Chapter 16

Optical Measurements of Secondary Electron Transfer in Photosystem I

Fabrice Rappaport*
Laboratoire de Physiologie membranaire et moléculaire du Chloroplaste, UMR 7141 CNRS-Univ. Paris 6, Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France

Bruce A. Diner
Central Research and Development, Experimental Station, E. I. du Pont de Nemours & Co., Wilmington, DE 19880-017, USA

Kevin Redding
Department of Chemistry, University of Alabama, Tuscaloosa, AL 35487-0336, USA

Summary ............................................................................................................................... 224
I. Introduction ....................................................................................................................... 224
II. Secondary Electron Transfer: Are the Two Phylloquinones Involved? .......................... 224
   A. Monophasic Versus Biphasic Kinetics ......................................................................... 224
   B. The Origin of the Biphasic Kinetics ............................................................................. 225
III. Uni-Directional or Bi-Directional Electron Transfer in Reaction Centers ..................... 226
IV. A Mutagenesis Survey of the Two Phases Ascribed to A1 Reoxidation ......................... 227
   A. Mutations Affecting the Quinone Binding Pocket ...................................................... 227
      1. The PsaA-W697 and PsaB-W677 Mutants ........................................................ 227
      2. More Mutants in the Quinone Binding Pocket .................................................... 228
   B. Mutations Along the Electron Transfer Branches Upstream of the Quinones ............... 230
      1. Mutations Affecting A0A or A0B ...................................................................... 230
      2. Mutations Upstream of A0 ............................................................................. 233
V. Spectroscopic Features Specific to the Spectra of the Fast and Slow Phase in the ....... 234
   320–540 nm Region ........................................................................................................... 234
   A. Spectroscopic Features Specific to the Spectra of the Fast and Slow Phase in the .... 236
      420–540 nm Region ........................................................................................................ 236
   B. Spectroscopic Features Specific to the Spectra of the Fast and Slow Phase in the .... 236
      320–420 nm Region ........................................................................................................ 236
VI. Energetic Picture of Quinone Reoxidation via Forward or Backward Electron Transfer .... 238
VII. Conclusions ..................................................................................................................... 241
Acknowledgments .................................................................................................................. 241
References .............................................................................................................................. 241

*Author for correspondence, email: rappaport@ibpc.fr

Summary

All known photosynthetic reaction centers have symmetric structures, using two similar or identical integral membrane subunits to form a dimeric core, which binds the cofactors through which electrons are shuttled across the membrane. This symmetric arrangement gives rise to two similar branches of cofactors, down which light-driven electron transfer could proceed. The first three members of each branch are chlorins, while the third is a quinone. It is known that the initial electron transfer occurs almost exclusively along one of the two branches in the well-characterized Type 2 reaction centers, although the origins of this strong asymmetry are still debated. Photosystem I is the best characterized representative of the Type 1 reaction centers, but many aspects of electron transfer directionality remain unresolved. Recent time-resolved absorption studies suggest that electron transfer can make use of both cofactor branches of Photosystem I at room temperature. Here, we will present the results that led to this proposal and discuss this model in the light of the recent studies aimed at testing its validity.

I. Introduction

In the past few years a considerable number of new results have improved and challenged our understanding of structure/function relationships in Photosystem I (PS I). The determination at atomic resolution of its three-dimensional structure is certainly not the least (see Fromme and Grotjohann, this volume, Chapter 6; Nelson and Ben-Shem, this volume, Chapter 7; Antonkine and Golbeck, this volume, Chapter 8). It set the basis for detailed calculations as well as experimental assessment of the excitonic coupling of the hundreds of chlorophylls that act as light-harvesting pigments (see Savikhin, this volume, Chapter 12; Karapetyan et al., this volume, Chapter 13; Schlodder and Renger, this volume, Chapter 35) and significantly boosted the site-directed mutagenesis strategy aimed at elucidating the physico-chemical properties, as well as the eventual participation, of the different redox cofactors involved in the various electron transfer (ET) reactions (see Webber and Ramesh, this volume, Chapter 14; van der Est, this volume, Chapter 25). In this article we will focus on the ET reaction between the quinones, which serve as secondary electron acceptors, and the iron–sulfur clusters, which serve as terminal electron acceptors. These reactions have been mostly studied by time-resolved absorption spectroscopy and transient EPR spectroscopy. We will focus on the results obtained with the former approach. As will be argued, we feel that this approach yields a fairly consistent picture but we refer the reader to Stehlik (this volume, Chapter 23) and Redding and van der Est (this volume, Chapter 24) for a comprehensive review of the results obtained by transient EPR spectroscopy, from which significantly different conclusions are drawn. Please note that unless stated otherwise, the numbering of the amino acids in the PsaA and PsaB subunits refers to that of Thermosynechococcus elongatus.

II. Secondary Electron Transfer: Are the Two Phylloquinones Involved?

The reoxidation kinetics of the quinone $A_1^-$ have been studied essentially by two techniques: optical spectroscopy, the time resolution of which is presently unequaled, and time-resolved EPR spectroscopy. Both techniques have been extensively used since 1988 and have provided an increasingly refined characterization of the kinetics of forward ET between the reduced phylloquinone and the Fe$_4$S$_4$ clusters.

A. Monophasic Versus Biphasic Kinetics

The first evidence for biphasic kinetics with half times of 20 and 150 nsec came from a study of the flash-induced absorption changes in the near UV in PS I complexes from spinach (Spinacia oleracea) (Sétif and Bretzel, 1993). The relative amplitudes of these two phases were found, however, to vary between 2:1 and 1:2 for different preparations. Since the relative amplitude of the fast phase rose with increasingly harsh solubilization treatment, it was proposed that the reoxidation of $A_1^-$ was intrinsically monophasic and slow, and that the fast phase was a consequence of the purification procedure. This hypothesis was consistent with