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PHOTOCHEMICAL TRANSFORMATIONS IN CHLOROPHYLL-LIKE MOLECULES VIA THE HOLE-BURNING TECHNIQUE*

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Summary

Hole burning was applied to chlorophyll and related molecules in frozen solutions at liquid helium temperature. Resonant holes were burnt in the 0–0 bands of the fluorescence as well as in the excitation spectra. Several kinds of burning mechanism were found. A photophysical mechanism, population transfer to the triplet state, is reversible within a few milliseconds. In chlorophylls, transformations within the inhomogeneously broadened band represent the mechanism for forming stable holes that are reversible under white light illumination. In metal-free chlorophyll (pheophytin) the formation of a new photoproduct was observed. This photochemical transformation was assigned to a tautomeric rotation of the central protons in the tetrapyrrole skeleton.

The absorption and emission spectra of chlorophyll and related molecules in solutions at room and liquid nitrogen temperatures consist of broad (about 300 cm^{-1} wide) bands which contain little information about vibrational frequencies or details of interactions with the environment. Vibronic spectra of molecular impurities in solids are known to consist of a purely electronic no-phonon line accompanied by a broad phonon wing and their replicas caused by intramolecular vibrational modes [1]. At low temperatures the homogeneous width of the purely electronic line (PEL) can be very small and can even reach the lifetime-determined limit, *i.e.* it can be less than 10^{-3} cm^{-1}. The integral intensities of the PEL and its phonon side band are comparable. This results in marked differences in peak intensities: the peak intensity of the PEL can be from four to six orders of magnitude higher than that of the phonon wing. This means that under a sufficiently narrow line excitation the resonant PELs dominate highly in absorption, which causes a number of specific effects.

However, in many cases, especially in glassy matrixes or frozen solutions, the inhomogeneous broadening of spectra leads to a complete lack of any sharp line structure. Some years ago it was shown by Szabo [2] for ruby and by Personov *et al.* [3] for molecules in glasses that such inhomogeneous broadening can be partly removed by using a selective laser excitation in the purely electronic transition region. However, even under monochromatic excitation the residual

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linewidth may be several orders of magnitude larger than the natural linewidth. Using monochromatic laser excitation at liquid helium temperature, we observed sharp lines in the fluorescence spectra of chlorophyll-a (Chl-a) and some of its derivatives [4 - 6]. Excitation in the vibronic region resulted in linewidths of 5 - 10 cm^{-1}, which is also the limit of accuracy of vibrational frequencies determined from such sharp line spectra.

Resonant 0–0 lines can be much more thoroughly investigated by the photochemical hole-burning technique first used by Gorokhovski *et al.* [7]. Homogeneous widths close to the lifetime limit have been observed by means of photochemical hole burning in phthalocyanine and porphyrins [7, 8] as well as in other molecules [9].

A narrow hole at the laser excitation frequency will appear in the inhomogeneously broadened band of the impurity molecule when optical excitation leads to some transformation (such as photochemical reaction, singlet-to-triplet conversion etc.) that changes the transition frequency [7, 10]. The hole profile and width depend on the hole formation kinetics [11] and are sensitive to the structure of the matrix [12]. The homogeneous 0–0 linewidth and its broadening due to the transition modulation with phonons were first observed via hole burning [13]. Recently, hole burning in the spectra of Chl-a and its closest derivatives has been performed [14, 15]. Here we present more detailed results of these studies.

Holes were observed in two kinds of spectra: fluorescence and excitation spectra. For the first case a double-beam excitation technique was used in order to enable on-resonance fluorescence measurements at the burning laser line to be made. A probe beam was used for the permanent non-selective excitation of the whole inhomogeneous fluorescence spectrum, while another laser beam was applied in the 0–0 region for "burning out" resonantly absorbing molecules. A phosphoroscope system was used for cutting off the stray light of the burning beam so that stationary measurements of holes in fluorescence spectra could be performed with a delay of about 1 ms. In such a way a triplet state bottle-neck

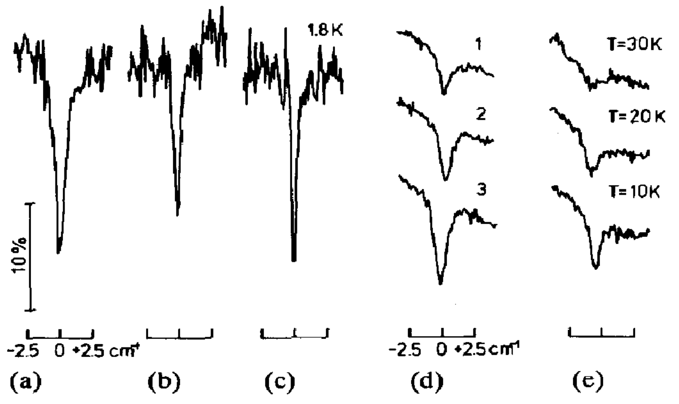


Fig. 1. Resonant holes in the fluorescence spectra of PChl in ether–butanol ((a), (b) and (c)) and ether ((d) and (e)), burnt at 6328 Å: $T = 5$ K, except where indicated. The probe beam (4416 Å) was attenuated by a factor of 10 in (d) from spectrum 1 to spectrum 3.

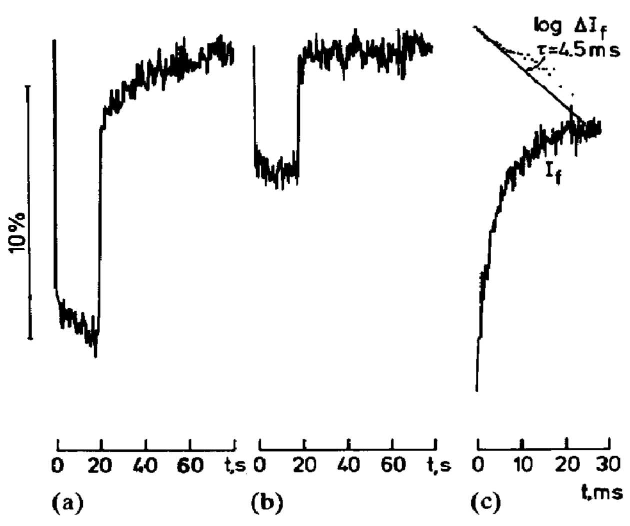


Fig. 2. Hole-burning and recovery kinetics of PChl in *n*-heptane at 5 K. The burning beam was switched on at $t = 0$ and off at $t = 20$ s: (a) $\lambda_f = 6328$ Å; (b) $\lambda_f = 6340$ Å; (c) $\lambda_f = 6328$ Å (the fast recovery is shown on an extended time scale ($t = 0$, "off")).

hole burning was observed for protochlorophyll (PChl) in ether solutions at 4.2 K. The hole of this type was filled in about 5 ms in accordance with the triplet state lifetime of PChl [16].

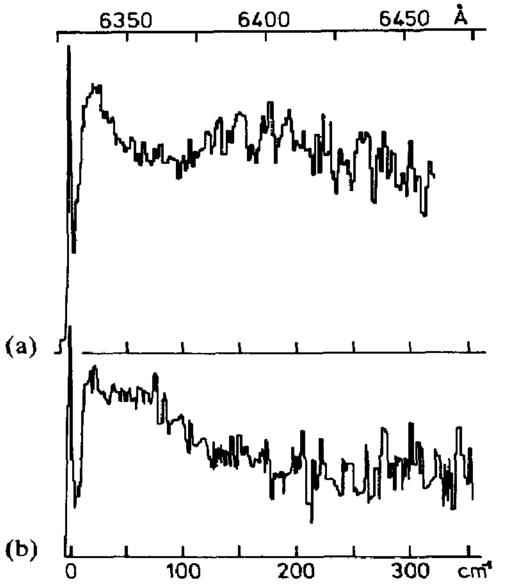
The instrumentally limited hole widths in the spectrometer recordings at 5 K were 0.5 cm^{-1}, but the hole broadened with temperature (Fig. 1). The hole depth was dependent not only on the burning beam but also on the probe beam intensity. This indicates the existence of light-induced reversibility in the hole-burning mechanism. This aspect was further considered by kinetic measurements (Fig. 2). Apart from the fast phase in the burning and in the recovery kinetics caused by the build-up and decay respectively of the triplet state, a slow phase was found. This is another manifestation of the above-mentioned reversible phototransformations, which can tentatively be classified as photochemical processes. Of course, the question of the origin of these processes arises. We found that the mechanism consists of site interconversions within the inhomogeneously broadened spectral band. The interconversion mechanism means that after photoexcitation to its excited singlet state S_1 the molecule changes its degree of interaction with the surrounding solvent molecules with a certain probability so that its 0–0 line is shifted to another wavelength within the inhomogeneous band. Under resonant monochromatic excitation this process results in hole burning at the laser wavelength. However, since the probe beam excitation is non-selective, it is able to excite all the molecules with almost the same efficiency, causing a large number of different "hops" of the 0–0 lines and leading to the recovery of the hole. Furthermore, partial recovery may be caused by the burning line itself if strong phonon wings exist.

We demonstrated the important role of phonon wings. They were measured during the fast phase of recovery. Two time gates were used for obtaining the difference spectra between 15 ms and no delay. The result can be seen in Fig. 3.

The main maximum is at 25 cm^{-1}, and overlapping with the intramolecular modes is possible in the high frequency tail.

Returning to the problem of site interconversions, we may note that molecules excited via their phonon wings tend to fill the hole produced by the excitation via the no-phonon line. Further, in the region of the phonon wing of the hole there is an approximate dynamic equilibrium between the incoming and the outgoing hops. As a result there are almost no slow changes in the phonon wing region, while on the resonance line both components contribute (Fig. 2).

The same mechanism of site interconversions was found to be mainly responsible for the hole burning for Chl-a. Here we used the second scheme where the hole was first burnt at a constant wavelength and then the excitation spectrum was measured near the line. Using a single-frequency laser we obtained a value of 0.01 cm^{-1} (300 MHz) for the width of the hole at 1.8 K (Fig. 4). Owing to the broad band recorded, the excitation intensity could be made very low and its actinic effect practically excluded. This enabled us to investigate dark processes. The holes burnt in the inhomogeneous profile of the spectra of Chl-a were found to be stable in the dark at 4.2 K. The elimination of light-induced as well as dark recovery enabled us to observe the evolution of the inhomogeneous profile as a function of burning time (Fig. 4). As can be seen, the greatest contributions to the transformations are from those species with small 0–0 electronic energy shifts. The main species of Chl-a in frozen solutions has been identified [17] as being monoligated to polar impurities at the magnesium atom. The dominating mecha-



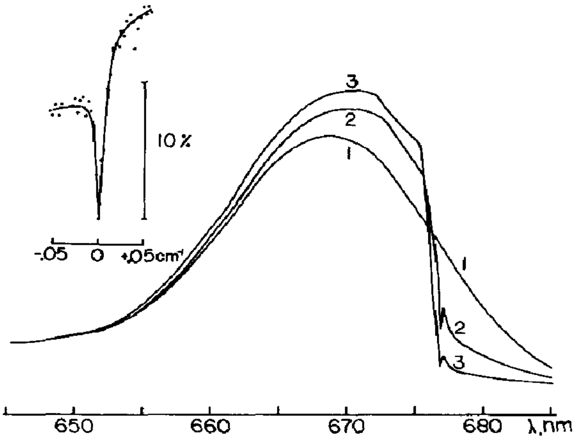


Fig. 3. Phonon wings accompanying the holes in the difference spectra of PChl at 5 K in (a) EB and (b) C_7.

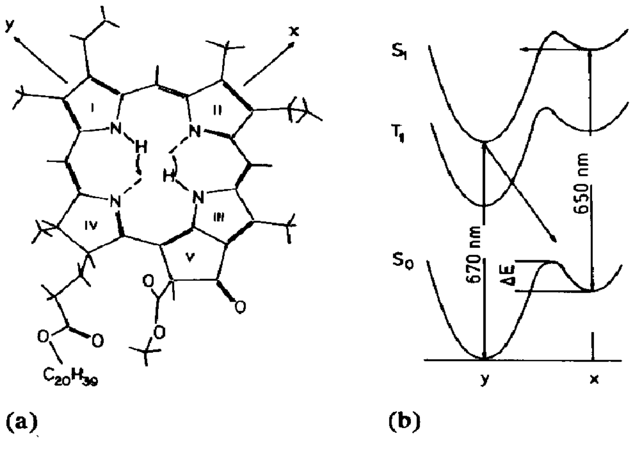
Fig. 4. Fluorescence excitation spectra of Chl-a at 5 K before burning (curve 1) and after hole burning with 250 mW cm^{-2} at 6764 Å for 3 min (curve 2) and 15 min (curve 3). In the inset a hole in the excitation spectrum of Chl-a at T = 1.8 K is shown measured by etalon scanning the single-frequency 6764 Å output of a CR-2000K krypton laser.

nism of site interconversions may be assigned to the changes in the strength of ligation to magnesium as well as to some peripheral groups.

We do not discuss further here the question whether the site interconversion is a photochemical or a non-photochemical process (see, for example, ref. 18), but we go on to another example which has been classified as photochemical hole burning. For metal-free porphyrins [7, 8] and chlorin [19] the hole burning has been explained as a result of hydrogen tautomeric transitions. For these systems a redistribution of intensity between sharp lines [7, 8] or the appearance of new photoproduct lines [19] has been observed after burning. A similar process should be possible in metal-free chlorophylls, *i.e.* pheophytins (Pheo), where the central magnesium atom is replaced by two hydrogen atoms. Indeed, after irradiation for a few minutes of Pheo-a at 4.2 K in its absorption band near 670 nm we succeeded in observing a new fluorescence maximum at 650 nm. However, the identification of this weak structureless band required new experiments.

Firstly, under an exciting probe beam the new band disappeared rapidly but could be observed steadily through a phosphoroscope system, so the process was reversible. The hypothesis about the tautomeric transformation was supported by the absence of a similar new band in the spectra of Chl-a after irradiation in its 0–0 band. Quite a large shift (about 500 cm^{-1}) of the 0–0 band of the new phototautomer from that of the initial form of Pheo-a can be explained if we take into account that in Pheo-a the two possible orientations of the inner hydrogen pair are not equivalent (Fig. 5). Analogously, in chlorin the tautomeric shift of the 0–0 transition is about 1500 cm^{-1} [19].

The fluorescence excitation spectrum of the photoproduct was measured in order to obtain some information about the higher excited states (Fig. 6). In the blue region an intense band (the so-called Soret band), slightly shifted to lower



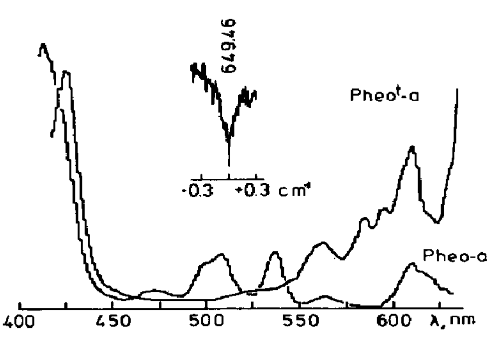


Fig. 5. (a) Molecular structure of Pheo-a; (b) hypothetical potential curves for the rotation of the inner hydrogen atoms.

Fig. 6. Fluorescence excitation spectra of Pheo-a (λ_f = 675 nm) and its photoproduct Pheo^t-a (λ_f = 645 nm) at 4.2 K. In the inset a part of the photoproduct band is shown after hole burning with 90 $\mu J\ cm^{-2}$ at 649.5 nm (λ_f = 700 nm).

energies, was observed. In the intermediate region the characteristic Pheo-a bands at 508 and 536 nm disappeared, while a new maximum at 584 nm arose which could be assigned to the $S_2 \leftarrow S_0$ transition. The maxima around 610 nm belong to the vibronic $S_1 \leftarrow S_0$ transitions. Monochromatic excitation in this region resulted in a quasi-line structure in the 650 nm fluorescence band. Further, narrow ($\delta\lambda \leqq 0.15$ cm^{-1}) holes could easily be burnt in the photoproduct 0–0 band of the excitation spectrum near 650 nm (Fig. 6).

An observation of the line structure and narrow holes proves the existence of well-defined potential minima for the hydrogen pair in the photochemically produced tautomer (Fig. 5). It has been shown theoretically [20] and by nuclear magnetic resonance (NMR) [21] that in the ground electronic state of the stable tautomer of pheophorbide-a (*i.e.* Pheo-a without the phytol chain) the NH–NH axis is along the y axis. This is caused by the higher electron density on the nitrogen atoms of rings I and III. The same argument is valid for the S_1 and T_1 excited states. We suppose that in the phototautomer both protons are moved to the adjacent nitrogen atoms lying along the x axis, although the NMR data do not exclude other configurations.

In Fig. 5 we have sketched a configuration coordinate potential curve for the tautomeric rotation in Pheo-a. The height of the potential barrier in the ground state was estimated from the temperature dependence of the dark decomposition of the photoproduct. The decays were clearly non-exponential, evidently as a result of the existence of tautomers with different activation barriers ΔE. From the slopes of the initial nearly exponential parts of the decay curves we obtained an estimate for the barrier height ΔE of approximately 50 cm^{-1}. Probably, the values of ΔE are inhomogeneously distributed over a wide range owing to the interaction with surrounding molecules, so the tautomerization is not a purely intramolecular process.

Since the photoproduct was found to be stable in the dark at 4.2 K, the dynamics of phototransformations could be investigated. By taking into account the extinction coefficient and the burning density in each measurement, the phototransformation efficiency could be estimated from the observed decay rates. For the formation of the phototautomer we obtained a yield of 10^{-3}. Since insufficient activation energy is available at low temperatures, a one-photon process cannot take place immediately in the S_1 excited state but must occur in the triplet state or in the ground singlet state.

The yield of the photodecomposition of the unstable tautomer appeared to be higher by almost two orders of magnitude. The higher probability of this transformation can be expected since such a process is possible as a tunnelling transition in any of the electronic states involved (S_1, T_1 or S_0). The lifetime τ_f of the S_1 state of the phototautomer was measured to be 4.4 ns, while for Pheo-a $\tau_f = 7.9$ ns. Most probably, the lifetime is shortened as a result of the increase in the rate of non-radiative processes.

Summarizing the results, we point out that three different kinds of reversible phototransformations leading to the hole burning were found for chlorophyll-like molecules: (1) population transfer to the lowest triplet state; (2) site interconversions within the inhomogeneous profile; (3) tautomeric rotation of the inner protons in the tetrapyrrole ring. Process (1) and especially process (3) are intramolecular, however, and are certainly influenced by the interaction with solvent molecules. Mechanism (2) is thought to be completely due to the variations in the solute–solvent interactions. Since reversible photochemistry is a primary step in photosynthesis, the investigation of analogous processes *in vitro* by the hole-burning technique is of special interest.

Acknowledgments

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