy transfer should increase with increasing protein concentration, if the transfer were to take place from free detergent molecules. This increase was, however, not observed, the efficiency showing practically no concentration-dependence. Therefore, it has to be assumed that the protein molecules interact with micelles, similarly as was found in the system of tween 20—lysozyme [10]. In Fig. 6 the ratio of the activities measured in the presence and absence of triton is plotted as a function of the logarithmic detergent concentration. When a great number of micelles are present, the activity of lysozyme

increases by about 60%. Formally we can think that the lysozyme molecule interacting with the micelles is oriented in such a way that its active part becomes more accessible for the substrate. An alternative explanation is offered by assuming the weakening of the β—1,4 glycoside linkages of the substrate due to the detergent, which would cause the lysis to become quicker.

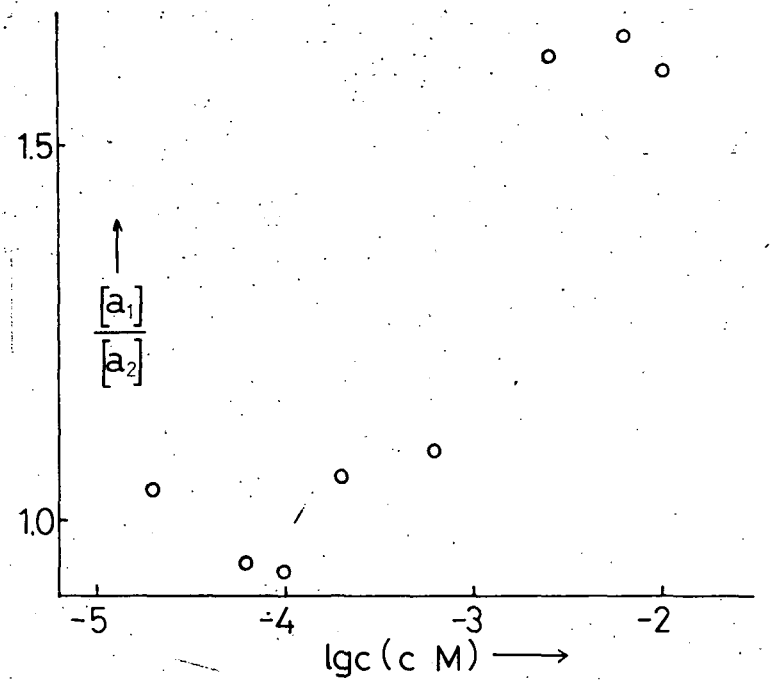

Fig. 6

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ПЕРЕНОС ЭНЕГРИИ ЭЛЕКТРОННОГО ВОЗБУЖДЕНИЯ В РАСТВОРАХ ПРОТЕИНА—ДЕТЕРГЕНТА

Э. Возари и Л. Салаи

Для системы лизоцим-тритон *X*—100 характерно, что спектры полгошения аддитивны, тогда как в случае альбумин-тритон *X*—100 аддитивность не выполняется при концентрации белков $2 \cdot 10^{-6}$—$8 \cdot 10^{-4} M$ и тритона $2 \cdot 10^{-5}$—$8 \cdot 10^{-3} M$. В обеих системах вследствие перекрытия спектров поглощения и флуоресценции происходит передача энергии электронного возбуждения от тритона на белок. Зависимость переноса энергии от расстояния молекул тритона *X*—100 и белка в растворах лизоцима и альбумина описывается закономерностью R^{-6} и R^{-5}, соответственно. Установлено, что ферментная активность лизоцима в растворах тритона *X*—100 увеличевается на 60%.