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Flavin-sensitized photooxidation of amino acids present in a parenteral nutrition infusate: Protection by ascorbic acid

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The visible light mediated photo-oxidation of amino acids present in a parenteral nutrition infusate was studied, using the sensitizing agents usually included in these solutions: riboflavin, flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), and the multivitamin mixture. Of the 14 amino acids studied (alanine, arginine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, and valine), only histidine, methionine, and tryptophan were photo-oxidized by the action of visible light in the presence of these sensitizers. When a mixture of the three photo-oxidizable amino acids was irradiated the photoconversion of tryptophan predominated. Riboflavin and FMN had about the same efficiency as sensitizers, whereas FAD was substantially less effective. The photo-oxidative efficiency of the multivitamin infusate on the amino acids, measured on the basis of observed molecular oxygen consumption, was greater significantly than that found in the presence of FMN. This difference is because of the antioxidative effect created by the vitamin C present in the multivitamin infusate, in relation to the pro-oxidative action of the flavin in its excited state. It was found that a solution of ascorbic acid and FMN, whose concentrations were equivalent to the one in a parenteral nutrition infusate, has the same rate of molecular oxygen consumption as a solution of the multivitamin infusate when irradiated with visible light. The generation of some oxidation products of the tryptophan, generated after irradiation of this amino acid in the presence of FMN, was monitored with emission spectroscopy. On completion of this same experiment, but adding vitamin C, it was observed that for an initial period of time no generation of tryptophan products occurred, after which, tryptophan was modified and had a rate of modification similar to that shown previously. Tryptophan is protected for the time necessary to consume all the vitamin C present in the solution. (J. Nutr. Biochem. 8:341–345, 1997) © *Elsevier Science Inc. 1997*

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Introduction

Many studies have been conducted on the physical and chemical stability of components of total parenteral nutrition (TPN). Visible light is one of the factors leading to instability of vitamins[1,2] and amino acids[3–8] in TPN admixtures.

The vitamin riboflavin (RF) or its conjugated form (flavin mononucleotide, FMN), plays an important role in sensitivity to visible light possessed by the TPN admixture.[4,5] Studies in animals have suggested that several amino acids, especially tryptophan (Trp), may play a role in the development of hepatic dysfunction,[5] one of the most common complications associated with parenteral nutrition (see references cited in Ref. 5). Two mechanisms have been proposed to explain sensitized photo-oxidation. In the type I mechanism, the substrate reacts initially with the sensitizer in the triplet state and then with molecular oxygen. In the type II mechanism, the excitation energy is transferred from the sensitizer in the triplet state to the molecular oxygen, giving rise to singlet oxygen ($^1O_2$), which reacts with the substrate. A mixed-type I-type II mechanism has been observed when using RF as a sensitizer in the photo-oxidation of Trp[9]. The photodegradation of this amino acid

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in oxygen-saturated aqueous solution sensitized by RF is accompanied by the generation of reactive oxygen species ($^1O_2$, $\cdot OH$, H_2O_2 and $O_2\cdot^-$) and the appearance of the following irradiation products: aggregate forms of RF, indolic products associated with flavins, indolic products of molecular weight higher than Trp, formylkynurenine, kynurenine, and other Trp photodecomposition products.[9] Recently, the generation of an adduct between RF and Trp after irradiation under anaerobic conditions has been described[10] and this compound has been proposed as one of the photoproducts that may be responsible for the observed hepatic dysfunction in parenterally fed patients[11] and cytotoxicity on tumor cells and preimplantation mouse embryos in daylight irradiated culture medium.[12,13] The subcellular targets and the mechanism involved in the induction of cytotoxicity have not been determined. The present study was undertaken to determine the effect of visible light on the amino acids usually present in a parenteral nutrition admixture sensitized by riboflavin or its conjugated forms (FMN and flavin adenine dinucleotide, FAD) or by a multivitamin infusate.

Methods and materials

To accomplish this study, as reference was used the final concentration of the amino acids that possesses a solution of parenteral nutrition prepared from a 10% commercial solution of amino acids obtained from Abbott. The latter has the following amino acid concentrations: 12.8 g/L alanine, 9.8 g/L arginine, 12.8 g/L glycine, 3.0 g/L histidine (His), 7.2 g/L isoleucine, 9.4 g/L leucine, 7.2 g/L lysine (acetate salt), 4.0 g/L methionine (Met), 4.4 g/L phenylalanine, 8.6 g/L proline, 4.2 g/L serine, 5.2 g/L threonine, 1.6 g/L Trp, and 8.0 g/L valine. It was assumed that 445 mL of this amino acids solution is carried to one liter of parenteral nutrition solution according to the following composition: 445 mL 10% amino acids solution, 300 mL 50% glucose, 20 mL 10% NaCl, 15 mL 20% KCl, 10 mL multivitamin infusate, and 210 mL distilled water. The final concentration of the amino acids in the parenteral nutrition is the following: 63.9 mM alanine, 25 mM arginine, 75.9 mM glycine, 8.6 mM His, 24.4 mM isoleucine, 31.9 mM leucine, 15.5 mM lysine (acetate salt), 19.2 mM Met, 11.9 mM phenylalanine, 33.2 mM proline, 17.8 mM serine, 19.4 mM threonine, 3.5 mM Trp, and 30.4 mM valine.

During photochemical treatment, the solutions were irradiated with a 150 W slide projector lamp in a 1 cm light path cuvette thermostated at 25°C. The solutions to be irradiated were prepared mixing a volume of the sensitizer (2.25×10^{-5} M) with two volumes of each amino acid studied or the set of these (12.9 mM His, 28.8 mM Met, and/or 5.3 mM Trp), obtaining the parenteral nutrition final concentrations of each one of these compounds (7.5×10^{-6} M FMN, 8.6 mM His, 19.2 mM Met, and 3.5 mM Trp). In the experiments were ascorbic acid was used, the final concentration of this vitamin in the solution was of 0.57 mM. When the MVI-1 infusate was used, 50 mL of this infusate were added to each 10 mL of the final solution to be irradiated. The solutions were irradiated without agitation to simulate the condition of a parenteral nutrition solution.

Oxygen consumption during irradiation was followed by a biological oxygen monitor (Yellow Springs Instruments model 5300). The absorption spectra were recorded on a Milton Roy, Rapid Scan Spectronic 3000 Diode Array spectrophotometer. Fluorescence measurements were performed with a Perkin Elmer 650-10S fluorescence spectrometer.

Ascorbic acid, flavin adenine dinocleotide, flavin mononucleotide, L-histidine, L-methionine, riboflavin, and L-tryptophan were purchased from Sigma Chemical Company (St. Louis, MO USA). All other reagents were analytical grade. The two ampoules (MVI-1 and MVI-2) that contain the multivitamin infusate added to a daily parenteral nutrition admixtures were obtained from Rhone Poulenc Rorer. MVI-1 (5 mL) contained: 100 mg vitamin C, 0.99 mg vitamin A, 5.0 μg vitamin D, 3.0 mg thiamine, 3.6 mg FMN, 4.0 mg vitamin B_6, 40 mg niacinamide, 15 mg pantotenic acid, and 10 mg vitamin E. MVI-2 (5 mL) contained: 60 μg biotin, 400 μg folic acid, and 5.0 μg vitamin B_{12}. Saline medium consisted of 0.03 M NaCl, 0.03 M KCl, and 0.02 M KH_2PO_4.

Results

Out of the 14 aminoacids (alanine, arginine, glycine, His, isoleucine, leucine, lysine, Met, phenylalanine, proline, serine, threonine, Trp, and valine) present in the studied parenteral nutrition infusate only His, Met, and Trp were associated with oxygen consumption after irradiation with visible light in the presence of RF (7.5×10^{-6} M), FMN (7.5×10^{-6} M) or FAD (7.5×10^{-6} M). The concentrations of these aminoacids (8.6 mM His, 19.2 mM Met, and 3.5 mM Trp) were equivalent to those found in typical parenteral nutrition infusates.[14] In all cases FAD was less efficient as a photochemical sensitizer than either RF or FMN, which have similar behavior (*Figure 1*). The oxygen consumption kinetics of the three amino acid mixture (His, Met, and Trp) is very similar to that observed upon irradiation of a solution containing only Trp. The spectral variations shown by the visible light irradiated solution of the three amino acid in the presence of FMN are the same as those found after a solution containing Trp alone was irradiated under the same conditions.

Of the two multivitamin infusate solutions, only MVI-1 showed absorption bands in the visible region corresponding to those of FMN. *Figure 2* shows the molecular oxygen

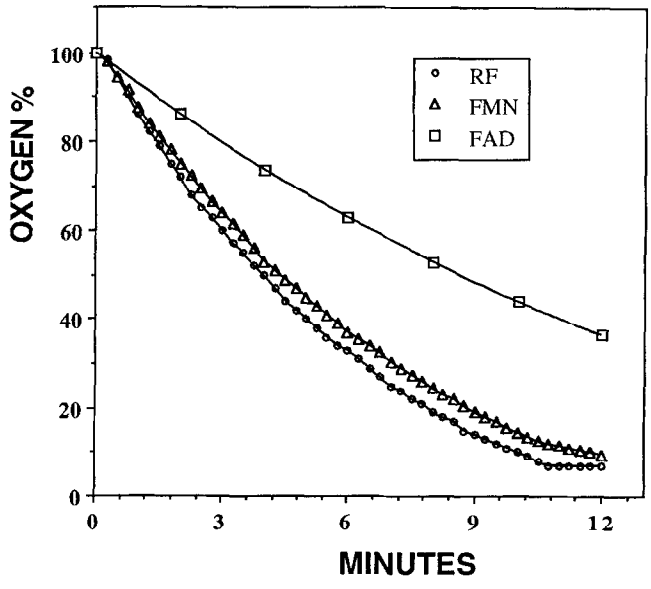

Figure 1 Oxygen consumption during irradiation with visible light of solutions composed of 8.6 mM His, 19.2 mM Met, and 3.5 mM Trp in the presence of 7.5×10^{-6} M RF, 7.5×10^{-6} M FMN or 7.5×10^{-6} M FAD, in saline medium.

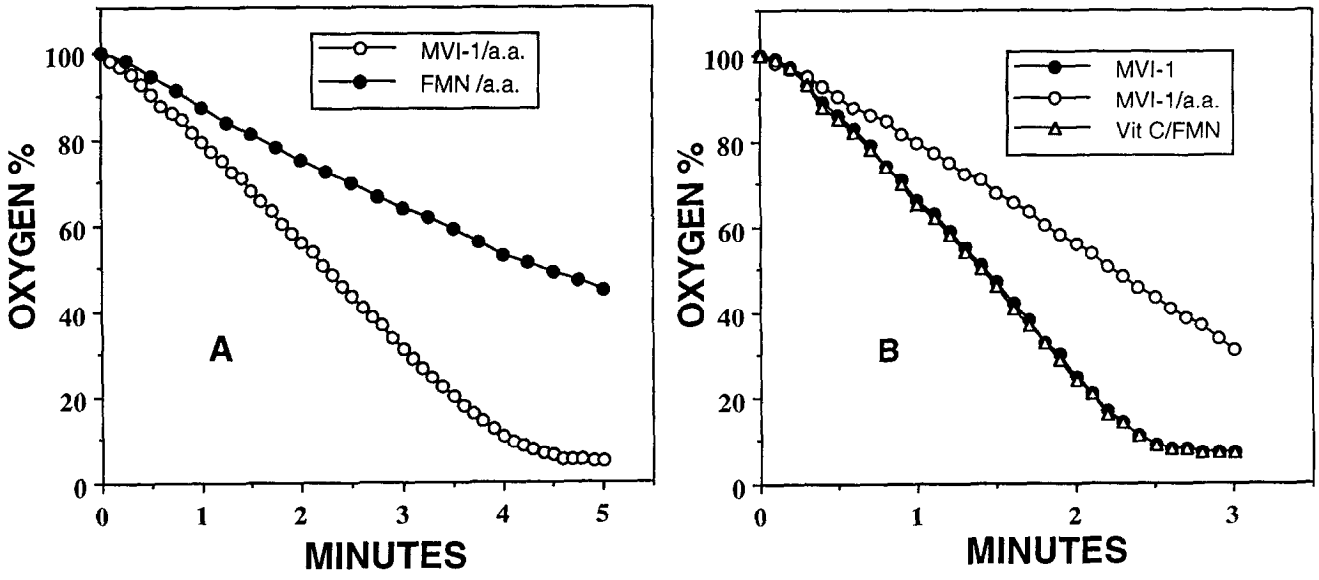

Figure 2 *A*, Oxygen consumption during the irradiation with visible light of solutions composed of 8.6 mM His, 19.2 mM Met and 3.5 mM Trp in the presence of MVI-1 (containing 7.5×10^{-6} M FMN) or 7.5×10^{-6} M FMN, in saline medium. *B*, Oxygen consumption during the irradiation with visible light of an MVI-1 solution (containing 7.5×10^{-6} M FMN and 0.57 mM ascorbic acid) in 0.05 M phosphate buffer, pH 7.0 (MVI-1), and of the same described solution in the presence of 8.6 mM His, 19.2 mM Met, and 3.5 mM Trp (MVI-1/a.a.). Also shown is the oxygen consumption of a visible light irradiated solution of 7.5×10^{-6} M FMN and 0.57 mM ascorbic acid, in 0.05 M phosphate buffer, pH 7.0.

consumption of the His (8.6 mM), Met (19.2 mM), and Trp (3.5 mM) solution after exposure to visible light in the presence of either MVI-1 or FMN at identical flavine concentrations (7.5×10^{-6} M). Molecular oxygen consumption is greater in the presence of MVI-1 than in the presence of FMN. FMN alone exposed to light does not consume molecular oxygen under the conditions used in this experiment. *Figure 2B* illustrates oxygen consumption by solutions of MVI-1 (containing 7.5×10^{-6} M FMN and 0.57 M ascorbic acid); His (8.6 mM), Met (19.2 mM), Trp (3.5 mM)/MVI-1, and Vit. C (0.57 mM)/FMN (7.5×10^{-6} M) when exposed to visible light. Molecular oxygen consumption by an irradiated solution of Vit. C (0.57 mM) and FMN (7.5×10^{-6} M), is the same as that shown by a solution of only MVI-1 (containing 7.5×10^{-6} M FMN and 0.57 M ascorbic acid), whereas it is greater than that observed after irradiation of the amino acid mixture in the presence of MVI-1.

The visible light irradiated solution of Vit. C/FMN decomposed, a process that could be followed spectrophotometrically, measuring the decrease in the absorbance of this compound at 265 nm (*Figure 3*). The vitamin C concentration (0.57 mM) was high enough, compared with that of FMN (7.5×10^{-6} M), to avoid the interference of the latter compound. The absorption bands of FMN in the visible region are hardly noticeable (*Figure 3*).

The fluorescent emission increases because of Trp photoproducts after visible light irradiation of a solution of this aminoacid (3.5 mM) and FMN (7.5×10^{-6} M), both in the presence and absence of vitamin C (0.57 mM) (*Figure 4*).

Discussion

Of all the amino acids present in a commercially available parenteral nutrition infusate only three (His, Met, and Trp), appear to be oxidized in a photosensitized process. Two additional photooxidizable amino acids,[15] tyrosine and cysteine, were not present in the studied infusate but are also found only at trace amounts in other parenteral amino acid mixtures.

When the amino acid and vitamin concentrations found in the studied parenteral nutrition infusate are irradiated with visible light, only the solution of the three amino acids previously mentioned were associated with molecular oxygen consumption. Of the three flavin derivatives used, RF and FMN had the same capacity as sensitizers and FAD was significantly less efficient. This difference reflects an intramolecular energy diffusion of the excited FAD.

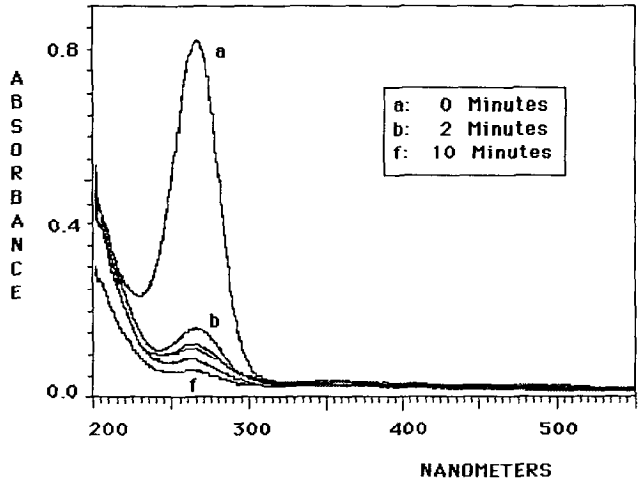

Figure 3 Absorption spectra of 0.57 mM ascorbic acid solutions irradiated for 0, 2, 4, 6, 8, and 10 min with visible light in the presence of 7.5×10^{-6} M FMN, in 0.05 M phosphate buffer, pH 7.0. The solutions were diluted 10 times before measurements.

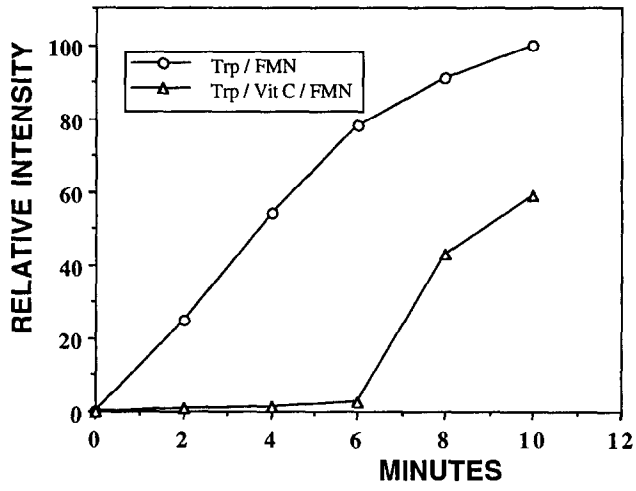

Figure 4 Trp-photoproducts fluorescence emission (λ_{exc} = 360 nm; λ_{em} = 410 nm) of Trp solutions irradiated for 0, 2, 4, 6, 8, and 10 min in the presence of 7.5 × 10^{-6} M FMN (Trp/FMN); and of 0.57 mM ascorbic acid and 7.5 × 10^{-6} M FMN (Trp/Vit C/FMN). The solutions were diluted 30 times previous to measurements.

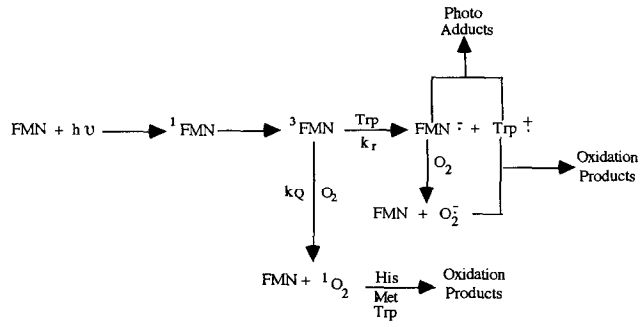

Figure 5 Photophysical and photochemical processes involved in the irradiation with visible light of a solution that contains the amino acids His, Met, and Trp, in the presence of FMN under aerobic atmosphere. $^1O_2$ represents singlet oxygen. FMN, ^{1}FMN, and ^{3}FMN represent the flavine acting as sensitizer, in the ground state, and in the excited singlet and triplet states, respectively.

In spite of the fact that oxygen consumption rate of His or Met solutions irradiated in the presence of FMN are greater than those of a Trp solution irradiated under the same conditions, the oxygen consumption kinetics of a solution containing the three amino acids together is practically the same as that obtained for a solution of only Trp. The fact that oxygen consumption is determined by Trp (although it is present in lower concentrations) is indicative that the other two amino acids are photo-oxidized through different processes (*Figure 5*). His is an amino acid that is photo-oxidized preferentially through singlet oxygen, obtained by the interaction of triplet flavin with molecular oxygen through a type II mechanism. From the results of this work we can conclude that His and Met are photo-oxidized following the same reaction route mediated by singlet oxygen and that Trp preferentially follows a different mechanism. Another alternative is that Trp interacts directly with the triplet flavin (type I mechanism)[9] reducing the population of this activated species and, consequently, producing less singlet oxygen. Figure 5 shows the reactions involved in these reaction mechanisms.

It is important to consider that the reaction of indole with the triplet state of a flavin (k_r = 3.7 × 10^9 $M^{-1}s^{-1}$, in the case of lumiflavin)[16] is approximately three times greater than the quenching constant of triplet lumiflavin by molecular oxygen (k_Q = 1.2 × 10^9 $M^{-1}s^{-1}$).[16] The molecular oxygen concentration in aqueous buffered medium equilibrated with air (0.237 mM), is 15 times smaller than that of the Trp (3.5 mM) used in this work and found in parenteral nutrition infusates. Under these conditions it is evident that the reaction of the triplet state of the flavin with Trp is favoured and, consequently, the type I mechanism will be preferred although the Met (19.2 mM) and His (8.6 mM) concentrations are greater than that of Trp.

After irradiation of a solution containing His, Met and Trp in the presence of the multivitamin complex (MVI-1) it was found that oxygen consumption was considerably greater than that observed in the presence of FMN (at the same concentration as that of this compound in MVI-1). The multivitamin complex MVI-1 had a significant molecular oxygen consumption even in the absence of the aminoacids under study. This finding indicates that some component of this complex has been photo-oxidized through a sensitized process. From this complex vitamin C and vitamin E were considered as the most likely compounds to consume molecular oxygen because of their anti-oxidant nature. Thus, after irradiation of a solution of vitamin C in the presence of FMN (mantaining the concentrations of these compounds as in the parenteral nutrition infusate) it was found that it presented the same oxygen consumption kinetics as the MVI-1 solution irradiated with visible light. On the other hand, an emulsion of vitamin E exposed to visible light in the presence of FMN did not show molecular oxygen consumption. In the study of the antioxidative effect of vitamin C on a Trp solution exposed to visible light in the presence of FMN it was found that this vitamin exerted a protective effect that lasted the time required for its photo-oxidation. After that period of time the photo-oxidation of Trp yielded a series of photoproducts, including kynurenine.[9] Considering that Trp is photoconverted preferentially through a type I mechanism, we can deduce that vitamin C exerts its antioxidative function by interacting with flavin in the triplet state, which is responsible for the electron abstraction and the oxidation of ascorbic acid. This mechanism is important for the photochemical stability of the aminoacids present in a parenteral nutrition infusate exposed to visible light which would be protected from a photo-oxidative effect for as long as there is vitamin C in the medium. It is important to emphasize that the oxidation products of vitamin C have the property of protein glycation (nonenzymatic glycosylation) and this reaction is important in the ocular lens because this kind of process can produce opacity and in extreme cases it can cause cataracts.[17–20]

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