Tracing Charge Separation Events in Photosynthesis: Anomalous Photovoltage Polarity Effects Explained

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Photoelectric techniques for tracing photoinitiated charge separation have been successfully exploited since the early 1980s to study primary events in photosynthetic membranes. However, unexpected, seemingly anomalous excitation wavelength-dependent inversions of the polarities of the measured photovoltages have been observed. In this issue (pp. 379–385), Paillotin et al. (1) demonstrate that these puzzling observations can be understood in terms of the basic principles of the propagation of light waves in lamellar membrane systems.

In photosynthetic membranes, light is collected by antenna chlorophyll molecules which channel the energy to a specialized chlorophyll-protein reaction center (RC) complex, where the primary charge separation reaction of photosynthesis occurs. The primary events, which involve the rapid, subnanosecond time scale migration of excitation energy from the antenna chlorophyll-protein complexes to the RC complexes, and the ensuing initial charge separation reactions, have been extensively studied by fluorescence and transient absorbance techniques. In the early 1970s, using microsecond-duration red light pulses to irradiate chloroplasts suspended between two metal electrodes, it was shown by Fowler and Kok (2) and by Witt and Zickler (3) that these processes result in macroscopically measurable photovoltages. The interpretation of this effect is based on the light-gradient across the strongly pigmented vesicular membranes, which results in a greater number of primary charge separation events in the side of each vesicle facing the light source than in the opposite side. As long as the light flash intensity was limited in order to avoid exciting all of the reaction centers (light saturation), a measurable photovoltage of the expected polarity was observed (2, 3). Becker et al. (4) showed that the magnitude of the photovoltage depended on the orientations of the chloroplasts relative to the light beam, and related these observations to differences in the mobilities of positive and negative ions in solution (Dember effect). In all cases, the basic origin was traced to the effects of the initial charge separation. It was already clear, even then, that this macroscopic photoelectric effect was of great potential interest for studying electrogenic phenomena in photosynthetic membranes.

Using light flashes of different wavelengths and of nanosecond and microsecond duration, Gräber and Trissl (5) noticed a puzzling, apparent anomaly: the polarity of the photovoltage was positive in regions of strong light absorption, and negative in regions of low light absorption. It quickly became evident that these photoelectric effects should be studied on faster time scales, closer to those of the primary charge separation processes. On longer, microsecond time scales, secondary effects, notably ion movements in response to the altered charge distributions, can affect the characteristics, even the polarity of the measured photovoltages (6). However, Meszéna et al. (7) reported that nanosecond pulse excitation in a region of high absorbance of chlorophyll (420 nm) yields the same photovoltage polarity as observed previously with longer light pulses (2, 3). On the other hand, Gräber and Trissl (5), using weakly absorbed (530 nm) nanosecond laser flashes, observed polarities of opposite sign.

Subsequently, Trissl, Leibl, Breton. and their co-workers extended these types of measurements into the subnanosecond time domain. In this range of time scales, the kinetics and characteristics of the observed photovoltage are more closely associated with the primary charge separation processes in photosynthetic membranes (see, for example Refs. 8 and 9 and references quoted in Paillotin et al. (1)). The apparently anomalous dependence of the sign of the photovoltage on the wavelength of excitation, or more precisely the magnitude of the absorption coefficient, was ignored or "swept under the rug" in these studies.

This anomalous wavelength dependence of the polarity of the photovoltage has been elegantly resolved in the paper of Paillotin et al. (1). This anomaly is explained in terms of the basic physics of the reflection of light waves at interfaces, and their propagation in a system of alternating layers of different, wavelength-dependent, complex refractive indices. By calculating the interference patterns at these lamellar interfaces, it is possible to predict correctly the inversion of the sign of the photovoltage when the wavelength is shifted from a region of strong absorbance to a region of weak absorbance. These results clearly emphasize that the simple Beer-Lambert law is not adequate for describing the attenuation of a light beam propagating through layers of biological membranes. It should be noted that this line of reasoning was already foreseen by Meszéna and Devault (10) who wrote in 1989: "Since the size of the thylakoid vesicle system is in the range of the wavelength of the light, it is incorrect to suppose that the one side of the vesicle simply shadows the other one. Within the lamellae system the light intensity has a complicated interference pattern rather than a light gradient derived from geometrical optics. It is impossible to predict the resultant polarity without complicated calculations of this pattern. Obviously, it can depend on the wavelength of the light."

The contribution of Paillotin et al. (1) has thus led to new insights into the macroscopic origin of the photovoltage effect, and has resolved an important paradox. It is likely that this method might see increasing use, particularly in photosynthesis research. New applications include not only studies of the primary charge separation processes, but also investigations of subsequent, slower, nonelectrogenic reactions. For example, using double-flash techniques, the kinetics of the electron steps leading to the protonation of the secondary quinone acceptors have been recently reported for *Rps. viridis* (11). The advantage of this method is its applicability to studies of intact, or nearly intact

systems, e.g., whole cells or organelles. However, the photovoltage signals tend to be small (typically of the order of 1 mV) and require specialized equipment for measuring kinetics on subnanosecond time scales with a good signal-to-noise ratio.

Much remains to be done in this area from both theoretical and experimental points of view. Future applications might include explicit calculations of photovoltages for multilamellar membranes (grana stacks) and spherical membranes (vesicles). It would also be of interest to extend these photoelectric studies to photosynthetic organisms with different types of organization of the thylakoid membrane to gain a better understanding of the effects of membrane stacking on light gradient-induced photovoltage phenomena.

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