Is it time to throw away your apparatus for chlorophyll fluorescence induction?

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Chlorophyll (Chl) fluorescence has been introduced about five decades ago into photosynthesis research. Based on the notion that it provides an intrinsic probe of photosynthetic activity and that it is highly sensitive to changes in the functional organization of the photosynthetic apparatus, Chl fluorescence has since been used in an ever increasing fashion as a powerful tool to study a large variety of different properties of photosynthetic systems (for reviews see references 1, 2). Fluorescence techniques used involve both steady-state and time-resolved methods, extending down to ultrashort time scales of pico- and femtoseconds (for a review see reference 3).

One of these techniques, Chl fluorescence induction, has become particularly popular (see references 2 and 4 for reviews). It had been recognized early that the intensity of Chl fluorescence is a strong function of the redox state of the reaction center (RC) of photosystem (PS) II, being low when RCs are open (F_0-value) and high when RCs are closed (F_{max}-value) (5). Thus by switching on an actinic light, RCs in a photosynthetic tissue can be closed photochemically. The usually sigmoidal time course of the Chl fluorescence, rising from the F_0- to the F_{max}-level, called the fluorescence induction curve, can thus be recorded. Today there exists hardly a photosynthesis research laboratory in the world that does not at least occasionally use fluorescence induction and for many laboratories this method has become one of their major tools. At first sight the method has many attractive features: It is noninvasive, i.e., it can be applied also in vivo, it requires relatively inexpensive equipment, it is highly sensitive and its analysis and interpretation seemed to be fairly straightforward so far. Furthermore, in recent years the method has seen an additional boost by commercial equipment that is light enough to be carried out into the fields allowing us to record in vivo data in ecological research on environmental plant stress factors, etc. (2).

The necessity of theoretical justification of the fluorescence induction phenomenon had been well recognized early on (6, 7). However, until recently the mechanisms and kinetics of the energy transfer processes in the antennae or the electron transfer processes in the RCs were largely unknown. For this reason any theoretical foundation of the Chl fluorescence induction phenomenon had to rely on then experimentally unproven assumptions about charge separation/recombination processes in the RCs which severely limited the applicability of these theories. Unfortunately, however, these limitations were often ignored when interpreting fluorescence induction phenomena. Even worse, the induction curves were interpreted often on the basis of even more uncertain empirical rules that, over the years, had become sort of a dogma in the field. This dogma involved such far-reaching statements as, e.g., (*a*) "a proportionality exists between the complementary area above the fluorescence induction curve and the number of electrons flowing to the acceptor side," (*b*) "an inverse proportionality between the complementary area and the photosynthetic quantum yield," and (*c*) "the degree of PSU connectivity can be determined from the curvature of the variable fluorescence plotted against the fraction of closed RCs."

Very recently, doubts on the validity of such concepts have been voiced in a couple of papers (3, 8). A paper by Trissl, Gao, and Wulf (9), published in this issue, now entirely wipes out the theoretical basis for the above-mentioned relationships assumed so far to analyze Chl fluorescence induction. Based on kinetic model simulations that for the first time rely on an experimentally well supported molecular model of the energy transfer and charge separation processes, the so-called exciton-radical pair equilibrium model in the PS II antenna/RC complex (10, 11), this paper shows that in fact all the above-mentioned empirical assumptions on the induction signals are invalid. It is quite clear that, at present, fluorescence induction phenomena cannot even be interpreted qualitatively, let alone quantitatively. Thus we face the probably rare case of an apparently well established and widely used experimental method, viz. Chl fluorescence induction, that lacks any solid theoretical justification.

According to a conservative rating, the number of papers devoted to and exploiting the fluorescence induction phenomenon up to date exceeds a thousand. The phenomena that have been studied extensively by fluorescence induction cover such diverse subjects, like, e.g., cooperativity of photosynthetic units, degree of membrane stacking, changes in membrane potential, cation effects on energy distribution, pH-dependence of photosynthetic reactions, heterogeneity of photosystems, physiological adaptation phenomena, light state transitions and many more. Following the paper by Trissl et al. (9) one must infer that the majority of the conclusions made in these papers, if based mainly on fluorescence induction, is most probably invalid. Does this mean that we should stop recording fluorescence induction curves en-

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tirely? Wait for a little while and do not throw your equipment away, despite the truly unpleasant situation given by the present lack of an adequate theoretical basis! We may hope that out of the clear results presented in the paper of Trissl et al. (9) it might be possible to develop new analysis methods that eventually may provide a solid basis for interpretation of fluorescence induction data. Given the huge body of available experimental data it is to be hoped that such a method will indeed be available soon. In the meanwhile, beware of any conclusions based on fluorescence induction data!

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