A Laboratory Exercise for Photochemistry and Photobiology: Production of Hydrogen Peroxide

Fernando Gallardo,* Susana Gálvez, Miguel Angel Medina, and Antonio Heredia

Departamento de Biología Molecular y Bioquímica, Facultad de Ciencias, Universidad de Málaga–Instituto Andaluz de Biotecnología, Campus de Teatinos, E-29071 Málaga, Spain; **fgallardo@uma.es*

Carlos Gómez-Moreno

Departamento de Bioquímica y Biología Molecular y Celular, Facultad de Ciencias, Universidad de Zaragoza, E-50009 Zaragoza, Spain

The photoproduction of fuels has been considered as an alternative to the use of fossil fuels owing to the increased energy demand in industrialized and developing countries. The photoproduction of fuels requires that light drive an endergonic reaction—a principle that is also the central feature of photosynthesis, the conversion of light energy into redox energy (*1*–*3*). The assimilation of this principle underlying life is difficult for many students because they have trouble understanding physicochemical concepts such as excitation of organic molecules, electrochemical potential, and electron transfer processes. In this laboratory exercise, students see a photochemical reaction that produces a fuel. They can learn the relevant concepts using a simple and inexpensive photochemical system. Like photosynthesis, the photochemical system presented in this work has three essential components:

1. The *photosensitizer*, the molecule excited by visible photons, making it a greater reductant or oxidant depending on its redox state (oxidized or reduced) before the excitation,
2. The *electron donor*, a compound that is able to reduce the photosensitizer, and
3. The *electron acceptor*, a substance that upon reduction by the photosensitizer results in a compound of interest.

The Laboratory

In this laboratory, riboflavin (F in Fig. 1) acts as a photosensitizer and its reduction potential decreases after excitation (F*) by blue photons (increasing its oxidation capacity); the photoexcitation allows the reduction of the oxidized isoalloxazine ring by weak electron donors or stable compounds such as thiourea, EDTA, or semicarbazide (Fig. 1). The flavin semiquinone form produced (FH) may transfer the acquired electron to molecular oxygen and, in a second photoexcitation, to the superoxide anion, to produce hydrogen peroxide. This energy-rich compound is of great interest as a fuel and antiseptic. Its synthesis can be detected after 20 min of illumination and monitored using a simple enzymatic analysis with horseradish peroxidase. Related analyses using horseradish peroxidase have been published in this *Journal* (*4*, *5*).

The photoreactor consists of a Petri dish situated 13 cm from the light source, a 60-W bulb from a desk lamp (Fig. 2). This basic equipment is suitable for medium-sized laboratory classes (e.g., 24 students working in groups of two) and allows the design of complementary experiments by changing two basic parameters, irradiance (the bulb's wattage and distance from the Petri dish) and the pH of the reaction mixture. In a unique class experiment, students may correlate the production of hydrogen peroxide with the irradiance and evaluate the

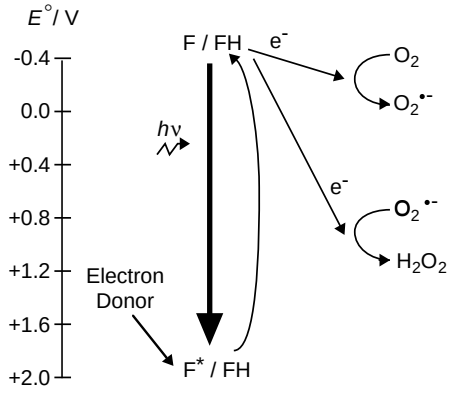

Figure 1. Electron transport in the photochemical system. $E°$ is redox potential. The increase in $E°$ of the flavin after photoexcitation (thick arrow) implies a higher oxidant capacity of the molecule (or a decreased reduction potential). Part of the energy absorbed during light excitation is used for chemical work.

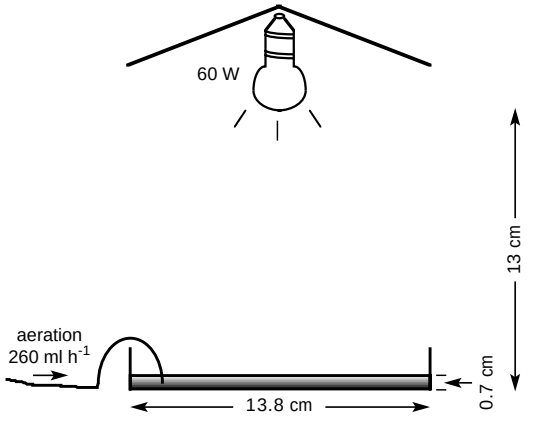

Figure 2. Scheme of the photoreactor. Light is supplied by a desk lamp with a 60-W bulb. The reaction mixture is placed in a Petri dish and aerated with a peristaltic or membrane pump. The aeration may be omitted in classes with a large number of students.

reactivity of flavin species formed at pH values for which the semiquinone form is neutral or present as the anionic radical (p*K* of 8.5). In our experiments, the reaction mixture is adjusted to pH 7.5 and 12.3. At pH 7.5 the concentration of hydrogen peroxide increases with the time of exposure, reaching a maximum in 2 h. At pH 12.5 more hydrogen peroxide is produced, and production is linear for an extended period of 8 h. The higher production of hydrogen peroxide at pH 12.3 is related to the fact that after the transfer of one electron to riboflavin (F), the semiquinone is present as a free radical ($F^{\bullet -}$), which is a more reactive species than the neutral semiquinone form (FH) predominant at pH 7.5 (Fig. 3 top). The production of hydrogen peroxide can be monitored by analyzing aliquots removed from the reaction mixture every 15–30 min; since differences in the rate of product synthesis at pH 7.5 and pH 12.3 can be observed after 40 min, the teacher may decide to carry out the light exposure for a shorter period than is shown in Figure 3, and perform the whole laboratory in one 3-h practical class, including the enzymatic determination of hydrogen peroxide.

During the experiment a slight decrease in the typical yellow coloration of the reaction mixture is observed. This change may be partly due to the deexcitation of the photosensitizer and emission of fluorescence, but also due partly to the photodegradation of the riboflavin. This degradation can also be monitored directly in the aliquots removed from the reaction mixture by measuring their absorbance at the characteristic absorption maximum of flavins at 450 nm. Photochemical degradation of the photosensitizer is produced as a consequence of the reactivity of the flavin molecule in the light-excited state, which can lead to inconvenient reactions that yield photochemically inactive side products. In our experiments a decrease of 20% of the initial absorbance at 450 nm was observed after 3 h in the reactions carried out at both pH values (7.5 and 12.3). This did not interfere with the quantification of hydrogen peroxide, since dilution of the aliquots removed from the photoreactor is required for quantification of their hydrogen peroxide content (see *Instructor notes* and *Student handouts*[W]).

The efficiency of the photochemical system can be improved by using other flavins (e.g., flavin mononucleotide), by increasing the irradiance, or by providing a high aeration of the reaction mixture (1300 mL/min) (*6*). The components used in this laboratory were selected because of their affordability for a large number of students. The reaction mixture can be aerated by a peristaltic or aquarium membrane pump, but this special equipment, which might not be available for teaching, can be omitted without great changes in the rate of hydrogen peroxide production described in Figure 3.

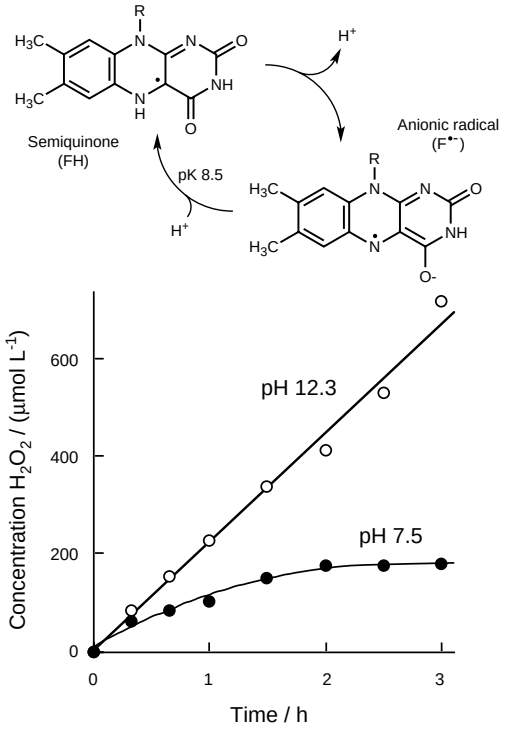

Figure 3. Top: Flavin semiquinone forms. Only two predominant semiquinone forms for pH 7.5 and 12.3 and the p*K* of N(5)H are shown. Bottom: The measured content of hydrogen peroxide in the reaction mixture at pH 7.5 and 12.3. Aliquots were removed at the indicated time and hydrogen peroxide was enzymatically determined with horseradish peroxidase and *o*-dianisidine.

Discussion

This laboratory is an example of the potential use of light in the production of synthetic fuels. Hydrogen peroxide is of interest in industry as an antiseptic and as a fuel because its decomposition releases a great amount of energy ($\Delta G^{\circ\prime} \cong$ 100 kJ mol^{-1}). In addition, this laboratory illustrates a number of photochemistry concepts. Since the experiments deal with biochemical topics such as light-excitation, changes in redox potential and transfer of electrons, and enzymatic analysis, it has been selected as part of a new course in Experimental Biochemistry for advanced undergraduate students at the Universidad de Málaga.

This photochemical system can also be used to illustrate the central feature of photosynthesis, the conversion of light energy into chemical work. For this special use it is relevant to note that photoexcitation of the flavin leads to a decrease in its reduction potential (Fig. 1), making it a stronger oxidant than in the basal state; while in photosynthesis, photoexcitation of chlorophylls at the reaction centers provokes an increase in their reductant potential (see a comparative figure in the *Instructor Notes*[W]). Although these differences in the behavior of the photosensitizer might be expected to confuse students, our experience with advanced undergraduates indicates rather the reverse. The understanding that an oxidized or a reduced species can respectively be a more powerful oxidant (flavins) or reductant (chlorophylls) after excitation is a general principle in photochemistry. The differences in the behavior of flavins and chlorophylls will concentrate the attention of students on the redox changes associated with the photoexcitation, and lead to a better understanding of in vivo photosynthesis.

Acknowledgments

This work was partially supported by the Instituto de Ciencias de la Educación, Universidad de Málaga, Spain. We gratefully acknowledge Ignacio Núñez de Castro and Francisco M. Cánovas for critical reading of the manuscript.

[W]Supplemental Material

Supplemental material for this article is available in this issue of *JCE Online*.

Literature Cited

1. Mathews, C. K.; van Holde, K. E. *Biochemistry*, 2nd ed.; Benjamin/Cummings: Menlo Park, CA, 1995.
2. Nobel, P. S. *Physicochemical and Environmental Plant Physiology*; Academic: San Diego, 1991.
3. Hader, D.-P.; Tevini, M. *General Photobiology*; Pergamon: Oxford, 1987.
4. Bateman, R. C. Jr.; Evans, J. A. *J. Chem. Educ.* 1995, *72*, A240–A241.
5. Pope, S. R.; Tolleson, T. D.; Williams, R. J.; Underhill, R. D.; Deal, S. T. *J. Chem. Educ.* 1998, *75*, 761.
6. Fontes, A. G.; de la Rosa, F. F.; Gómez-Moreno, C. *Photobiochem. Photobiophys.* 1981, *3*, 355–364.