ELSEVIER

Rapid Communication

Visible light-induced photooxidation of glucose sensitized by riboflavin

Eduardo Silva, Ana María Edwards, and Danilo Pacheco

Facultad de Química, P. Universidad Católica de Chile, Santiago, Chile

We conducted this study to evaluate the oxidation of glucose induced by visible light in the presence of sensitizers such as methylene blue and flavins (i.e., flavin mononucleotide and riboflavin). The concentration of the sensitizers was similar to that of flavin in parenteral nutrients. The photooxidation of glucose sensitized by flavin mononucleotide or riboflavin was greater than that which was observed in the presence of methylene blue, whereas the isotopic effect of deuterium oxide (D_2O) was enhanced more substantially in the presence of methylene blue than in the presence of flavins. These results show that methylene blue exerts its action through singlet oxygen and that at a high substrate concentration (as was used in this work) flavin mononucleotide and riboflavin act preferentially as type I sensitizers. In the flavin photosensitized processes, the presence of hydrogen peroxide, superoxide anion, and hydroxyl radical was demonstrated. The photooxidation of glucose is favored by an increase in pH, and it also depends on the energy absorbed by the system. By using a specific reagent for glucose (i.e., o*-toluidine), it was possible to quantify the photoconversion of glucose. The results obtained in this work should be considered in the management of glucose-containing parenteral nutrients that are exposed to visible light in the presence of a multivitamin complex containing flavin mononucleotide.* (J. Nutr. Biochem. 10:181–185, 1999) *© Elsevier Science Inc. 1999. All rights reserved.*

Keywords: riboflavin; glucose; photosensitized; photooxidation

Introduction

Most types of molecules of biological importance are relatively insensitive to direct effects of visible light because they do not absorb radiation in this wavelength range. However, a variety of biological systems are subjected to damage and destruction by light in the presence of appropriate photosensitizers and molecular oxygen (O_2). These dye-sensitized photooxidation reactions are commonly termed *photodynamic action*.[1–4]

Vitamin B_2 riboflavin and its derivative flavin mononucleotide are customary components at the cellular level. They are also present in foods and in parenteral nutrient infusates that are exposed to the action of visible light. In the presence of visible light, the flavins can sensitize photooxygenation processes. The dye-sensitized photooxidations are classified into two mechanisms: type I, which involves electron transfer that leads to the generation of the superoxide ion, and type II, which involves energy transfer that leads to the formation of singlet O_2.[5] Flavin-sensitized reactions include both pathways. In the type I mechanism, the substrate reacts first with the sensitizer in the triplet state and then with molecular O_2 (or one of its active species) via a radical intermediate. In the type II mechanism, the excitation energy is transferred from the sensitizer in the triplet state to molecular O_2, which then gives rise to singlet oxygen ($^1O_2$). Previous studies have described the effect of visible light sensitized by flavins on the lipidic[6] and aminoacidic[7,8] components of the parenteral nutrients infusates. In this article we examined the behavior of glucose solutions at parenteral nutrition concentrations exposed to visible light in the presence of riboflavin or flavin mononucleotide.

Methods and materials

During photochemical treatment, the solutions were irradiated with a 150-W slide projector lamp in the cylindrical cuvette of the O_2 monitor that was set at 25°C. The fluence rate at the level of the

This work was supported by grant No. 1980874 from FONDECYT.
Address correspondence to Dr. Eduardo Silva, Facultad de Química, P. Universidad Católica de Chile, Casilla 306 Correo 22, Santiago, Chile
Received June 12, 1998; accepted October 27, 1998.

© Elsevier Science Inc. 1999. All rights reserved.
655 Avenue of the Americas, New York, NY 10010
0955-2863/99/$–see front matter
PII S0955-2863(98)00093-X

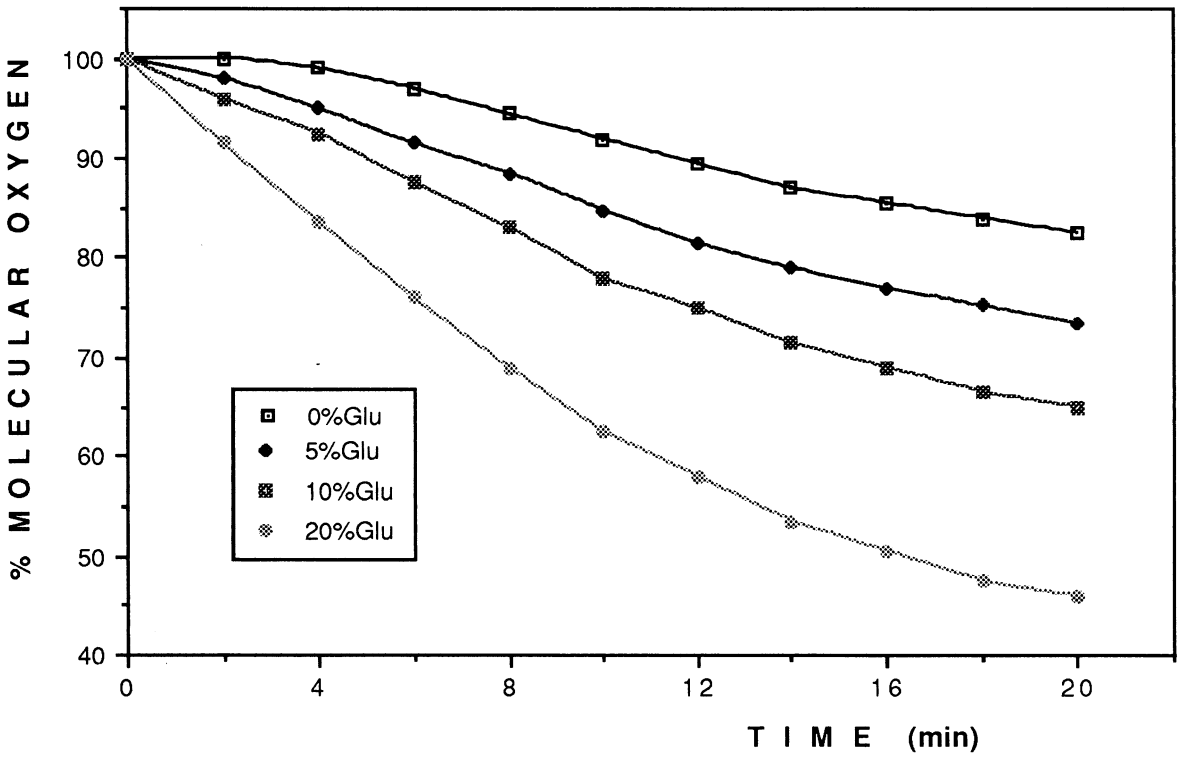

Figure 1 Oxygen consumption during the visible light irradiation of solutions composed of 3.5×10^{-5} M riboflavin and glucose at different concentrations (0, 5, 10, and 20% w/v) in 0.05 M phosphate buffer, pH 7.0, at 25°C.

irradiated solution (160 J/m^{2}seg) was measured with a YSI-Kettering 650 A radiometer (Yellow Springs Instrument Co., Inc. Yellow Springs, Ohio USA). The solutions to be irradiated (3 mL) were prepared mixing one volume of the sensitizer (1.05×10^{-4} M) with two volumes of a 30% w/v glucose solution, both in 0.05 M phosphate buffer, pH 7.0, at 25°C. The study of the pH effect was performed using 0.05 M phosphate buffer solutions at pHs of 4.0, 7.0, and 10.0.

O_2 consumption during irradiation was monitored by a biological O_2 monitor (Yellow Springs Instruments model 5300, Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio USA).

The glucose concentration after irradiation was determined by measuring the absorbance increase at 630 nm of a solution consisting of 3.0 mL of an *o*-toluidine solution and 100 mL of the glucose irradiated solution. The *o*-toluidine solution was prepared by mixing 0.45 g thiourea, 282 mL acetic acid (glacial), and 18 mL 9.15 M *o*-toluidine.

Glucose, catalase, D_2O, flavin mononucleotide, methylene blue, *o*-toluidine, and riboflavin were purchased from Sigma (St. Louis, MO USA). All other reagents were of analytical grade.

Results

Figure 1 shows the molecular O_2 consumption that was observed for solutions of different glucose concentrations when irradiated with polychromatic visible light in the presence of riboflavin (3.5×10^{-5} M) in 0.05 M phosphate buffer, pH 7.0, at 25°C. An increase in the molecular O_2 consumption with increasing glucose concentrations was observed.

Molecular O_2 consumption can be increased by modifying the fraction of the light absorbed (1-T) by the sensitizer. This was achieved by varying the concentration of the sensitizer. The transmittance (T) was measured at 450 nm (*Figure 2*). The pH of the reaction medium also played an important role in the molecular O_2 consumption rate: 3.79×10^{-3}, 8.30×10^{-3}, and 5.50×10^{-2} mM $O_2 \times$ min^{-1} at pHs of 4.0, 7.0, and 10.0, respectively. When methylene blue was used as sensitizer, a substantially lower molecular O_2 consumption occurred than was observed in the presence of flavins. This consumption increased approximately 4.7 times when water of the buffer was changed to D_2O. D_2O did not produce any effect in the case of riboflavin.

The addition of catalase to the system (20% w/v glucose and 3.5×10^{-5} M riboflavin) produced a decrease in the molecular O_2 consumption when exposed to light. When the same system was irradiated in the absence of catalase—and this enzyme was added only after ending the irradiation—a recovery of the consumed molecular O_2 level is observed (*Figure 3*). The molecular O_2 consumption was reduced slightly when the irradiations were performed in the presence of Cu^{2+} and practically were annulled when the experiments were performed in the presence of $K_3[Fe(CN)_6]$.

Applying Dubosky's method,[9] it was possible to determine the photoconversion that a solution of 1.1×10^{-3} M glucose underwent when it was irradiated with polychromatic visible light in the presence of 1.1×10^{-4} M riboflavin (*Figure 4*).

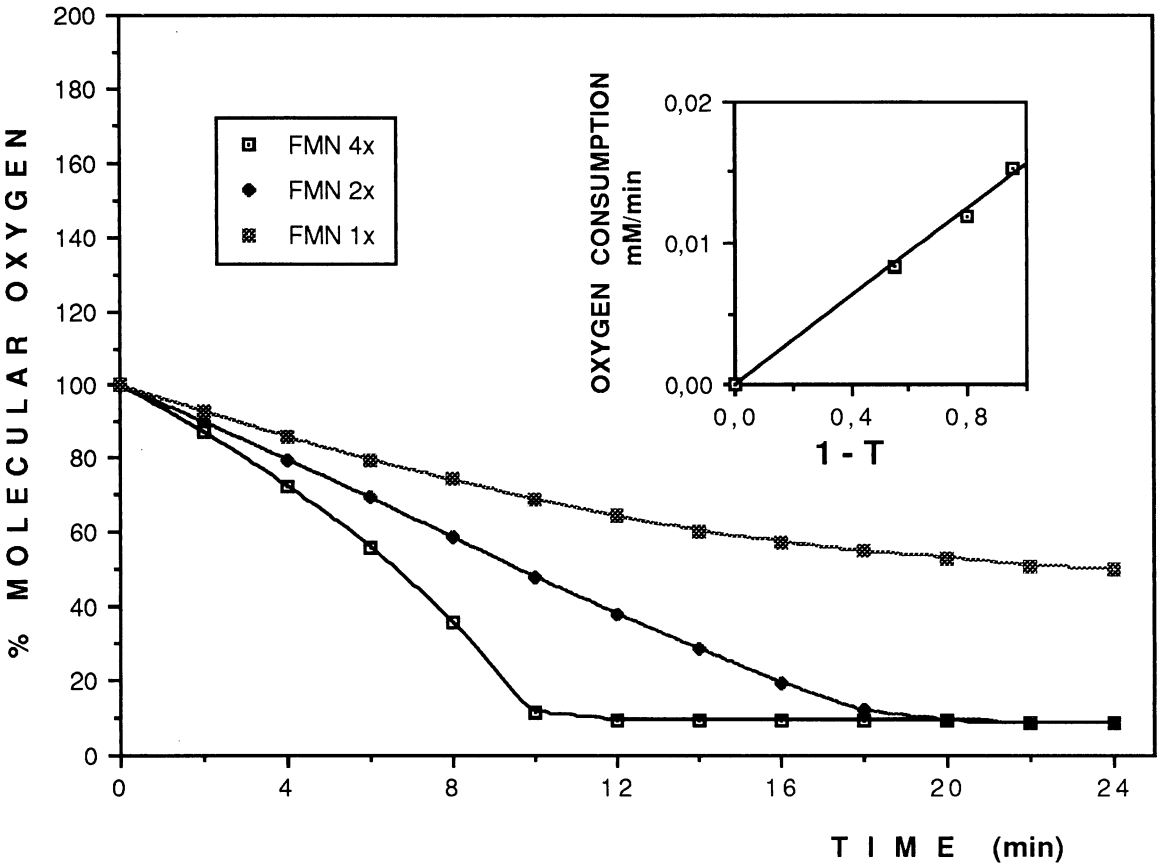

Figure 2 Oxygen consumption during the irradiation with visible light of solutions composed of 20% w/v glucose and flavin mononucleotide at different concentrations (3.5×10^{-5}, 7.0×10^{-5}, and 1.4×10^{-4} M) in 0.05 M phosphate buffer, pH 7.0, at 25°C. *Inset:* Oxygen consumption rate expressed as the fraction of the energy absorbed (1-T) by the sensitizer. The transmittance (T) was measured at 450 nm.

Discussion

Glucose is photooxidized by the action of visible light in the presence of flavinic sensitizers such as riboflavin and flavin mononucleotide. The extent of the photoconversion is directly proportional to the sensitizer concentration in the excited state, which is estimated indirectly by the fraction of energy absorbed by the system studied. The rate of flavin-sensitized glucose photooxidation is substantially greater than that observed in the presence of methylene blue at the same concentration, even though methylene blue absorbs an energy percentage that is greater than that of the flavins. Methylene blue is a sensitizer that, in the excited state, generates essentially singlet O_2. The presence of singlet oxygen species was demonstrated in this work by the increased effect that was observed when the aqueous buffer was changed by a buffer in D_2O. The half-life of $^1O_2$ is 53 to 68 μs in D_2O,[10,11] compared with the half-life of approximately 4 μs in H_2O.[12] Thus, neglecting solvent isotope effects for photooxidation, the reaction rate should increase in transition from H_2O to D_2O.

The flavins have the ability to sensitize through a mixed type I–type II mechanism.[13] In our system of 20% w/v glucose, 3.5×10^{-5} M riboflavin, and visible light, the lack of effect in the presence of D_2O indicates that $^1O_2$ does not play an important role in this case. This result is in agreement with the diminished photoconversion of glucose that is observed when the experiment is performed in the presence of the electron acceptor $K_3[Fe(CN)_6]$.[13] This suggests that electron transfer processes play an important role, because it is typical of a type I mechanism. The fact that a type I mechanism prevails in this experiment correlates well with the high glucose concentration employed, favoring the direct interaction between the excited flavin and the sugar. The efficient type I process also can be explained on energetic grounds. The reduction potential of riboflavin is −0.3 V[14] (vs. the Standard Hydrogen Electrode) at pH 7.0. The redox potential of a triplet flavin is estimated to be shifted to 1.7 V,[5] which is high enough to oxidize the glucose.

Because catalase produces a decrease in the molecular O_2 consumption during the irradiation of a glucose solution in the presence of riboflavin, we can postulate the presence of hydrogen peroxide (H_2O_2) in our system. The generation of H_2O_2 as a consequence of the exposure to light also was demonstrated when catalase was added to the system after irradiation, and an increase of the molecular O_2 was observed. This enzyme transforms the H_2O_2 produced into H_2O and O_2, compensating for the consumption of molecular O_2. A decrease in the consumption of O_2 also was

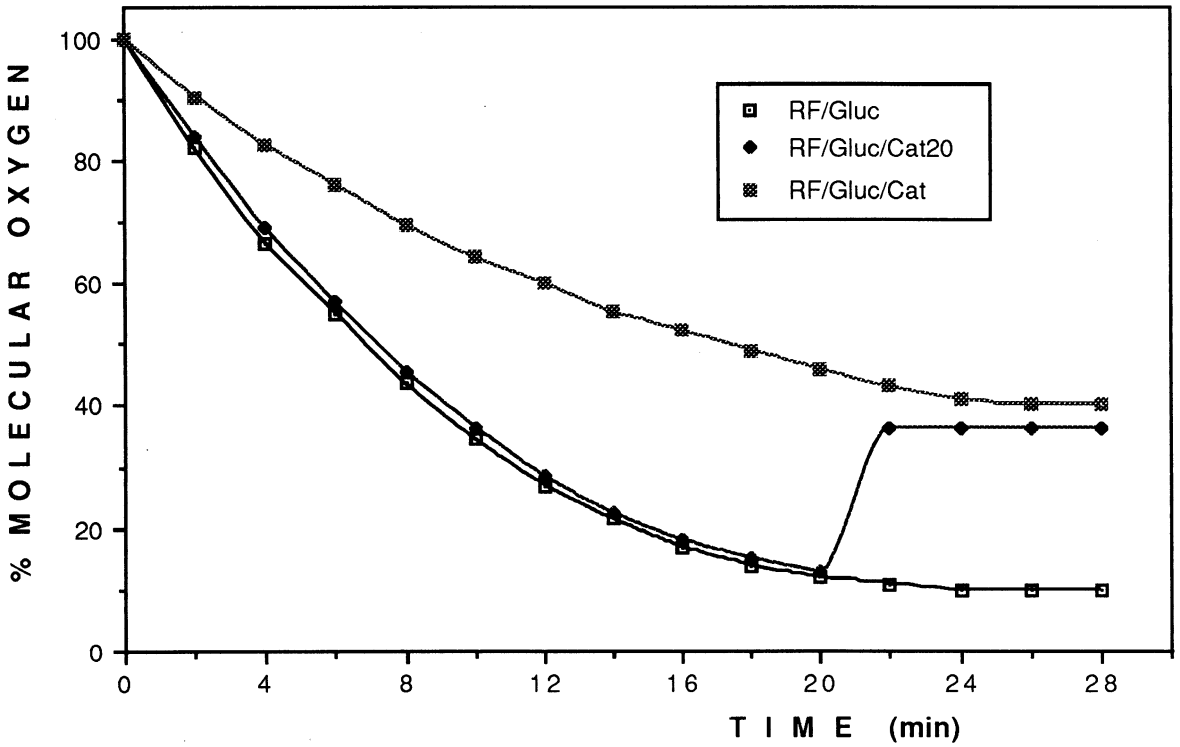

Figure 3 Oxygen consumption during the irradiation with visible light of solutions composed of 3.5×10^{-5} M riboflavin and 20% w/v glucose (RF/Gluc); 3.5×10^{-5} M riboflavin, 20% w/v glucose, and catalase (300 units; RF/Gluc/Cat); and 3.5×10^{-5} M riboflavin and 20% w/v glucose. In this last case, catalase (300 units) was added in the absence of light and after an irradiation time of 20 minutes (RF/Gluc/Cat20). The irradiation was performed in 0.05 M phosphate buffer, pH 7.0, at 25°C.

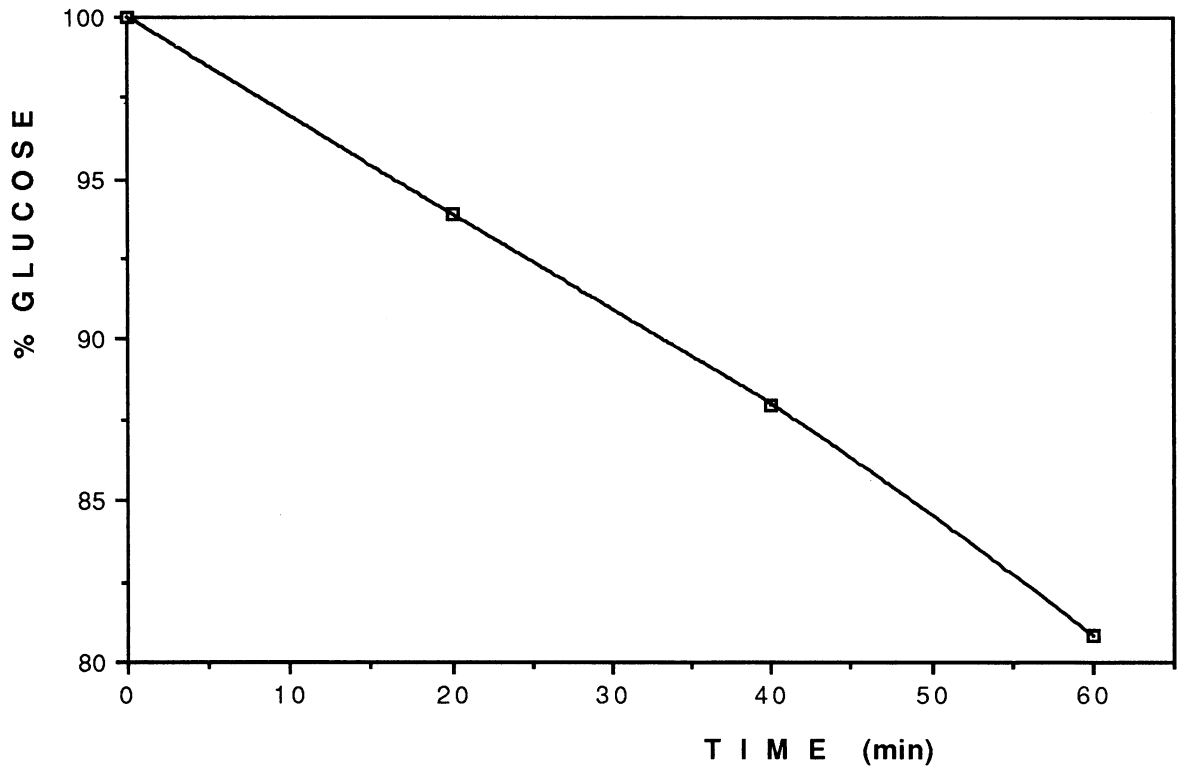

Figure 4 Glucose photoconversion when a solution of 1.1×10^{-3} M glucose is irradiated with polychromatic visible light in the presence of 1.1×10^{-4} M riboflavin in 0.05 M phosphate buffer, pH 7.0, at 25°C.

observed when Cu^{+2} was added to the system. Cu^{+2} could be reduced in the presence of O_2.- to produce Cu^{+1} and molecular O_2. The O_2.- arises from the reaction between O_2 and the flavin radical, which is produced as a consequence of a charge transfer from glucose to the flavin in the excited state. The following reaction sequence describes the different processes mentioned above:

$$RF + h\nu \rightarrow {}^1RF \rightarrow {}^3RF$$
$${}^3RF + Gluc \rightarrow RF.^- + Gluc.^+$$
$$RF.^- + O_2 \rightarrow RF + O_2.^-$$
$$2RF.^- + 2H^+ \rightarrow RF + RFH_2$$
$$RFH_2 + O_2 \rightarrow RF + H_2O_2$$
$$Cu^{2+} + O_2.^- \rightarrow Cu^{1+} + O^2$$
$$Cu^{1+} + H_2O_2 \rightarrow Cu^{2+} + OH^- + .OH$$
$$Gluc.+ .OH \rightarrow Gluc.^+ + OH^-$$
$$Gluc.^+ + O_2 \rightarrow GlucO_2 + H^+$$

When a high glucose concentration (20% w/v) related to riboflavin (1.5×10^{-5} M) was employed, it was not possible to determine any glucose photoconversion. This measurement was only possible when the [Gluc]:[RF] ratio was equal to 10:1. This indicates that the direct interaction between the sugar and the excited flavin plays an important role. The measured glucose photoconversion was less efficient compared with the results reported for aminoacids[7] and lipids.[6] The determination of glucose in the reaction medium was carried out employing *o*-toluidine, because the H_2O_2 generated during the irradiation does not permit the use of the glucose oxidase test.

Conclusion

The photochemical reactions of glucose sensitized by riboflavin or flavin mononucleotide should be considered in systems that contain concentrated solutions of glucose in the presence of a flavin and are exposed to visible light, such as the parenteral nutrition infusates.

References

1 Tsai, C.S., Godin, J.R.P., and Wand, A.J. (1985). Dye-sensitized photooxidation of enzymes. *Biochem. J.* **225,** 203–208

2 Roberts, W.G. and Berns, M.W. (1989). Cell biology and photochemistry of photodynamic sensitizers. *Proc. SPIE-Intl. Soc. Opt. Eng.* **1065,** 175–181

3 Spikes, J.D. (1991). The origin and meaning of the term "photodynamic" (as used in "photodynamic therapy" for example). *J. Photochem. Photobiol. B: Biol.* **9,** 369–371

4 Silva, E. (1996). Sensitized photo-oxidation of amino acids in proteins: Some important biological implications. *Biol. Res.* **29,** 57–67

5 Yoshimura, A. and Ohno, T. (1988). Lumiflavin-sensitized photooxygenation of indole. *Photochem. Photobiol.* **48,** 561–565

6 Silva, E., González, T., Edwards, A.M., and Zuloaga, F. (1998). Visible light induced lipoperoxidation of a parenteral nutrition fat emulsion sensitized by flavins. *J. Nutr. Biochem.* **9,** 149–154

7 García, J. and Silva, E. (1997). Flavin-sensitized photooxidation of amino acids present in a parenteral nutrition infusate: Protection by ascorbic acid. *J. Nutr. Biochem.* **8,** 341–345

8 Donoso, M., Valenzuela, A., and Silva, E. (1988). Tryptophan-riboflavin photo-induced adduct and hepatic dysfunction in rats. *Nutr. Rep. Inter.* **37,** 599–606

9 Kaplan, L.A. (1987). Glucose. In *Methods in Clinical Chemistry* (A.J. Pesce and L.A. Kaplan, eds.), pp. 105–112, Mosby, St. Louis, MO, USA

10 Lindig, B.A. and Rodgers, M.A.J. (1979). Laser photolysis studies of singlet molecular oxygen in aqueous micellar dispersions. *J. Phys. Chem.* **83,** 1683–1688

11 Ogilby, P.R. and Foote, C.S. (1983). Chemistry of singlet oxygen: 42. Effect of solvent, solvent isotopic substitution, and temperature on the lifetime of singlet molecular oxygen. *J. Am. Chem. Soc.* **105,** 3423–3430

12 Rodgers, M.A.J. and Snowden, P.T. (1982). Lifetime of $^1O_2$ in liquid water as determined by time-resolved infrared luminescence measurements. *J. Am. Chem. Soc.* **104,** 5541–5543

13 Silva, E., Ugarte, R., Andrade, A., and Edwards, A.M. (1994). Riboflavin-sensitized photoprocesses of tryptophan. *J. Photochem. Photobiol. B: Biol.* **23,** 43–48

14 Meisel, D. and Neta, P. (1975). One-electron reduction potential of riboflavin studied by pulse radiolysis. *J. Phys. Chem.* **79,** 2459–2461