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HOLE BURNING IN BIOPOLYMERS

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Abstract The technique of spectral hole burning has increasingly been used to investigate biopolymers. It can be used to measure extremely fast relaxation times, for instance in photosynthesis, but it can also be used to measure slow structural relaxation in the groundstate. I review various activities in this field.

INTRODUCTION

It seems to be a characteristic feature of biopolymers that they appear simultaneously as ordered and disordered entities. For example, the highly regular X-ray diffraction patterns, which are observed for quite a large series of biopolymers[1], are a direct indication of structural order. Yet, on the other hand, X-ray diffraction shows clearly that biopolymers are structurally disordered, too: The mean deviation from the equilibrium geometry, as obtained from the Deby-Waller-factor, may be as large as 0.5Å and is, in addition, subject to large variations along the polymer backbone[1,2]. Hence, there are segments which are characterized by a rather large local free volume, so that conformational changes can occur. The number of conformational substates is incredibly huge. For a protein, such as myoglobin, with 150 aminoacid residues it is on the order of 10^{40}. Some of these states may be in fast equilibrium, others are not. As a consequence, biopolymers are very often non-ergodic systems. In this respect, they resemble very much glasses. Also, like dye-doped glasses, their optical transitions are inhomogeneously broadened, so that a straight forward high resolution spectroscopy is impossible [3,4]

BASIC FEATURES OF SPECTRAL HOLE BURNING

Hole Burning is a very sensitive technique which allows for high resolution spectroscopy in the presence of structural disorder[5]. The limit of the resolution is determined by the natural linewidth of the transition investigated. Hole burning needs a long-lived intermediate where population can be stored, so that a groundstate depletion occurs for some time at the laser frequency. In case of photochemical hole burning the intermediate is a stable photoproduct, hence, the associated hole is persistent. In case of photophysical hole burning the intermediate is due to an optically induced change in the interaction between dye and surrounding matrix. Photophysical holes are persistent, too. Transient hole burning occurs when population is stored in a state with a finite lifetime. For example, hole burning in photosynthetic reaction centers occurs by storing population in the charge separated state with a lifetime on the order of 270μs [6].

A series of interesting informations can be extracted from a hole-burned spectrum:

a) Fast relaxation times associated with the homogeneous width, e.g. energy transfer times, electron transfer times, vibrational relaxation times, dephasing times.
b) Extremely slow relaxation times in the groundstate associated with the relaxation of the hole itself [3,7]
c) The chromophor-lattice coupling associated with the Debye-Waller-factor
d) Vibrational fine structure in the excited state.

In the following I will review some of the hole burning work on biopolymers.

HOLE BURNING IN CHROMOPROTEINS OF PHOTOSYNTHESIS

The fact, that sharp zero-phonon holes do occur in the absorption of chromophores embedded in proteins, was first shown for pigments of bacterial photosynthesis, namely phycoerythrin and phycocyanin[8]. These pigments show also sharp holes when embedded in phycobilisomes (Fig. 1). Phycobilisomes are supramolecular aggregates with a molecular weight on the order of 5000kD. They are highly organized, so that light harvesting and energy transfer to the reaction center is optimized.

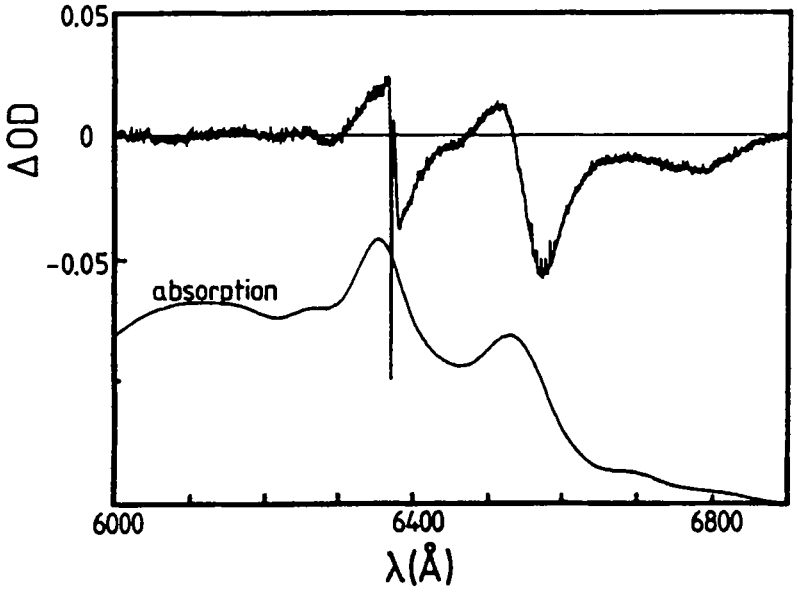

FIGURE 1 Low temperature absorption and ΔOD spectrum of phycobilisomes of *Mastigocladus laminosus*. Laser excitation was performed at 6380Å [9].

The lower trace in Fig. 1 represents the absorption of the phycobilisomes. The peaks around 6530Å and 6315Å originate form allophycocyanin and phycocyanin, respectively. The upper trace represents the ΔOD spectrum after hole burning at 6380Å. ΔOD > 0 characterizes regions where photoproduct appears. Since product appears in the immediate neighborhood of the zero-phonon line, we conclude that at least part of the reaction must be photophysical in nature. In this case the chromophore itself remains unchanged. It is evident from the ΔOD spectrum that most of the reaction occurs in the allophycocyanin chromophore which acts as an energy acceptor. Also, energy selectivity is lost to a high degree due to the transfer process.

The transfer process from phycocyanin to allophycocyanin in the phycobilisome assembly is very efficient, as can be inferred from Fig. 2. Two holes are shown both burnt into phycocyanin. However, the upper trace stems from the isolated pigment, whereas the lower trace from the phycobilisomes. We attribute the much larger width in the phycobilisomes

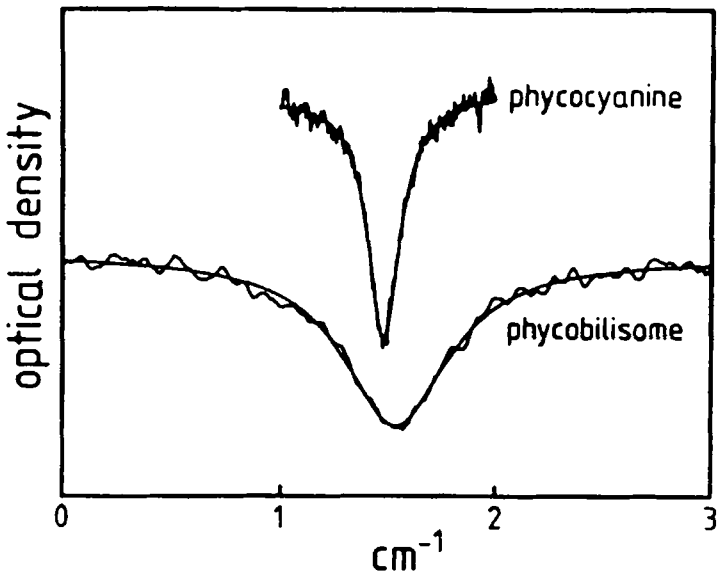

FIGURE 2 Zero-phonon hole of isolated phycocyanin trimers and phycobilisomes [9].

to fast energy transfer processes to allophycocyanin. The transfer time is estimated to be larger than 16ps.

I stressed above the similarity between proteins and glasses. This similarity is most obvious in the dependence of the so-called "quasihomogeneous" linewidth on the temperature. In glasses, it has been shown by numerous experiments that this width depends almost linearly on temperature [10]. The reason for this behavior is attributed to the influence of structural disorder. An example for a chromoprotein, namely phycocyanin trimers, is shown in Fig. 3. The temperature exponent in this case is 1,16. Around 1K the linewidth is on the order of 270MHz, which is rather close to the lifetime limited value (100MHz).

In recent years the hole burning technique was used by several groups to elucidate the details of the very first steps in the electron transfer process of photosynthetic reaction centers. Investigations were performed on the primary donor states of *Rhodobacter spheroides* (P870), *Rhodopseudomonas viridis* (P960) photosystem I (P700) and photosystem II(P680). This hole burning activity was triggered by a paper by Boxer et al [12]. These authors

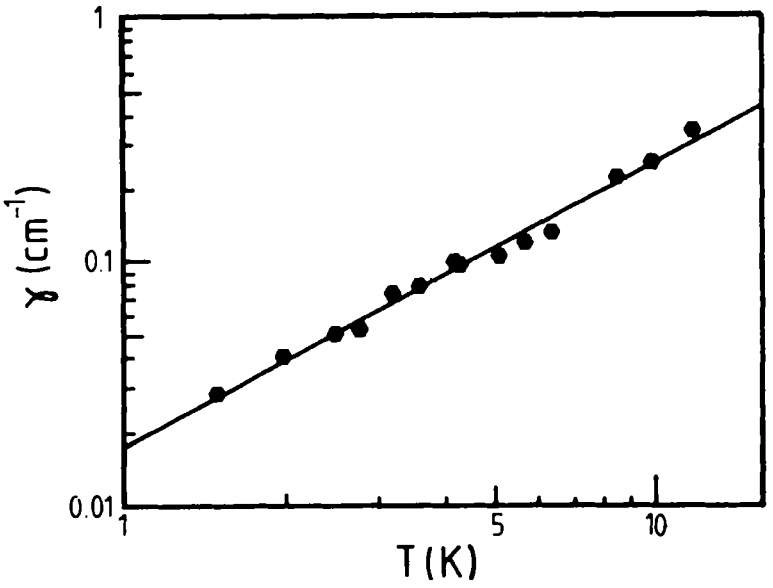

FIGURE 3 Hole width (extrapolated to zero fluence) as a function of temperature. Sample: C-phycocyanin trimers in ethyleneglycol/water [11].

arose whether these broad holes are due to ultrafast relaxation processes in the femtosecond time domain or to strong electron-phonon coupling. Time domain experiments by Meech et al. [13] showed that a very fast relaxation process is indeed involved. Also, very recent hole burning experiments by Shuvalov et al. [14] were interpreted in this way. In their experiments they found sharp holes with a width on the order of a wavenumber when the electron transfer chain was blocked. Otherwise very broad holes were measured. G. Small and his group could come to an interpretation which fits the time domain as well as the frequency domain experiments [6]: The extremely fast relaxation observed is most probably due to vibrational relaxation which establishes thermal equilibrium before electron transfer. The broad features of the hole, which are observed only above a certain excess energy, can be perfectly interpreted by assuming a moderately strong coupling to a vibration of the special pair ("pair marker mode") which in turn couples moderately strongly to the phonons. If burning is carried out at the very red edge of the absorption, sharp zero-phonon holes could even be

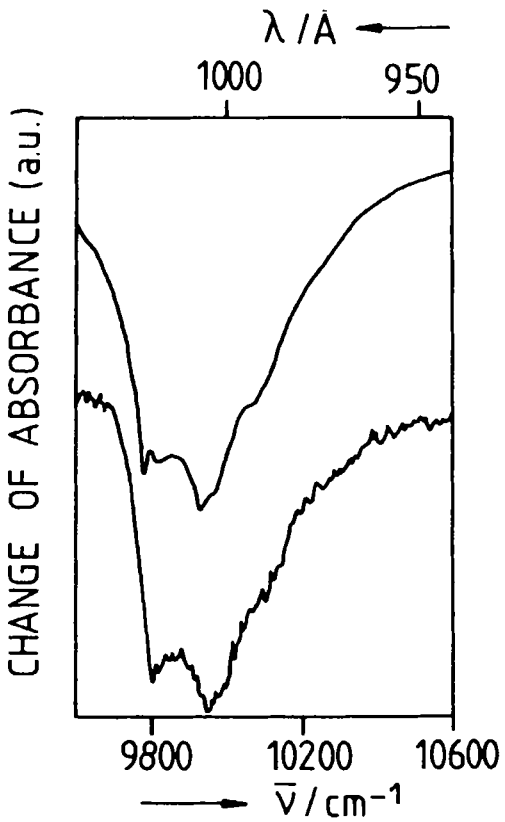

FIGURE 4 Hole-burned spectrum of P960* of *Rhodopseudomonas viridis* [6].

observed. Their widths are in line with an electron transfer time on the order of 1ps. This view fits also quite well to the recent data by Holzapfel et al. [15]. Fig. 4 shows a hole-burned spectrum with red edge excitation of the reaction center of *Rhodopseudomonas viridis* (P960*). Note the sharp zero-phonon feature around 9800cm^{-1}, where excitation was performed. Also the pair marker mode with a 150cm^{-1} spacing can be indentified. The upper trace shows a simulated spectrum.

I stress, that the hole-burned spectra of photosystem I and II-reaction centers look quite different. They can, however, be interpreted quite satisfactorily on the basis of the electron-phonon coupling theory [16,17].

HOLE BURNING IN HEME PROTEINS

The simplest heme protein is myoglobin. It stores and transports oxygen in muscles. Myoglobin serves in many cases as a prototyp of a protein. In a series of papers it was shown that the absorption of the heme molecule is indeed inhomogeneously broadened due to a distribution of conformational substates. It is the kinetics of binding and rebinding of O_2 and CO to the heme molecule which is sensitive to the actual conformational substate which the protein occupies. Hence, this kinetics can be used to probe the conformational inhomogeneity. For example, it was found that the near IR-band around 760nm of deligated CO-myoglobin shows a temporal shift to the blue and a narrowing as the ligand rebinds [18]. Obviously there is a correlation between reaction rates and site energy in the sence that the low energy sites have faster reactive rate constants. Hence, the reaction leads to frequency selective changes in the band, a fact which has been called "dynamic hole burning" [19]. The inhomogeneity was estimated to be on the order of 20% of the total band. It is this small amount of inhomogeneity which makes a straight forward photochemical hole burning experiment difficult. The homogeneous width of the transition is obviously very large, probably due to fast intersystem crossing processes induced by the central iron atom. Recently, Pahapill and Rebane succeeded in determining the homogeneous linewidth of the visible band of oxymyoglobin by performing a straight-forward hole burning experiment (Fig. 5 [20]). It is on the order of $65cm^{-1}$ which corresponds to a T_1-lifetime on the order of 80fs.

HOLE BURNING IN DNA-STRANDS:

So far hole burning spectroscopy on biopolymers has been almost totally confined to chromoproteins. Most biopolymers, however, lack intrinsic dyes, and the question arises whether or not this category of biopolymers is beyond any application of the hole burning technique or not. There seem to be two possibilities: either to burn holes into the UV where the backbone absorbs or to use proper dye molecules which bind or intercalate to the biopolymer. As far as I know, the first possibility has never been tried, the second possibility however `has. It was used` by Flöser and Haarer to burn

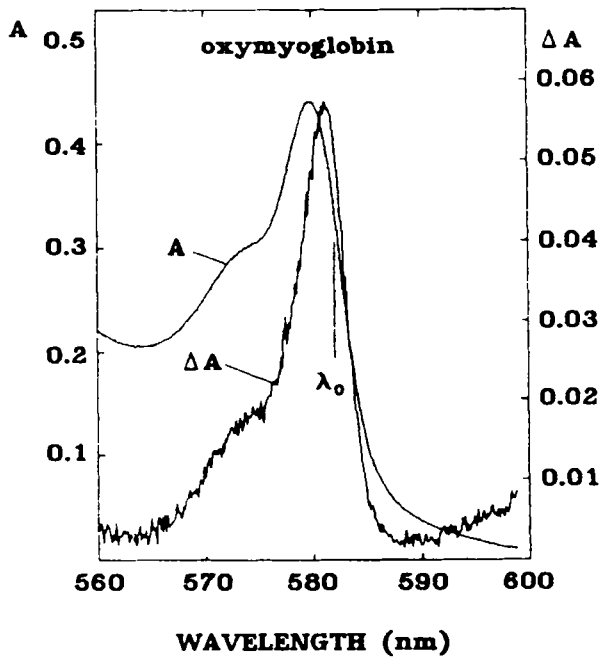

FIGURE 5 Absorption A and hole-burned spectrum ΔA of oxymyoglobin at 4.2K. Laser irradiation was carried out at λ_o=582.0nm [20].

holes into DNA-intercalation complexes [21]. The probe dye was daunomycin which is well known as an anticancer drug. Its chromophoric group is very similar to quinizarin which has been shown to undergo very efficient hole burning via proton rearrangement reactions [5].

Fig. 6 shows an intercalation complex (daunomycin-d(CGTACG)) as determined from X-ray diffraction [22]. Fig. 7 shows the absorption of daunomycin intercalated into a DNA oligonucleotid. Hole burning was performed right at the peak of the long wavelength band. Several interesting conclusions could be drawn from these experiments: The fact, that hole burning does occur, shows that the absorption bands are inhomogeneously broadend. Hence, similar to proteins, microscopic disorder prevails. The hole burning process is most likely of photophysical nature. Most surprisingly, the reaction yield was found to differ dramaticly for different

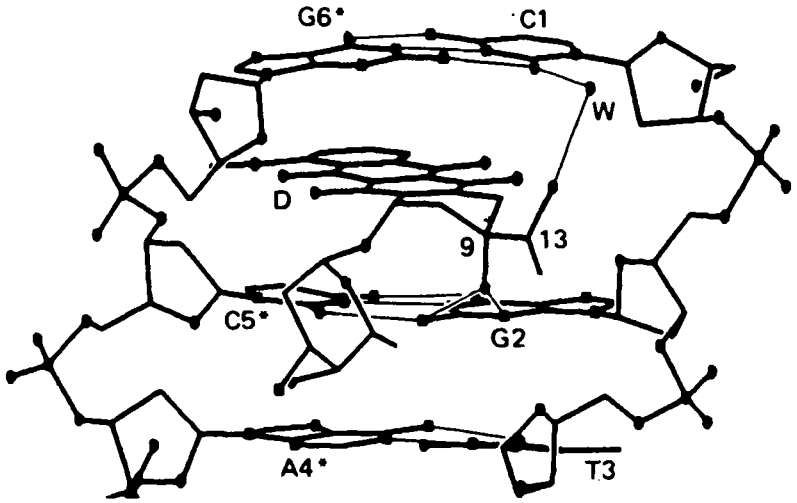

FIGURE 6 Structure of a DNA-intercalation complex (daunomycin-d (CGTACG)) after Wang et al [22].

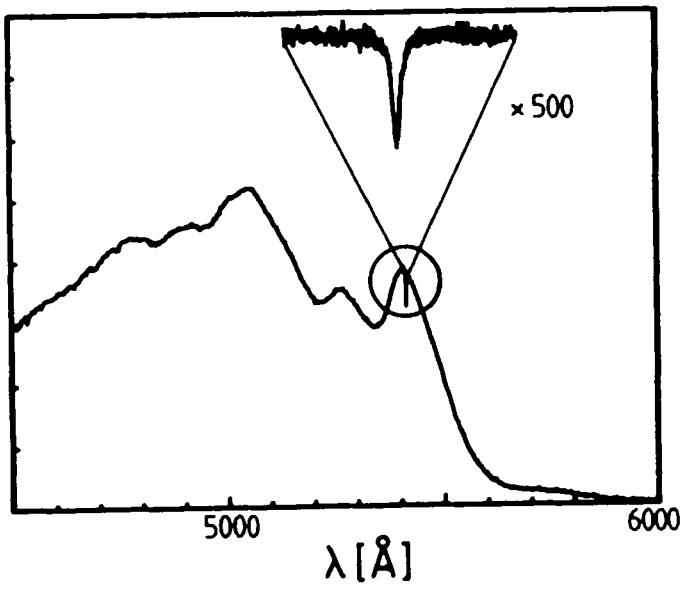

FIGURE 7 Hole Burning of a daunomycin-d $(AT)_5$ intercalation complex [21].

oligonucleotids. Also, in this case, the groundstate relaxation of the hole was observed over a long time scale. It was found that the decay function follows closely a log t-behavior, a feature which has also been observed for a variety of organic glasses [3].

INVESTIGATION OF GROUNDSTATE PROPERTIES OF BIOPOLYMERS VIA PERSISTENT SPECTRAL HOLES

The molecular weight of biopolymers may cover a weight of several 10 kDa to several thousand kDa. The phycobilisomes of *Mastigocladus laminosus*, for example, have a molecular weight on the order of 5000 kDa. Though, for spectroscopic purpose, these proteins are dissolved in a glassy matrix, it seems that the chromophores do not significantly interact with the glassy solvent since they seem to be well shielded by the huge biopolymer. Hence, the chromophores probe the proteinaceous environment rather than the host glass. A spectral hole burnt into the absorption of a specific chromophore can be considered as a characteristic label for a specific protein state. As the temperature is changed or as time goes on, this state undergoes conformational relaxation which is reflected in a broadening and a recovery of the burnt-in hole. Hence, relaxation processes of the whole protein in its groundstate can be investigated with a high level of precision just by observing the changes of a burnt-in hole as a function of a proper parameter. In this application mode the hole burning technique is rather unique because there is almost no other spectroscopic technique with the capability of measuring groundstate relaxation processes of structurally disordered materials (for a review, see [3]).

An example is shown in Fig. 8. The insert shows part of the absorption of the core pigment, namely allophycocyanin, of intact phycobilisomes together with a burnt-in hole at 6573 Å (see also Fig. 1). This hole serves as a label for the starting state of the protein. The actual experiment is a temperature cycling experiment, which is performed in the following way: The hole is always measured at the starting temperature where it was burnt. In the case considered here, this temperature is 4.2 K. The protein is, however, exposed to temperature cycles, where the so-called cycling temperature (abscissa in Fig.8) is steadily increased in steps of, say 1 K, or so. When the temperature is increased the protein relaxes into a manifold of

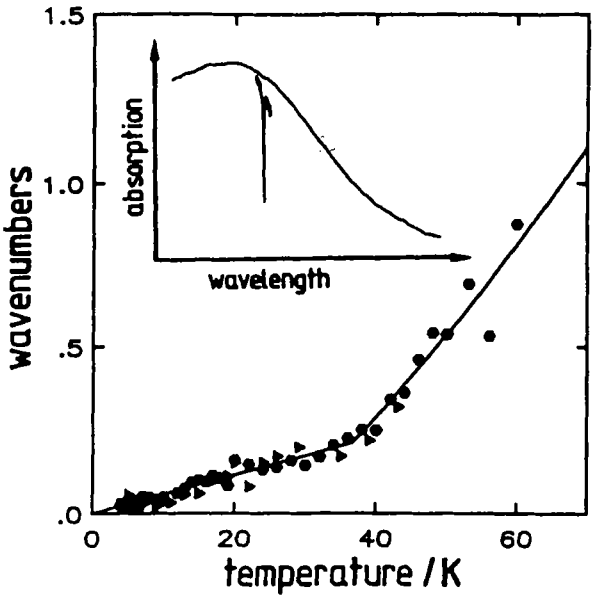

FIGURE 8. Thermally irreversible broadening of an optical hole (insert) as measured by a temperature cycling experiment.
Sample: phycobilisomes of *Mastigocladus laminosus* [23].

new conformational states and eventually gets trapped in one of them when the temperature is lowered again. The change in the microenvironment results in slight changes in the interaction between protein and chromophore which in turn leads to a broadening of the burnt-in hole. I think that an experiment of this type is the most direct demonstration that a protein undergoes indeed relaxation between conformational substates, even at temperatures as low as a few K. The change of the holewidth as a function of cycling temperature can be modeled within the frame of spectral diffusion theories modified in a proper way to account for thermal irreversibility [3,7]. The data can be accounted for by assuming that the conformational relaxation processes occur by tunneling at temperatures below 35 K and are activated above. Tunneling processes lead to a linear increase of the holewidth whereas activated processes lead to an increase proportional to $T^{3/2}$. These temperature laws essentially results from a

distribution of conformational barrier heights and associated energies of the conformational states as a consequence of local structural disorder. In this respect, as has been stressed several times, proteins are very similar to glasses.

Summary

Hole burning offers attractive and unique possibilities in the spectroscopy of biopolymers. Though the focus in this field is still on chromoproteins of photosynthesis, it was applied to other materials, such as heme proteins or DNA-intercalation complexes, as well. In the investigation of groundstate properties hole burning is unique. There is no other technique with the capability of measuring groundstate relaxation processes with high sensitivity in a straight forward way.

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