BIOLOGICAL IMPLICATIONS OF RIBOFLAVIN SENSITIZED PHOTOPRODUCTS OF TRYPTOPHAN. A REVIEW.

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A review of the effect of riboflavin sensitized visible light on the essential amino acid tryptophan is performed in this work. The biological implications of the formed products in connection to hepatic and cytotoxic effects associated with light exposition of parenteral nutrients and celular culture media respectively are discussed. The participation of riboflavin sensitized tryptophan photoproducts in protein aggregation processes of the ocular lens, a tissue that is regurarly exposed to visible light, is also analyzed.

Keywords: riboflavin; tryptophan; photo-toxicity.

INTRODUCTION

Most types of molecules of biological importance are relatively insensitive to direct effects of visible light since 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 appropiate photosensitizers and molecular oxygen. These dye-sensitized photooxidation reactions are commonly termed photodynamic action.[1-3] Two mechanisms, classified as Type I and Type II processes, have been proposed to explain sensitized photo-oxidation.[4-6] In the Type I mechanism radical species are involved and initially the substrate reacts directly with the sensitizer in the triplet state and then with molecular oxygen. Many other dye-sensitized photooxygenation reactions have been regarded as Type II processes where singlet oxygen formed in the reaction between the triplet state of dyes and oxygen is the reactive intermediate.

The vitamin B_2, riboflavin (Rb) is a photosensitizer that, in water solutions and in the presence of oxygen, leads to substrate oxidation both through Type I and Type II mechanisms.[7] The role of free and protein-bound tryptophan in the light sensitivity of biological systems has prompted numerous studies of the photoprocesses of this essential amino acid, either following the direct absorption of light by the indole group or promoted by compounds acting as sensitizers.[8] Of the sensitized photoprocesses, those involving the riboflavin-tryptophan system are of particular interest due to the endogenous nature of both compounds.

The sensitized photooxidation of tryptophan has been the subject of a number of studies.[8-13] Three pathways involving a common intermediate (hydroperoxy indol alanine) have been proposed to explain the generation of two important oxidation products of tryptophan: kynurenine and N'-formylkynurenine,[11,14] whose fluorescent characteristics have been reported by Fukunaga et al.[15] In turn, Nakagawa et al.[16] have shown that pH not only exerts an influence on the Trp photooxidation rate, but that it also influences the formation of other photoproducts, such as 5-hydroxyformylkynurenin at pH higher than 7.0 and a tricyclic hydroperoxide in the 3.6 to 7.1 pH range.

An exceptional efficiency for the photo-oxidation of Trp has been found when the vitamin riboflavin was used as sensitizer.[17] This process is characterized by higher quantum yields than those observed with other sensitizers such as methylene blue or rose bengal which involve a Type II photo-oxidation mechanism. This behaviour suggested that riboflavin may act preferently through a Type I mechanism. This fact has been demonstrated by the lumiflavin-sensitized photooxygenation of indole.[7] Yoshimura and Ohno[7] found, that the semiquinone anion radical of lumiflavin and the half-oxidized radical of indole were formed by the reaction between triplet lumiflavin and indole. The semiquinone anion radical of lumiflavin reacted with oxygen to form superoxide. They also demonstrated, that the quantum yield of the lumiflavin-sensitized photooxygenation of dilute indole via radical processes was much higher than that via $^1O_2$ processes, though appreciable amounts of $^1O_2$ was formed via a Type II process. In this same respect, it was previously reported[18] that irradiation of lysozyme with visible light in the presence of riboflavin and molecular oxygen, not only produced the photooxidation of some amino acid residues of that enzyme, but also generated a binding of this sensitizer to the protein. This riboflavin-lysozyme photo-binding can also be obtained in an anaerobic atmosphere, thus avoiding photooxidative Type II processes and allowing the Type I process in it first stage, that do not require the presence of molecular oxygen.

In subsequent studies, after obtaining peptides from lysozyme[19] it was demonstrated that a Trp residue of the enzyme was specifically involved in the binding between riboflavin and lysozyme.[20] Through the anaerobic irradiation of the essential amino acid tryptophan in its free form, in the presence of Rb, it was possible to isolate and characterize spectrophotometrically at least two types of photoadducts, according to the degree of modification of the flavin.[21] The effects of pH and ionic micelles on the rates of product formation following irradiation of riboflavin in the presence of tryptophan has also been investigated by absorption and fluorescence spectroscopy.[22] Under anaerobic conditions, formation of riboflavin-tryptophan adducts is inhibited in acid solutions and by the addition of anionic (sodium dodecylsulphate) and cationic (cetyltrimethyl ammonium bromide) micelles. The oxidation of tryptophan photoinduced by riboflavin is considerably faster in basic solutions.

It is worth noting that a binding of riboflavin-lysozyme and riboflavin-tryptophan have also been obtained in the dark. When riboflavin and lysozyme are added to the 2-methylpropanal/peroxidase/O_2 system, which generates triplet acetone, an adduct is formed to a small extent.[23] The adduct can be separated by gel filtration and is similar to that prepared by irradiating riboflavin in the presence of lysozyme.[18] When riboflavin and tryptophan are added to this system a riboflavin decomposition product (of lumichrome type), formylkynurenine and an adduct between the riboflavin and the tryptophan are formed.[24]

RIBOFLAVIN SENSITIZED PHOTOPRODUCTS OF TRYPTOPHAN AND HEPATIC DYSFUNCTIONS

Self-administration of large doses of the amino acid Trp has become widespread for the treatment of insomnia.[25-31] This amino acid is exceptional in its diversity of biological functions. It contributes importantly to normal growth and protein syntheses in a number of tissues and regulates numerous physiological mechanisms. Despite these facts, tryptophan is considered to be one of the agents involved in the pathogenesis of hepatic encephalopathy.[32] The administration of tryptophan in high doses produces ultrastructural changes of the liver in rats.[33] The presence of tryptophan and riboflavin has also been related to hepatic dysfunctions produced during parenteral nutrition.[34] Hepatic dysfunction is one of the most common complication associated with parenteral nutrition. The spectrum of hepatic dysfunction ranges from elevations of several liver enzymes to the development of acute or chronic liver disease. The presence of tryptophan and riboflavin has been related to hepatic dysfunctions produced by parenteral nutrition.[34] Studies in animals have suggested the role of the riboflavin photosensitized oxidation of tryptophan in the pathogenesis of hepatic dysfunction in neonatal gerbils.[35] Considering that the action of visible light on solutions containing tryptophan and riboflavin generated not only photo-oxidation products, as a consequence of the production of the active oxygen species $O^{\cdot-}$ and $^1O_2$ during irradiation, but also indol-flavin and indol-indol photoadducts,[36] it became of interest to study the possible toxicity of these compounds. In this way a decrease in the gain of weight and an increase in the activity of serum γ-glutamyl transpeptidase was found in rats receiving intraperitoneally, for 12 days, both anaerobic and aerobic light-exposed tryptophan-riboflavin solutions compared to controls. Concentrations of γ-glutamyl transpeptidase were higher in animals receiving the anaerobic irradiated solutions than in the other groups.[37] This enzyme is usually employed in clinical studies, as an indicator of hepatic canalicular membrane function and integrity, thus an increase of γ-glutamyl transpeptidase at the plasma level is associated with hepatic dysfunctions.

CYTOTOXIC EFFECT OF THE RIBOFLAVIN SENSITIZED PHOTOPRODUCTS OF TRYPTOPHAN

Wang et al.[38] have reported that when mammalian cells in tissue-culture medium (Dulbecco's Modified Eagle's Medium) were irradiated with the near-UV light emitted by black-light tubes, the cells were killed both physiologically and reproductively. In another report, they showed that the killing effect was due to formation of toxic photoproducts from riboflavin, tryptophan, and tyrosine in the medium.[39] Cell damage does not occur when these components are withdrawn from the medium prior to irradiation.[40] Considering, that at the experimental conditions described by these authors, tryptophan riboflavin and indol-indol bindings also occur, it was of interest to compare the cytotoxic effect of tryptophan-riboflavin solutions irradiated under either anaerobical or aerobical conditions. In the first case, the adducts are preferentially generated, and in the oxygenated medium these compounds, are accompanied by products of tryptophan photooxidation. When the products of the anaerobic irradiation of a tryptophan-riboflavin solution were added to cell culture media seeded with teratocarcinoma F-9 cells, it was observed that these products inhibited both cellular adhesion to the substrate and the natural proliferation process.[41] This same effect was found in the presence of a mixture of the tryptophan photo-oxidation products and the adducts, when using solutions previously irradiated with visible light in an O_2 atmosphere. A cytotoxic effect was also observed with embryos incubated in the presence of a tryptophan-riboflavin adduct, in the latter case necrosis and embryo development arrest occured.[41]

RIBOFLAVIN-PHOTOSENSITIZED ANAEROBIC MODIFICATION OF RAT LENS PROTEINS

A number of chemical and/or structural modifications occur in lens proteins during aging or cataratogenesis: an increase in insoluble protein,[42-45] the formation of high-molecular-weight protein,[46-48] the production of a yellow to brown colouration,[42,49-51] and the formation of blue fluorescence.[52-56] Several workers have attempted to associate the action of light with these changes on lens proteins. Furthermore, cross-linking of crystallins caused by photo-oxidation has been induced by photosensitizers, such as methylene blue,[57-59] 8-methoxypsoralen,[60-61] promazines,[62] kynurenine derivatives,[58,63-67] rose bengal[67] and riboflavin.[59,68]

Since riboflavin is normally present in the ocular lens, a tissue permanently exposed to light, the study of the behaviour of lens proteins irradiated with visible light in the presence of riboflavin is of interest. Bose et al.[69] have presented evidence which show that the riboflavin-sensitized conformational changes of a-crystallin are very different from those sensitized by methylene blue or N-formylkynurenine.

It was previously reported[21] that the photo-binding between free tryptophan and riboflavin may also be obtained in an anaerobic medium where oxygen-mediated processes can be suppressed. Because the *in situ* lens has a low oxygen concentration (less than 10^{-5} M),[70] it is probable that aerobic and anaerobic photoprocesses contribute simultaneously to the photoinduced damage of lens proteins. When ^{14}C-riboflavin enriched lenses were exposed to visible light, a photo-induced binding between riboflavin and a water insoluble protein fraction of the lenses was observed.[71]

The irradiation of rat lens homogenate or its soluble protein fractions in the presence of riboflavin leads to a modification in the chromatographic elution pattern with an increase in the high-molecular-weight fraction.[36] In a simultaneosly aging study with rats, it was shown that the proportion of the high-molecular-weight protein fraction significantly increased with age, whereas the proportion of the low-molecular-weight protein fraction concomitantly decreased.[36] It was postulated that aging produced an increase in the accessibility of the tryptophan residues of the lens proteins, as established from iodide-quenching experiments, which would be more susceptible to the interaction with excited riboflavin, with generation of radical species that could be responsible for the initiation of the aggregation processes.

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