Inhibition of Blue Light-Induced, Flavin-Mediated Membraneous Redox Transfer by Xenon

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Xenon as a potential triplet quencher is capable of inhibiting blue light-induced, flavin-mediated transport of redox-equivalents across vesicle membranes by xenon. These data call for the reinterpretation of results obtained with xenon as a potential inhibitor of phototrophic curvature of grass coleoptiles, which were taken to suggest the flavin singlet as active species.

Introduction

The present paper continues our series on vesicle-associated flavins [1—5], dealing with the inhibition of blue light-induced flavin-mediated transport of redox-equivalents across vesicle membranes by xenon gas. Our basic goal studying these completely artificial model systems is the elucidation of the clear-cut identification, purification and — as a result of studies on vesicles — reconstitution. The present paper describes are based on a model system developed recently. Ferricytochrome c as a potential electron acceptor is trapped in the lumen of unilamellar lipid vesicles, EDTA as potential electron donor added to the bulk medium.

Flavin- and Cyt c-loaded single-shelled vesicles have been described previously [4]. Fluorescence of flavin was obtained with xenon as a potential inhibitor of phototrophic curvature of grass coleoptiles, which were taken to suggest the flavin singlet as active species.

Materials and Methods

The description of the synthesis of amphiphilic flavins has been published previously [10]. FMN was obtained as a gift from Hoffman La Roche, Basel. DML (N42803) was purchased from Fluka, Buchs, Neu-Ulm. EDTA (0.1 × Titrilex III, 8431 or 8418) and Cyt c (Art 24804) were obtained from Merck, Darmstadt, and xenon gas from Deutsche Edelgas, Stuttgart (XE N30). The preparation of flavin- and Cyt c-loaded single-shelled vesicles has been described previously [4]. Fluorescence of flavin was monitored with an absolute-fluorimeter [11], absorption was measured with a home-made single-beam [12] and home-made dual wavelength spectrophotometer [13]. Photoreduction of Cyt c or flavin was induced with a 150 W xenon lamp (ORIEL 6253), yielding near uv-light of 100 W/m² through a 365 nm interference filter (exciting the S0 → S2 transition of flavin).

Results and Discussions

Most photochemical and photobiological reactions are initiated by the triplet state of the responsible photoreceptor pigments. Nevertheless, in recent years evidence has accumulated suggesting the involvement of the first excited singlet state of chlorophylls in primary reactions of photosynthesis of plants and bacteria. Whether this holds true for the phototropism of corn coleoptiles (and other blue light responses) is still a matter of debate. Schmidt et al. [14] reported specific inhibition of phototropism in corn coleoptiles by iodide, azide and phenylacetic acid which are preferential triplet quenchers of flavins [3, 4]. In contrast to these findings, Song [15] and As a first approach a completely artificial isotropic electron transport chain as driven by blue light via a flavin moiety had been explored by us some years ago [9]. It included EDTA, flavin, oxygen (optionally) and Cyt c. On this basis a more advanced anisotropic model system was designed in order to study blue light-mediated transport of redox-equivalents across vesicle bilayers [5]. Single-shelled vesicles were prepared with Cyt c trapped in their aqueous lumen as potential electron acceptor, and amphiphilic flavin bound to the vesicle-membrane as photoreceptor pigment. EDTA as potential electron donor for the membrane-bound flavin was added to the bulk medium.
Vierstra et al. [16] failed to establish any inhibiting influence of xenon. Xenon as a water-soluble noble atom gas is — in contrast to iodide — chemically inert, but isoelectronic with iodide and therefore assumed to be a photophysically equivalent and excellent quencher of the first flavin triplet state. Therefore these authors concluded that the flavin singlet rather than the triplet is the most likely state mediating phototropism of corn coleoptiles. However, such an interpretation appears to be premature in light of the data reported below.

Even under saturating partial pressure of xenon (4.8 mM at standard conditions, i.e. a flavin/xenon ratio of ~ 1/5000) and isotropic/anaerobic conditions we failed to detect significant quenching of flavin photochemistry (less than 10%, Fig. 1; quenching of the first excited flavin triplet by the minute concentration of Cyt as ironprotein is safely excluded, cf. Schmidt [4, 9]). In fact, our observation is essentially consistent with that by Vierstra et al. [16]: if their data are analyzed on the basis of total inhibition, xenon has only a negligible influence of less than 8%, compared to about 50% as they arbitrarily deduced from the initial slopes of the photooxidation kinetics of NADH (their Fig. 1). The absolute number of molecules quenched appears to be a physiologically more relevant measure than kinetic data, which strongly depend on the particular mechanisms involved. In addition, they report that flavin fluorescence under isotropic conditions is not affected at all by xenon, a finding which also agrees with our observation. Summarizing, the available data clearly demonstrate that xenon is a rather poor quencher of both the first excited flavin singlet and triplet state under aqueous isotropic conditions.

However, to our surprise we found that xenon efficiently inhibits flavin-mediated, bluelight-induced transmembrane redox transport (Fig. 1): 4.8 mM xenon inhibits Cyt c photoreduction in absolute terms by as much as 50%, but fluorescence is not significantly quenched. Similarly, in our previous study (Fig. 8B in Schmidt [4]) we had shown that for membrane-bound flavin (DPL/AFl 3) the same concentration of iodide (4.8 mM) is capable of quenching flavin chemistry completely (i.e. the first triplet state), but — again — leaving fluorescence unaffected (i.e. the first excited singlet state). The reason for this “activation” of xenon as quencher of flavin triplets by the membrane is probably caused by its higher solubility in hydrophobic environments. Unfortunately, relevant physico-chemical data are not available.

Taking the protective role of the membrane into account, i.e. its (potential) capability of shielding membrane-bound pigments from bulk components (cf. Fig. 12 in Schmidt und Hemmerich [3] and Fig. 7 in Schmidt [4]), the presented and available data do not justify to exclude the flavin triplet as photoactive species of phototropism. As long as the precise nature of cryptochrome, its site of action and the primary sensory transduction steps are unknown, it rather remains the most likely candidate.

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