Photosynthetic Light-Harvesting Systems

Organization and Function

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LOW TEMPERATURE SPECTROSCOPY OF CYANOBACTERIAL ANTENNA PIGMENTS

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Abstract

Disorder on a microscopic scale leads to inhomogeneous line broadening of the optical spectra of chromophores, which prevents high resolution spectroscopy in a straightforward manner. In this paper emphasis is put on hole burning experiments on C-phycocyanin (PC) and phycobilisomes (PBS) of Masticogladus laminosus. This technique is capable of resolving the zero phonon fine structure in spite of disorder. From the measured hole profiles energy transfer times within a broad frequency range of the phycobilisome absorption could be estimated. From fluorescence line narrowing experiments combined with hole burning, details about the level structure and the loss of correlation in energy transfer processes could be elucidated. From temperature dependent hole filling experiments the distribution of conformational barriers of the chromophore attached proteins could be measured.

Introduction

In the case of antenna pigments the knowledge of the details of the electronic-vibrational level structure is a prerequisite for understanding the

Abbreviations: PE phycoerythrin, PC phycocyanin, APC allophycocyanin, PBS phycobilisome

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energy transfer mechanisms and, hence, the functioning of these pigments in the photosynthetic process (1,2). Spectroscopy at liquid Helium temperatures can usually provide this information in organic molecular systems. This results from two facts: First, the linewidths may narrow dramatically as the temperature is lowered, and second, most of the intensity may be confined to the so-called 'zero phonon transitions'. Hence, low temperatures principally allow for highly resolved spectra with very good signal to noise ratio (3).

Unfortunately, disorder on a microscopic scale prevents straightforward high resolution spectroscopy in the pigments studied, e.g. PC and PBS from M. laminosus, because of the concommitant inhomogeneous line broadening. Inhomogeneous line broadening not only obscures all the information contained in the homogeneous line shape function but also wipes out the vibrational pattern to a high degree. Here we wish to report how some of these drawbacks can be overcome by spectral hole burning and fluorescence line narrowing techniques.

How disorder on a microscopic scale obscures spectroscopic information

To understand the kind of information one can obtain from a homogeneous line shape function of guest molecules in a host matrix we assume that the host-guest system is perfectly ordered and the concentration is low enough so that each guest molecule has the same microscopic environment. In this case the absorption line shape has the typical form shown in Fig. 1. It consists of a narrow so-called zero phonon line and a broad so-called phonon side band. The relative intensity in the narrow zero phonon line determines the Debye-Waller-factor, a characteristic parameter of the considered host-guest system, which is, for many systems on the order of 0.4-0.8 for temperatures around 4K. Above 40 K, or so, it becomes extremely small so that the zero phonon structure tends to vanish. The phonon side band is an outcome of the Franck-Condon-principle applied to the vibrations of the host material: The excited guest molecule has a different charge distribution. Hence, the matrix is polarized and tends to assume a new equilibrium configuration while the guest molecule is in the

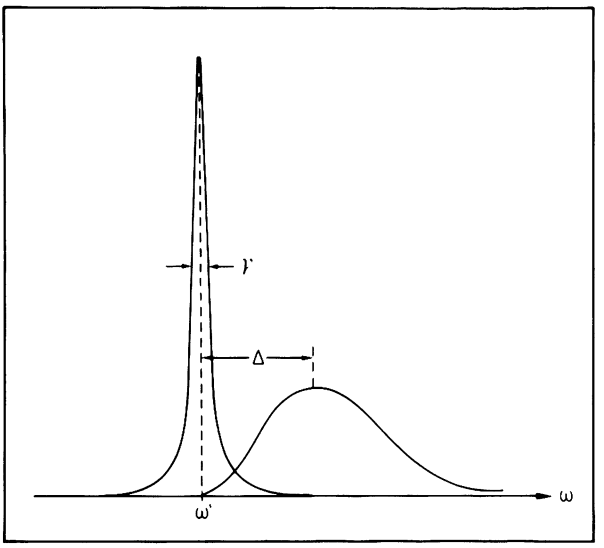

Fig. 1. Zero phonon line with homogeneous width γ and phonon sideband. Δ is half the Stokes-shift

excited state. This is equivalent to say that the matrix vibrates as a consequence of the guest excitation. Matrix vibrations are usually called phonons. If the matrix or, strictly speaking, the immediate environment of the excited chromophore is very rigid, the coupling of the electronic excitation to the matrix environment (to the lattice) is small and, hence, the phonon side band carries little oscillator strength and the Debye-Waller-factor will be close to 1. The spectrum is then dominated by the narrow and intense zero phonon lines.

The zero phonon line is a purely electronic transition, i.e. the vibrational state of the lattice does not change during this kind of excitation. Since the lifetime of an electronic level is orders of magnitude longer than that of a vibrational level, its width γ is, according to the uncertainty relation, much narrower, and, hence, its peak intensity may be orders of magnitude higher than the phonon side band. Hence, we have a typical absorption line shape as depicted in Fig. 1. Along these lines of reasoning it seems to be clear that one can determine the lifetime of an electronic

level in case one succeeds in measuring its homogeneous zero phonon line shape. This, however, is not an easy task because of several reasons: First, only at extremely low temperatures on the order of 1 K is the width determined by the true lifetime of the electronic level excited. With increasing temperatures the dynamics of the host matrix broaden the line without changing its lifetime. Second, many dye molecules have lifetimes on the order of 1 nsec, hence the width will be on the order of 10 to 100 MHz (0.0003 - 0.003 cm^{-1}) and the required resolution of the spectrometer has to be on the order of 10^7. Third, and most important, the presence of microscopic disorder on a molecular level obscures the homogeneous line shape function. Hence, a straightforward spectroscopy is not possible (for a review, see (1)). How disorder changes the spectral properties of a chromophore in a host matrix is discussed in the following paragraph.

Fig. 2 symbolizes a disordered matrix. The solvent cage of molecule 1 is different from that of molecule 2 and 3. Hence, as schematically shown

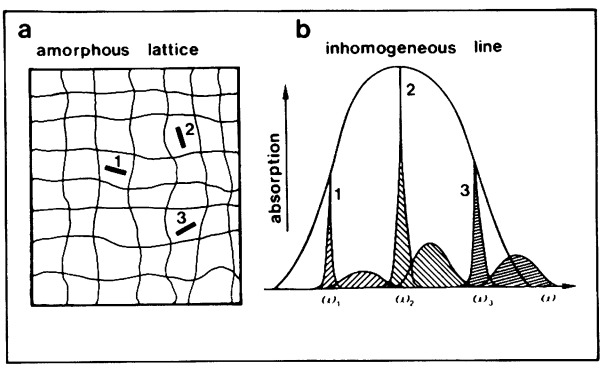

Fig. 2. Schematic representation of inhomogeneous line broadening (b) as a result of microscopic disorder (a)

in Fig. 2b, these molecules absorb at different frequencies. As a consequence, spatial disorder leads to a spread of absorption frequencies, which is called inhomogeneous line broadening. At low temperatures, the inhomo-

geneous width may be larger than the homogeneous width by more than 4 orders of magnitudes. Hence, all details of the homogeneous molecular line shape are wiped out due to disorder.

Fluorescence line narrowing (FLN) and hole burning (HB) spectroscopy

Both FLN and HB are high resolution techniques designed to overcome inhomogeneous line broadening. The basic idea of FLN is sketched in Fig. 3a. A narrow bandwidth laser with frequency ω_L excites only those centers in the inhomogeneous band which are accidentally tuned with their absorption freuqency to ω_L. Then, only molecules absorbing in a frequency range on the order of the homogeneous width around ω_L can emit fluorescence. If the fluorescence is detected with a high resolution spectrometer, the lines will show a structure as shown in Fig. 3a. A drawback of FLN is the fact that it is very difficult to observe resonance fluorescence and, hence, the lines are broadened by vibrational relaxation and other processes.

Hole burning is shown in Fig. 3b. It always works in case the guest molecules are photoreactive, and the zero phonon line carries enough oscillator strength. Then, those molecules excited within a frequency range

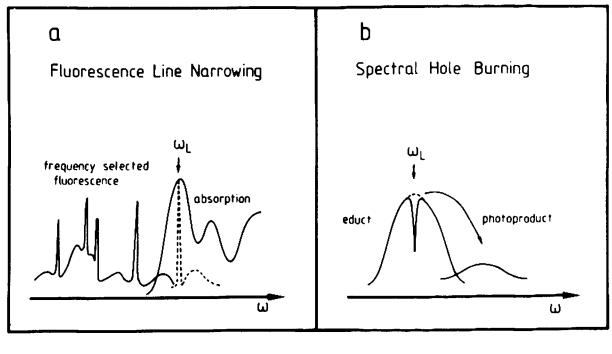

Fig. 3. Schematic representation of fluorescence line narrowing (a) and spectral hole burning (b).

of the homogeneous linewidth γ around ω_L are transformed to a product, hence, the number of molecular absorbers at ω_L is diminished and a hole appears in the spectrum. Contrary to FLN, HB allows for a resonant detection at the laser frequency; therefore, this technique has the capability of measuring the homogeneous linewidth. One has, however, to be careful to rule out all slow relaxation processes such as spectral diffusion, because the time scale of HB-experiments is slow and a lot of processes may occur which broaden the hole.

Hole burning experiments on phycobilisomes and spectrally resolved energy transfer times

Fig. 4 shows an absorption spectrum of phycobilisomes of M. laminosus at a temperature of 4 K in a saccharose/phosphate buffer solution. The

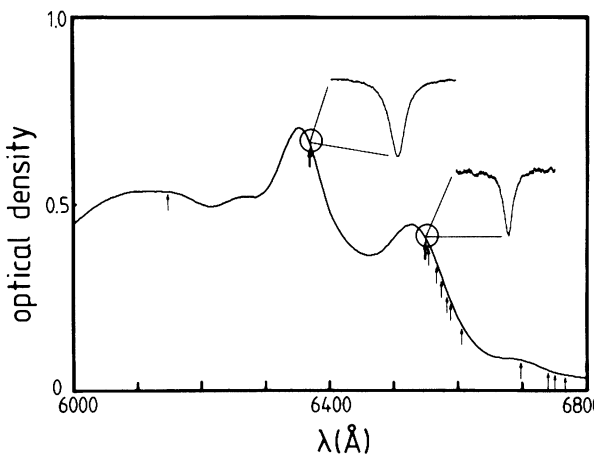

Fig. 4. Hole burning in PBS of M. laminosus. Burning positions are indicated by arrows. Typical hole shapes obtained in the PC and APC-peaks are shown on an enlarged scale. PBS preparation, modified from Nies and Wehrmeyer (4), was made in 0.9 M phosphate buffer (pH 6). Centrifugation was repeated twice. Isolated PBS solution was saturated with saccharose, to ensure coupling in a low temperature glass.

spectrum is characterized by a clearly resolved structure which originates from the various pigments which build the highly organized phycobilisome assembly APC, PC, PE. In spite of the highly organized structure, there is a remarkable amount of disorder as documented by the inhomogeneous linewidths. It is a question of great interest whether the observed disorder is an intrinsic property of the chromoprotein or of the proteinaceous environment. In case of the PBS, most of the chromophores are known to interact strongly with the protein. Consequently, we believe that disorder is intrinsic and reflects the different conformational states of the latter (5). The arrows in Fig. 4 indicate the positions where hole burning was performed. Typical holes are shown in the same figure as insets. Their width is on the order of 0.4 cm^{-1}. It is interesting to compare this width with that measured for isolated phycocyanine (6). This is done in Fig. 5 for phycobilisome and PC in saccharose/buffer solution. The hole width in

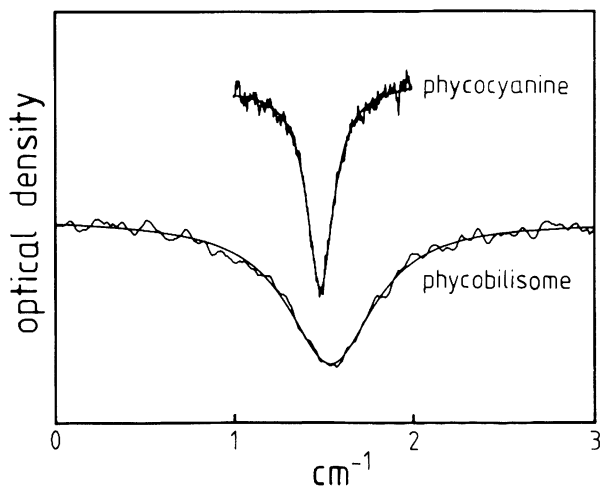

Fig. 5. Comparison of spectral holes in isolated PC and phycobilisomes (PC-peak).

the phycobilisome system is much larger than in the isolated PC. We interprete this finding in the following way: Hole burning on the PC-trimer occurs effectively only on the red edge of the visible band. Hence, it seems that it is only the fluorescing chromophore(s) (or chromo-

phore states) which are effectively burnt. In these chromophores, the fluorescence lifetime is mainly governed by intramolecular decay processes, which occur on the order of nanoseconds. At sufficiently low temperatures, where dephasing is small, these decay processes determine the width of the hole. In the phycobilisomes there is, apart from these decay processes, a fast energy transfer to acceptor chromoproteins, like APC. In case these energy transfer processes dominate the dynamics of the excited level, the transfer times can be determined from the measured hole width. From our results we would estimate transfer times on the order of 30 ps. It is interesting to note that these results do not depend very much on the position of excitation (see Fig. 4).
We conclude this section with a few remarks on the advantages and disadvantages of the hole burning method in determining lifetimes. Unlike fluorescence detection methods, which suffer from overlapping contributions from the various pigments, hole burning is a resonant method which directly yields information on the burnt state. No kinetic model is necessary in evaluating this information. However, the width of the hole is not only determined by the lifetime of the state considered, but depends also on the pure dephasing time. It is difficult to determine the latter one separately and, hence, the hole burning results have to be considered as lower bounds for the transfer times.

Spectral distribution of the photoproduct

The nature of the hole burning reaction is, as of yet, not clear. Basically one discerns between photochemical - and photophysical reactions. In the first case it is assumed that photochemical changes of the dye molecule itself (i.e. of the chromophore) occur (e.g. bond breaking, proton transfer reactions, etc.). In the second case it is assumed that the dye molecule itself is unchanged whereas the surrounding changes (e.g. a change in the conformational state of the protein). Photochemical reactions usually lead to large spectral shifts, while photophysical reactions lead to small spectral shifts (7). In many cases (including chromoproteins) it may be difficult to distinguish both types of reactions in a clear cut way. Fig. 6 shows (together with the absorption spectrum) the difference in optical

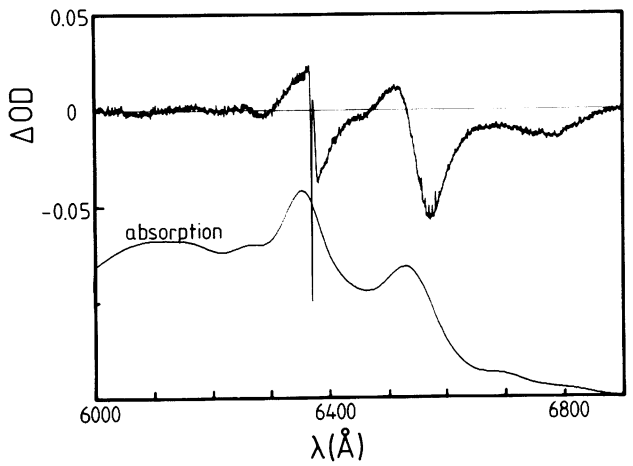

Fig. 6. Difference in optical density (ΔOD) before and after burning the phycobilisome spectrum around 6370 A.

density before and after burning. Note, that most of the product occurs in a rather narrow range around the educt which favours a photophysical hole burning reaction. An interesting observation is the phototransformation which occurs in the APC-range around 6560 A. Note, that laser irradiation is performed at 6370 A in the PC-range. As obvious, the spectral range burnt in the APC-spectrum is almost as broad as the inhomogeneous band. Hence, frequency selection is lost in this case. Interestingly, only the long wavelength edge of the APC- band is burnt. It seems, that only this long wavelength range of the band is photoreactive.

Conformational barriers

In case the reaction product is determined by the interaction between probe molecule (i.e. chromophore) and environment, we expect that, due to the disorder of the system, the reaction barriers are not well defined, but are rather characterized by a broad distribution. Hole burning offers a possibility for directly measuring this distribution of barriers (5,7). To

this end we make use of temperature cycling experiments: A hole is burnt at a low temperature T_b ('burn'). Then, the temperature of the sample is raised to some value T (the 'excursion temperature') and cycled back to T_b, where the hole is measured again.
In our case burning was performed at the APC-peak, near 6550 A (see Fig. 4). T_b was 4 K. The changes of the hole area during such a cycle is a measure for the number of product molecules having returned to their educt state. The experiment is performed as a function of the excursion temperature T. For a fixed excursion temperature T, all those centers return to the educt state characterized by barriers

$$V_T = kT \ln R_o \tau \qquad (1)$$

R_o is the attempt frequency and τ the experimental time needed to drive the system through one cycle. $\ln R_o\tau$ is on the order of 30. Centers with barriers higher than V_T stay unaffected. The variation of the excursion temperature enables a sampling of the distribution of barrier heights. Fig. 7a shows the result for PBS. For comparison, the results obtained for a series of organic glasses are also shown.

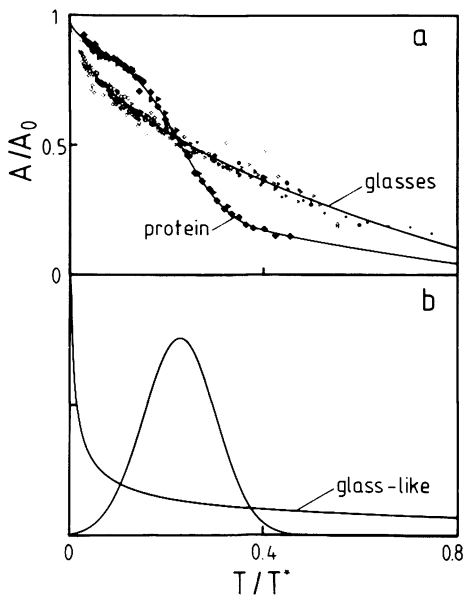

Fig. 7. Annealing of a spectral hole burnt into the PBS absorption (a). For comparison the same behavior for organic glasses is also shown. The distribution of reaction barriers can be well described by a superposition of glass-like states and a discrete feature distributed in a Gaussian fashion (b).

The experiments on the protein sample can be perfectly fitted by assuming that the distribution of barrier heights consists of a superposition of a glass-like distribution and a Gaussian distribution which is centered around 650 cm^{-1} and which has a width of 210 cm^{-1}. We interprete these findings in the following way: We assume that hole burning is due to a conformational change of the protein environment around the irradiated chromophore (photophysical HB- mechanism). On one hand, a protein is very much like a glass, i.e. it is disordered on a microscopic scale and can exist in many conformational states with different barriers. We have recently shown that in a glass the probability of finding a barrier with height V is proportional to $1/\sqrt{V}$ (5,7). On the other hand a protein has also very specific conformational states, i.e. 'functionally important states', which are related to well defined motions, say of a large part of the peptid chain (8). It is plausible that the barrier height of such a specific conformational state depends on the actual substate of the glass-like states which the protein occupies, and, hence the barrier is spread around some mean value, which in our case is 650 cm^{-1}. The decomposition of the experimental results in a glass-like distribution and a symmetrically broadened discrete feature is shown in Fig. 7b.

Fluorescence line narrowing experiments and level structure in C-phycocyanin

The hole burning experiments document well the zero phonon nature of the optical transitions in chromoproteins (9-11) in contrast to the results obtained for reaction centers of Rhodopseudomonas viridis and Rhodopseudomonas sphaeroides (12-14). However, the fluorescence emission is broad irrespective of the mode of excitation and detection. A simple explanation of this phenomenon is the assumption of a loss of energy correlation due to transfer processes, that is, a specific level with a sharp frequency in a sensitizing state populates the whole inhomogeneous band of the fluorescing state. The idea was to narrow the fluorescence by resonant excitation of the fluorescing state. Fig. 8 shows that this idea did not work out. There is no sharply structured fluorescence even in case excitation is carried out far in the red edge of the PC-absorption. This phenomenon can be under-

stood on the basis of the special level structure involved (15) and the loss of energy correlation. As to the special level structure we have to assume that the fluorescing state consists in reality of (at least) two states with strongly overlapping inhomogeneous bands. The loss of energy corre-

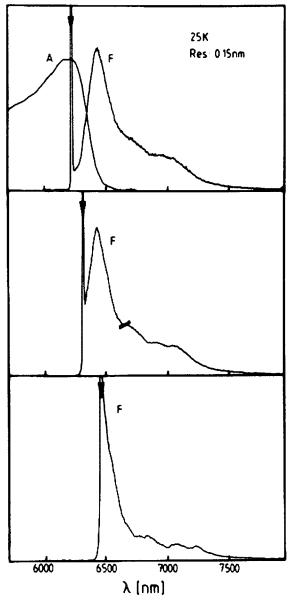

Fig. 8. Selectively excited fluorescence of PC-trimer as a function of excitation wavelength.

lation means that, though the inhomogeneous bands are strongly overlapping, the energy spacing between the two states in an individual molecule can have any value between zero and (roughly) the inhomogeneous width. Since these states are coupled via energy transfer processes the maximum of the fluorescence does not shift with excitation frequency and, as the frequency is scanned into the red, the fluorescence shows a characteristic cut off at the laser frequency. Another consequence of this special level structure is a wavelength dependent hole burning efficiency: As long as the excitation frequency is far enough to the blue of the states involved, there is a high probability that the second level is lower in energy. Hence, the probability for energy transfer is high and, corres-

pondingly, the probability for hole burning or resonance fluorescence is low. As the laser frequency is turned to the red, the probability of finding an acceptor state with lower energy decreases, hence, the efficiency for hole burning and resonance emission increases. Both phenomena can indeed be observed.

Acknowledgement

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