Lietuvos fizikų draugija
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Lietuvos aukštosios mokyklos

LIETUVOS FIZIKOS ŽURNALAS

LITHUANIAN JOURNAL OF PHYSICS

ЛИТОВСКИЙ ФИЗИЧЕСКИЙ ЖУРНАЛ

Mokslinis žurnalas

34

Nr. 4

VILNIUS "FISICA" 1994

LIETUVOS FIZIKOS ŽURNALAS

до 1993г.

LIETUVOS FIZIKOS RINKINYS

ЛИТОВСКИЙ ФИЗИЧЕСКИЙ СБОРНИК

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2600 Vilnius, A. Goštauto 12,
Lithuanian Physical Society,
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2600 Вильнюс, ул. А. Гоштауто 12,
Литовское физическое общество,
тел. 611-980

ISSN 0024–2969

PROCEEDINGS OF

INTERNATIONAL WORKSHOP ON

PIGMENT-PROTEIN COMPLEXES: STRUCTURE AND SPECTRAL PROPERTIES

(May 12-16, 1994, Preila, Lithuania)

Tarptautinio simpoziumo

Pigmentų baltymų kompleksai: Sandara ir spektrinės savybės

D A R B A I

1994 m. gegužės 12-16 d., Preila, Lietuva

FISICA
in cooperation with
ALLERTON PRESS, INC.

Lietuvos fizikos žurnalas, 1994, 34, Nr. 4, 339–343

SUBSTRUCTURE ANALYSIS OF THE BACTERIAL ANTENNA LH II BY NONLINEAR POLARIZATION SPECTROSCOPY IN THE FREQUENCY DOMAIN

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For the nonlinear polarization spectroscopy in the frequency domain (NLPF), line shape formulas are given for the cases of a homogeneously broadened as well as an extremely inhomogeneously broadened absorption band, for the intermediate case, and for a superposition of several bands. As experimental examples of the latter case, NLPF line shapes for the 850 nm part of the light-harvesting antenna LH II of *Rh. sphaeroides* are presented. At room temperature at least 3 subbands are clearly resolved for the first time. Their phase relaxation times are about 30 fs, corresponding to homogeneous widths around 300 cm^{-1}.

1. Introduction

Substructures of absorption bands of photosynthetic pigment-protein complexes are of considerable interest. They can be used to distinguish various binding sites and contain information on excited-state dynamics. Although the subject has been investigated for decades, there is up to now a lack of methods which can give direct evidence of subbands (or their absence) at physiologically relevant room temperature. We are currently exploring the potential of a method, which can yield this information, viz. nonlinear polarization spectroscopy in the frequency domain (NLPF). In the following, a summary is given on the principal information content of NLPF spectra, and results obtained in the 850 nm band of the bacterial antenna LH II (B800–850) are discussed.

2. NLPF: method and its information content

The principle of the NLPF is shown in Fig. 1. The sample is probed by a linearly polarized highly monochromatic probe beam at a fixed wavelength λ_2^{fix} located in the spectral region of the absorption band to be analysed. This beam reaches the sample via a polarizer and is blocked behind it by a perpendicularly oriented polarizer. The sample is pumped by a second linearly polarized, highly monochromatic (pump) beam polarized at an angle of 45° with respect to the polarization of the probe beam and with its wavelength λ_1^{var} located somewhere in the region of the same electronic transition. It crosses the probe beam nearly parallel in the opposite direction within the sample. At sufficient intensity of the pump beam, nonlinear

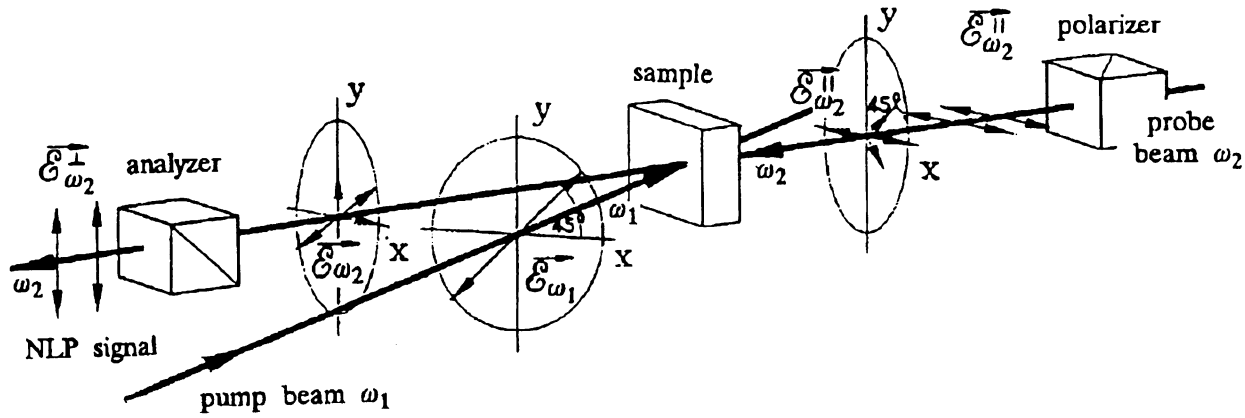

Fig. 1. The principle of nonlinear polarization spectroscopy in the frequency domain. The probe wave is linearly polarized at 45° to the linearly polarized pump beam. The pump beam induces dichroism and birefringence in the sample. The dichroism and birefringence lead to a component orthogonal to the initial probe polarization. This orthogonal component of the probe wave is the signal of the nonlinear polarization spectroscopy

interaction in the sample induces dichroism and birefringence and gives rise to a certain polarization component at λ_2^{fix} orthogonal to the original polarization. The intensity of this signal is measured behind the second polarizer. The pump beam λ_1^{var} is tuned over the spectral range of the absorption band under investigation including the resonance case $\lambda_1^{var} = \lambda_2^{fix}$ to yield the NLPF spectrum. Both beams are obtained from tunable dye lasers, whose pulse durations are greater than the longest excited-state relaxation time of the system under investigation (stationary NLPF). The principle of the experimental set-up is given in [1], for the present measurements it was improved by using (i) a pair of selected polarizers, (ii) narrow band dye lasers, and (iii) a photomultiplier instead of a photodiode as detector. The details will be given elsewhere (Voigt et al., in preparation).

The information content of the NLPF line shapes can be analysed for model systems by solving the equations of motion of the density matrix of interest by the third-order perturbation theory [2–4]. In the following, a short description is given of the theoretical results for four exemplary situations, viz. a single homogeneously broadened absorption band, an extremely inhomogeneously broadened band, the intermediate case of moderately inhomogeneous broadening, as well as the case of several overlapping, homogeneously broadened bands (heterogeneous broadening). The latter case is of special interest with respect to the substructure analysis of the 850 nm absorption band of LH II.

In the following the theoretical line shape functions are given for these four cases. Also some general rules are given for the shape and the information content, which are derived from these formulas.

The following notation will be used: $\gamma = T_1^{-1}$, $\Gamma = T_2^{-1}$, $\omega = 2\pi c\lambda^{-1}$, ω_1 is the pump beam frequency (variable), ω_2 is the probe beam frequency (fixed), ω_0 is the centre frequency of the electron transition, ω_L is the peak frequency, δ is half of FWHM of the distribution function of centre frequencies of the electron transition, $\Delta = \omega_1 - \omega_2$, $\omega_{ab} = \omega_a - \omega_b$, $\Gamma_+ = \Gamma + \delta$, and α is a factor independent of ω_1, ω_2, and Γ.

2.1. *Case a: single, homogeneously broadened absorption band*

$$S^{(a)}(\Delta;\omega_{02},\Gamma) = \frac{\alpha}{\Gamma^2+\omega_{02}^2}\left|\frac{1}{\gamma}\left(\frac{1}{\Gamma+i\omega_{01}}+c.c.\right)+\frac{1}{\gamma+i\Delta}\left(\frac{1}{\Gamma+i\omega_{02}}+\frac{1}{\Gamma-i\omega_{01}}\right)\right|^2$$

An absorption band of an ensemble of species is called homogeneously broadened, if the bandshape of an individual species is identical with the shape of the global absorption band, which in this case is of the Lorentzian type.

If the maximum of this absorption band is located at λ_0 and $\lambda_2^{fix} \neq \lambda_0$, then the NPLF line shape usually shows two maxima, one at resonance $\lambda_1^{var} = \lambda_2^{fix}$, the other at $\lambda_1^{var} = \lambda_0$. An example of a theoretical NLPF line shape in the case of homogeneous brodening is shown in Fig. 2*a*. It

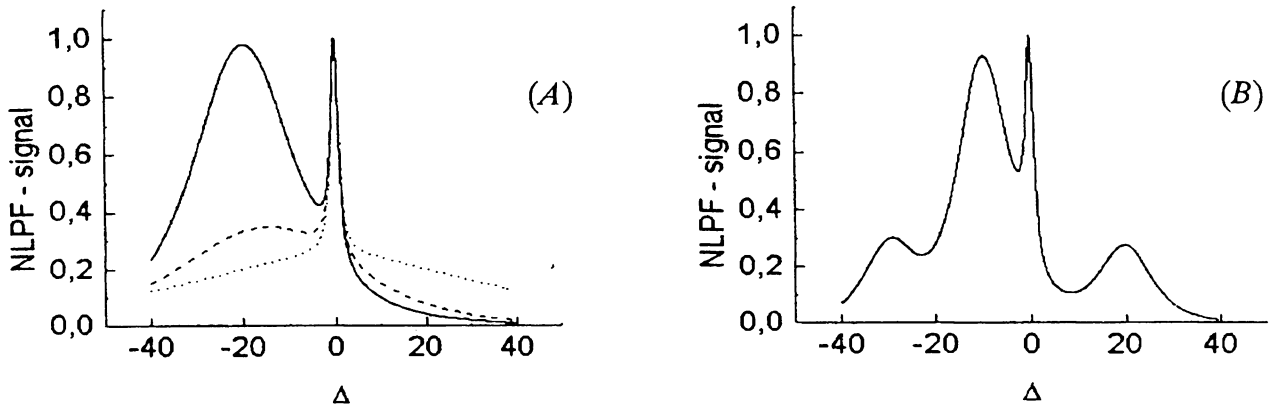

Fig. 2. Examples of NLPF line shapes, calculated according to the formulas given in the text (see text for denotations). (*A*): ($\gamma = 1$, $\Gamma = 20$, $\omega_0 - \omega_2 = -20$), —— single, homogeneously broadened band (case *a*), · · · single, extremely inhomogeneously broadened band (case *b*), and – – – single, moderately inhomogeneously broadened band (case *c*, with $\delta = \Gamma$). (*B*): heterogeneous broadening (case *d*) with $\gamma = 1$ and $\omega_{01} - \omega_2 = -30$, $a_1 = 1$, $\Gamma_1 = 10$; $\omega_{02} - \omega_2 = -10$, $a_2 = 0.7$, $\Gamma_2 = 10$; and $\omega_{03} - \omega_2 = 20$, $a_3 = 0.5$, $\Gamma_3 = 10$ (frequencies are given in 10^{12} s^{-1}

varies distinctly with changes in the location of λ_2^{fix}; if in particular λ_2 is located at the half width of the absorption band, then both NLPF maxima are of the same height.

2.2. *Case b: single, extremely inhomogeneously broadened band*

$$S^{(b)}(\Delta) = \alpha \frac{4\gamma^2 + \Delta^2}{\left(4\Gamma^2 + \Delta^2\right)\left(\gamma^2 + \Delta^2\right)}$$

The overall absorption band of an ensemble of species is called extremely inhomogeneously broadened, if the width of the distribution function of the centre (maximum) wavelengths of all individual absorption transitions is much larger than the homogeneous width of each of them. The overall absorption band then is of the Gaussian type.

In this case, the NLPF line shape consists of a single, symmetric signal centred at $\lambda_1^{var} = \lambda_2^{fix}$, and there is no 'fingerprint' of the overall absorption band maximum (Fig. 2*a*). In this simplest case of a NLPF line shape, the energy relaxation (longitudinal relaxation) time T_1 is reflected in the half width of the signal, and the phase relaxation (transverse relaxation) time T_2 in the wings. Especially, in the common case of $T_1 \gg T_2$, in particular the energy relaxation time can be obtained directly from the full width at half of maximum (FWHM) of the NLPF signal:

$$(T_1)^{-1} = (2\sqrt{2})^{-1} \times (\text{FWHM}).$$

In this and the following case it is assumed that there is no spectral cross-relaxation ($T_3 = \infty$).

2.3. *Case c: single, moderately inhomogeneously broadened band*

$$S^{(c)}(\Delta; \omega_{2L}) = \frac{\alpha}{\Gamma_+^2 + \omega_{2L}^2} \left| \frac{1}{\gamma} \frac{1}{\Gamma_+ - i\omega_{1L}} + \frac{1}{(\gamma + i\Delta)(\Gamma_+ - i\omega_{2L})} + \left(\frac{1}{\gamma} + \frac{1}{\gamma + i\Delta}\right) \frac{2\Gamma_+ + i\Delta}{(\Gamma_+ + i\omega_{1L})(2\Gamma + i\Delta)} \right|^2$$

We call the overall absorption band of an ensemble of species moderately inhomogeneously broadened, if the distribution function of the centre wavelengths of the individual transitions has properties between those of the limiting cases *b* and *a*. (In the latter case, the distribution function is degenerated to a delta-function). In this intermediate case, the overall absorption band shape is of the Voigt type, i. e. a convolution of a Gaussian with a Lorentzian.

The possible NLPF line shape in case *c* varies between the limiting cases of the NLPF line shapes for *a* and *b*, an additional characteristic is the width of the distribution function. For mathematical reasons, a Lorentzian distribution function was used [4]. An example of a NLPF line shape for case *c* is shown in Fig. 2*b*.

2.4. *Case d: heterogeneous broadening*

$$S^{(d)}(\Delta) \approx \sum_{k=1}^{m} a_k S^{(a)}(\Delta; \omega_{0k} - \omega_2, \Gamma_k)$$

This line shape formula holds as a good approximation for superposition of m homogeneously broadened components with centre frequencies $\omega_{01}, \omega_{02}, \ldots, \omega_{0m}$ and weights $a_1, a_2, \ldots, a_m$. An example is shown in Fig. 2*a*. Notice that case *d* does not include any energy transfer. To simulate an experimentally obtained NLPF line shape in a definite way according to case *d*, it is absolutely necessary to have signals at several values of λ_2^{fix} (this is very useful in all other cases, too).

Without going into details, it should be mentioned that highly resolved NLPF spectra around resonance ($\lambda_1^{var} = \lambda_2^{fix}$) can give information on T_3, which is very valuable, e. g., for an unequivocal identification of moderately inhomogeneous broadening near the limit of case *a*.

3. NLPF signals from the bacterial antenna LH II

NLPF signals have been investigated at room temperature in the spectral region of the 850 nm absorption band of the isolated pigment-protein complex B800–850 from the photosynthetic purple bacterium *Rhodobacter sphaeroides* as well as of whole chromatophores. The preparation of the samples is described in [1]. First NLPF results with these samples obtained at lower resolution with an earlier version of the experimental set-up (cf. above) have been described in [1, 5], and a summary of other spectroscopic results concerning a possible substructure of the 850 nm band is given in [1, 6, 7].

The pump beam was tuned over the wavelength range from 8250 Å to 8650 Å with a mean photon flux density of 10^{24} photon/(cm^2·s).

NLPF signals were measured at three selected probe wavelengths: 8351.4 Å, 8452.0 Å, and 8602.0 Å. The results for the latter case are shown in Fig. 3. For comparison, in this figure also the room temperature absorption spectra of both samples are shown, which give no hint to any substructure.

From these NLPF line shapes it is obvious that the 850 nm band is heterogeneously broadened for both the isolated B800–850 complex and the chromatophores. Three subbands can be clearly identified, and there are indications of a fourth one with its maximum outside the measuring range. The analysis of the NLPF spectra is still incomplete. Preliminary results for the location of the maxima of these subbands are:

B800–850 complex:	830	845	855	(874)	nm;
chromatophore:	825	839	854	(874)	nm.

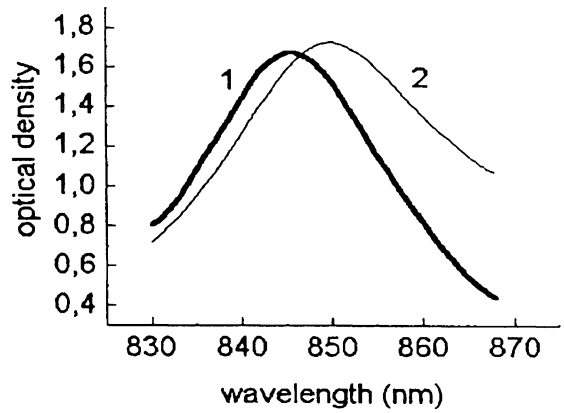

Fig. 3*a*. Absorption spectra (*1*) of the B800–850 complex from *Rb. sphaeroides* / LDAO and (*2*) of chromatophores from *Rb. sphaeroides* in the spectral range of NLPF investigations

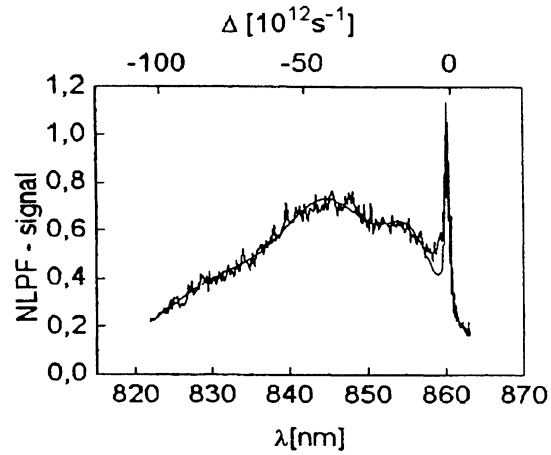

Fig. 3*b*. NLPF line shape of the B800–850 complex from *Rb. sphaeroides* probed at 8602.0 Å. Points, measurement; full line, simulation according to case *d*

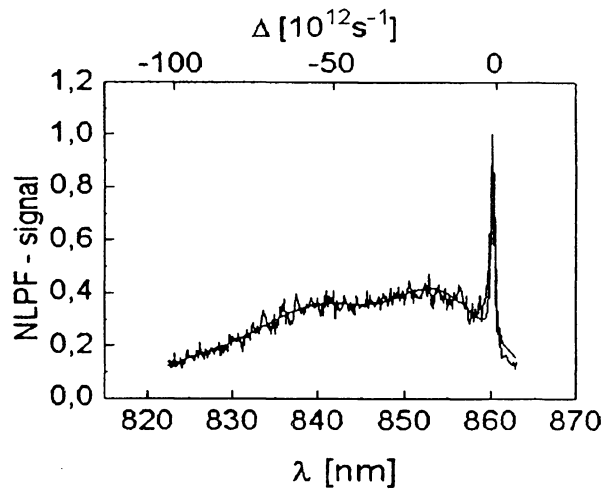

Fig. 3*c*. NLPF line shape of chromatophores from *Rb. sphaeroides* probed at 8602.0 Å. Points, measurement; full line, simulation according to case *d*

The values of T_2 and the corresponding homogeneous width of these subbands are 30 fs and 300 cm^{-1}, respectively. 'Preliminary' in this context means that these values were obtained from only a few selected but representative sets of measurements out of larger series and fitted individually according to the line shape formula of case *d*. A global analysis is in progress to fit the NLPF line shapes for the several probe wavelengths simultaneously. A quantitative analysis, in particular with regard to the error limits for the centre wavelengths of the subbands and definite differences in the spectroscopic substructures between the complex and the chromatophore, is therefore pending.

There is further information in these NLPF signals. Measurements with the highest spectral resolution in the pump beam region around $\lambda_1^{var} = \lambda_2^{fix}$ show that the NLPF maximum is not located exactly at the resonance of both beams, but rather shows a minor (~1 Å) but distinct shift. This is true for the B800–850 complex as well as for the chromatophores. This shift is an indication of spectral cross-relaxation [4], which means that the subbands are not purely homogeneous, but there is a contribution of inhomogeneous broadening. Consequently, each of the subbands has to be described by case *c*, and the complete line shape analysis for the complex as well as the chromatophores has to be based on a superposition formula analogous to case *d*. This is attempted presently in our global analysis, too. A preliminary qualitative result with both samples is that for each subband the inhomogeneous width is smaller than the homogeneous width.

Last but not least, it should be pointed out that NLPF line shapes also contain information on the following problems of special interest for photosynthetic antennas (Leupold et al., in preparation):

(i) energy transfer between the species represented by the subbands,

(ii) whether a subband belongs to an 'isolated' species or participates in excitonic interactions,

(iii) exciton annihilation and/or biexcitonic excitation in the subbands (substructures).

The results concerning (i) and (ii) can be derived by proper location of the probe beam wavelengths, those concerning (iii) by proper variation of the pump beam intensity.

4. Concluding remarks

As has been shown above, the NLPF method is well suited to characterize the type of absorption band broadening of organic molecules and to analyse a possible band substructure in the case of complex, composite samples. This is especially valuable for biological materials, because the NLPF method works at room temperature. Therefore, extensions of the work to modified and other antennas, also from higher plants, are currently under investigation. Another interesting route of investigation would be to bridge the gap to the well-established low-temperature method of hole burning (for review see [8]).

Acknowledgements

This work was supported by Deutsche Forschungsgemeinschaft, Bonn (AZ Sche 140/13-1 and Le 729/1-1). F. N. is indebted to organizers and sponsors of the Preila workshop for a travel grant.

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