Chapter 18

FTIR Studies of the Primary Electron Donor, P700

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Summary

Over the last two decades FTIR difference spectroscopy has emerged as a prominent technique to investigate the electronic structure and the bonding interactions of P700, the primary electron donor of photosystem I. In this chapter, the advances in the field during this period are reviewed and discussed in the light of the structural model of P700 derived from X-ray crystallography. The effect on the FTIR difference spectra of mutations of the axial ligands of the chlorophyll molecules in P700 as well as of amino acid residues in hydrogen bonding interaction with the carbonyl groups of P700 is analyzed. The results of both global and selective isotope labeling studies are

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presented. Special emphasis is given at analyzing the agreements and the discrepancies between the assignments of the various vibrational bands in the FTIR difference spectra recorded upon P700 photooxidation that are available in literature. The reasons behind the strong disagreement between the results of FTIR spectroscopy and of magnetic resonance techniques such as ADMR and ENDOR with regard to the localization of the triplet character in $^3$P700 and the extent of charge versus spin distribution in P700$^+$ are critically discussed.

I. Introduction

The key steps of photosynthesis occur in specific membrane proteins called reaction centers (RCs), where the initial separation of electric charges and their subsequent stabilization across the membrane take place. This process starts from the excited state of a special dimer of chlorophyll (Chl) or bacteriochlorophyll (BChl) molecules called primary electron donor (P). Owing to this important role, the structure of the primary donors in their neutral, triplet, and cationic states has been investigated in great detail using optical, vibrational, and magnetic resonance spectroscopic techniques. These studies have led to models for the organization of the pigments within the dimer and the distribution of charge and spin in the oxidized and triplet states of the primary donors.

In the last two decades, X-ray crystallographic models of RC proteins have provided an invaluable source of information to complement the data obtained by spectroscopy alone, notably, when the resolution of the structures becomes sufficient to identify the amino acid side chains interacting with the pigments through hydrogen bonds to the carbonyl groups or axial ligation to the central Mg atoms of the (B)Chl molecules. In this case, site-directed mutagenesis becomes the method of choice to perturb selectively the pigment–protein interactions, therefore providing RCs with modified primary donors, the structure and function of which can be probed by spectroscopy. While X-ray crystallography provides key information on the identity of the H-bond partners and some details on the structure of these H-bonds in the ground state of the primary donors, it remains silent on several important aspects of the electronic structure of the special pair after charge separation occurs, such as the localization of the triplet state in $^3$P or the hydrogen bonding status of the Chls and the charge distribution in P$^+$.

II. FTIR Studies of P700 Prior to the High-Resolution X-Ray Structure of PS I

A. Early Studies

The first light-induced FTIR difference spectrum of P700 photooxidation was obtained at room temperature on PS I particles isolated from pea (Tavitian et al., 1986). A control of the reaction was performed in parallel on the same sample by measuring the kinetics at 706 and 820 nm. In the absence of IR spectra of isolated Chl$^+$, the interpretation of the P700$^+$/P700 FTIR difference spectra was limited to that of the negative bands, i.e., the bands that pertain to the Chl(s) in P700. A large negative band at $\sim$1,700 cm$^{-1}$ was proposed to correspond to the 9-keto C=O group of the Chl$^+$, while the two negative bands at 1,749 and 1,735 cm$^{-1}$ were tentatively assigned to ester C=O groups. To assist the assignment of the bands in the P700$^+$/P700 FTIR difference spectra (Fig. 2a), cation-minus-neutral FTIR difference spectra of Chl$^+$ and pyroChl$^+$ (Chl$^+$ lacking the 10a-ester carbonyl) in organic solvents were generated electrochemically in

**Abbreviations:** PS – photosystem; P – primary electron donor; P700 – primary electron donor of PS I; (B)Chl – (bacterio)chlorophyll; RC – reaction center; FTIR – Fourier transform infrared; ADMR – absorption detected magnetic resonance; ENDOR – electron nuclear double resonance; Hfcs – hyperfine coupling constants.