New and Notable

A New Spectroscopic Tool for Analyzing Excitonic Structure and Dynamics in Pigment-Protein Complexes

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The group of Graham Fleming at Berkeley has pioneered a method to investigate light-harvesting in pigment-protein complexes (PPCs) in much greater detail than was possible in the past. Through two-dimensional photon echo spectroscopy, which is an extension of nuclear magnetic resonance and two-dimensional infrared spectroscopy to the wavelength region of visible light (1), the echo signal is recorded along with the delay times between three 45 fs optical pulses. A detailed analysis of the signal involving Fourier transforms along certain time axes, defined by the delay times, reveals information about the electronic structure and dynamics of the complex.

Read et al. (2), in this issue of the *Biophysical Journal*, report on how, in addition to the delay times, the polarization of the femtosecond pulses can be varied to obtain information about the geometry of optical transition dipole moments of the Fenna-Matthews-Olson (FMO) protein. As in almost all photosynthetic PPCs, the excited states in the FMO protein are delocalized over a number of pigments, and hence it is a nontrivial task to relate the structure to the optical properties and the function of the complex. The FMO protein furnishes an excellent model system for the development and application of a new method, because it is well characterized by structural, spectroscopic, and theoretical studies; it is the first PPC for which a crystal structure was determined, more than 30 years ago (3). The monomeric subunit of the FMO protein binds seven bacteriochlorophyll *a* (BChl) pigments which, in green sulfur bacteria, transfer excitation energy from an outer antenna system to the reaction center, where the excitation energy is converted into chemical energy.

The Coulomb coupling between optical transitions of the BChls (excitonic coupling) leads to the formation of partially delocalized excited states, the so-called exciton states. As a consequence, a pigment has contributions in more than a single band of a spectrum and the position of a band is different from the energy, at which a pigment in its binding site in the protein would absorb, if it was not coupled to other pigments. This hypothetical energy is called the site energy of a pigment. To obtain the exciton states of the FMO protein, one has to diagonalize a matrix that contains, in the diagonal, the site energies of the seven BChls and in the off-diagonal, the excitonic couplings between them. The latter can well be described by considering the point-dipole couplings between optical transition dipole moments. The information about the orientation of local transition dipole moments is obtained from the crystal structure and information about the dipole strength from measurements on BChl in solution. Hence, the only unknown quantities are the site energies.

Nevertheless, after the crystal structure was published, it took more than 20 years of research to arrive at a set of site energies that describes the experimental linear absorption, linear dichroism, and circular dichroism spectra. Louwe et al. (4) finally recognized that using a smaller effective dipole strength for the BChl transitions in the calculation of excitonic couplings allows one to find a suitable set of site energies. A quantitative explanation of the low effective dipole strength of the pigments in the FMO protein was given recently by taking into account the effect of the electronic polarizability of the protein in electrostatic calculations of the excitonic couplings (5).

Of course a more complete understanding of the building principles of this complex requires one not only to know the values of the site energies of the pigments, but also the mechanism by which the protein has tuned them. This problem was solved recently by Müh et al. (6) using a combined quantum chemical/electrostatic approach. Interestingly, the electric fields of two α-helices determine the sink of the excitation energy at a particular pigment and thereby direct excitation energy flow in the FMO protein. As indicated by the report of Read et al. (2), the exciton Hamiltonian, determined by Müh et al. (5) solely on the basis of the crystal structure, also describes the two-dimensional photon echo spectra.

Besides a critical check of an exciton Hamiltonian, the new two-dimensional spectroscopy has the potential to teach us about the role of protein dynamics in excitation energy transfer reactions. The protein is more than a rigid scaffold that holds the pigments and tunes their site energies. It dynamically modulates the site energies and, in this way, dissipates the excess energy of excitons during relaxation. Because the pigments contribute to more than one exciton state, there is a correlation between the fluctuations of different exciton state energies. This correlation determines the rate constants of exciton relaxation between the states and the intensity of certain peaks (the cross peaks) in the two-dimensional photon echo spectra. Thus, the spectra provide direct information about relaxation routes between different delocalized states and, if combined with a theoretical analysis, about the spatial pathways of the excitation energy transfer.

The new spectroscopy can be expected to provide the basis for the development of more realistic theories of excitation energy transfer in photosynthetic proteins. Two common approximations of existing theories that might not be valid are: 1), to assume an independent fluctu-

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ation of site energies at different sites; and 2), to describe exciton relaxation as a completely incoherent process. It will be exciting to see how this new spectroscopic tool, in combination with new theory, solves these and other problems.

REFERENCES

1. Hochstrasser, R. M. 2005. Spectroscopy at a stretch. *Nature*. 434:570–571.
2. Read, E. L., G. S. Schlau-Cohen, G. S. Engel, J. Wen, R. E. Blankenship, and G. R. Fleming. 2008. Visualization of excitonic structure in the Fenna-Matthews-Olson photosynthetic complex by polarization-dependent two-dimensional electronic spectroscopy. *Biophys. J.* 95:847–856.
3. Fenna, R. E., and B. W. Matthews. 1975. Chlorophyll arrangement in a bacteriochlorophyll protein from *Chlorobium limicola*. *Nature*. 258:573–577.
4. Louwe, R. J. W., J. Vrieze, A. J. Hoff, and T. J. Aartsma. 1997. Toward an integral interpretation of the optical steady-state spectra of the FMO-complex of *Prosthecochloris aestuarii*. 2. Exciton simulations. *J. Phys. Chem. B*. 101:11280–11287.
5. Adolphs, J., and T. Renger. 2006. How proteins trigger excitation energy transfer in the FMO complex of green sulfur bacteria. *Biophys. J.* 91:2778–2797.
6. Müh, F., M. E. Madjet, J. Adolphs, A. Abdurahman, B. Rabenstein, H. Ishikita, E. W. Knapp, and T. Renger. 2007. α-Helices direct excitation energy flow in the Fenna-Matthews-Olson protein. *Proc. Natl. Acad. Sci. USA*. 104: 16862–16867.