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Effect of phosphate buffer on the complexation and photochemical interaction of riboflavin and caffeine in aqueous solution: A kinetic study

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ABSTRACT

A study of the photodegradation of 5×10^{-5} M riboflavin (RF) in 0.2–1.0 M phosphate buffer in the presence and absence of 2.50×10^{-4} M caffeine at pH 6.0–8.0 has been carried out. RF in phosphate buffer is photodegraded simultaneously by normal photolysis (photoreduction) and photoaddition reactions giving rise to lumichrome (LC) and cyclodehydroriboflavin (CDRF) as the main final products, respectively. RF and its photoproducts, formylmethylflavin (FMF), lumiflavin (LF), LC and CDRF in degraded solution have been determined by a specific multicomponent spectrophotometric method with an accuracy of ±5%. The apparent first-order rate constants for the photodegradation of RF and for the formation of LC and CDRF are $5.47–15.05 \times 10^{-3}$ min^{-1}, $1.06–8.30 \times 10^{-3}$ min^{-1} and $4.31–8.05 \times 10^{-3}$ min^{-1}, respectively. An increase in phosphate concentration leads to an increase in the rate of formation of CDRF and alters the photodegradation of RF in favor of the photoaddition reaction. This photoaddition reaction is further enhanced in the presence of caffeine which results in a further decrease of the fluorescence of RF in phosphate buffer. Caffeine may facilitate the photoaddition reaction by suppression of the photoreduction pathway of RF.

1. Introduction

Buffers are considered as an important component of chemical and pharmaceutical systems to achieve optimum stability of a compound or to perform a reaction under controlled condition of pH, buffer concentration and ionic strength. In many cases, the buffers affect the stability of pharmaceutical compounds in aqueous solution [1–6]. Several studies have been carried out to evaluate the effect of buffer species on the photolysis of riboflavin (RF). These studies include the catalytic effect of acetate [7] and phosphate buffers [8–14] and the inhibitory effect of borate [15] and citrate buffers [16]. It has been shown that the monovalent phosphate ions ($H_2PO_4^-$) exert a catalytic effect on the normal photolysis or photoreduction (side-chain cleavage) of RF [11,17] and the divalent phosphate ions (HPO_4^{2-}) on the photoaddition (side chain cyclization) reactions of RF [9–14]. These two major pathways of the photodegradation of RF occur simultaneously depending on the pH, buffer concentration and light intensity and wavelengths [9–14]. The rate of photoaddition reaction of RF at pH 7.0 is more than twice of the photolysis reaction [11].

RF is known to form molecular complexes with caffeine [18–24] which causes the stabilization of RF and thus inhibits its rate of chemical [25] and photodegradation reactions [26,27] in aqueous solution. However, the previous studies deal only with the individual effect of caffeine (CF) [27], or the phosphate [11–14] on the photodegradation of RF. These studies do not provide information on the combined effect of CF and phosphate on the reaction.

CF molecules may enter the human body from various food sources in large amounts and, thereby, may influence RF function in organism. Hence, the knowledge of photochemical interaction of CF–RF is important for understanding their interaction in a biological system. The knowledge of the set of factors altering RF photodegradation in solution is also important in view of the positive synergistic effect of blue light and RF with respect to suppression of tumor cells growth in vivo [28] and the blue

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light-mediated DNA repair by photolysate enzymes containing flavin adenine dinucleotide (FAD) as cofactor [29].

The present work is based on the evaluation of the effects of CF and phosphate together on the photodegradation of RF and the role of CF vis-a-vis phosphate in altering the rates of the reactions. The study provides kinetic evidence to support the view that CF is involved in modifying the role of phosphate to enhance the photodegradation of RF. This would facilitate the understanding of the interaction of CF–RF in phosphate buffer and its influence on structural orientation to cause a particular change in the mode of degradation reactions.

2. Materials and methods

RF, LC, LF and CF were obtained from Sigma–Aldrich Chemicals Co. (St. Louis, MD, USA). FMF, CMF and CDRF were prepared by the previously described methods, respectively [30,31]. All the reagents and solvents were of analytical grade or of the purest form available from Merck & Co. (Whitestone Station, NJ, USA).

2.1. Photolysis of RF

The photolysis of 5×10^{-5} M RF solutions, containing 2.5×10^{-4} M CF was carried out at $25 \pm 1\,^{\circ}C$ in the presence and absence of 0.2–1.0 M Na_2HPO_4 at pH 6.0–8.0. The pH of the solution was adjusted with 1.0–5.0 M HCl/NaOH solution. The solution (100 ml) was irradiated in a dark chamber using a Philips 125 W high pressure mercury vapor fluorescent lamp (emission at 405 and 435 nm), fixed horizontally at a distance of 30 cm from the center of the flask. The 435 nm band of the radiation source overlaps the 445 nm absorption maximum of RF [11] while CF (λ_{max} 273 nm) [32] does not absorb in the visible region. The solution was stirred by bubbling a stream of air into the flask. Samples were withdrawn at appropriate intervals for chromatographic examination and spectrophotometric determination. The photolysis of RF was also carried out under the same conditions in the presence of 1 M phosphate and $0.625–2.50 \times 10^{-4}$ M CF at pH 7.0.

2.2. Thin layer chromatography

The identification of the photodegradation products of RF was carried out by thin-layer chromatography (TLC) using 250-μm silica gel G plates (Merck) and the solvent systems: (a) 1-butanol–acetic acid/water (40:10:50 (v/v), organic phase) [33], and (b) chloroform–methanol (9:2 (v/v)) [9]. The detection of RF and its photodegradation products was performed by their characteristic fluorescence emission under UV (365 nm) excitation (RF, FMF, CMF – yellow green; LC, sky blue) using a Uvitech lamp (Cambridge, UK) or by visual examination (CDRF – red color). The progress of the photolysis reactions was monitored by observing the intensity variations of the spots of different products.

2.3. Spectrophotometric assay

The assay of RF and its side-chain cleavage photoproducts (FMF, LC, LF) and cycloaddition photoproduct (CDRF) was performed by a five component spectrophotometric method of Ahmad et al. [11]. This method has previously been used in a number of photodegradation studies of RF in the presence of phosphate buffer [11–14]. It involves the pre-adjustment of the photodegraded solutions to pH 2.0 (0.2 M HCl/KCl buffer), followed by the extraction of LC and LF with chloroform and determination of the chloroform residue at pH 4.5 (0.2 M acetate buffer) by a two-component assay at 356 and 445 nm, the respective absorption maxima of LC and LF [11]. The other three compounds (RF, FMF, CDRF), present in the aqueous phase, are determined by a three-component assay at 385, 410, 445 nm, corresponding to the absorption maxima of FMF, CDRF and RF, respectively [11]. The absorption maxima of RF and FMF (445 nm, pH 7.0) can be distinguished at pH 2.0 where FMF exists in a protonated form (pK_a 3.5) [34] and thus can be assayed by this method. CMF is a minor product of the oxidation of FMF [13,17,35] and has not been considered in the assay scheme.

2.4. Spectral measurements

The spectral and absorbance measurements on RF and its photolyzed solutions were performed on a Shimadzu UV-1601 recording spectrophotometer using quartz cells of 10-mm path length.

2.5. Fluorescence measurements

The fluorescence of RF solutions was measured at room temperature (25 °C) with a Spectromax 5 fluorimeter (molecular devices, Sunnyvale, CA, USA) in the end point mode using 374 nm as the excitation wavelength and 525 nm as the fluorescence wavelength [36]. The fluorescence was measured in relative fluorescence units using a pure 0.05 mM RF solution as the standard.

2.6. Light intensity measurement

The measurement of the intensity of Philips HPL-N 125 W high pressure mercury vapor fluorescent lamp was performed using potassium ferrioxalate actinometry [37]. The value of the intensity of the lamp was determined as $1.16 \pm 0.098 \times 10^{17}$ quanta s^{-1}.

3. Result and discussion

3.1. Composition of photodegraded solution of RF

It is necessary to ascertain the nature of products formed in the photodegraded solutions of RF in the presence of phosphate buffer and CF at pH 6.0–8.0. TLC of the solutions using solvent system (a) showed the presence of the compounds (R_f values in parentheses): undergraded RF (0.36), FMF (0.61), LC (0.67), LF (0.42) and CMF (0.26), with their characteristic fluorescence emission (mentioned in Section 2 under thin layer chromatography), and solvent system (b): undergraded RF (0.37), LC (0.82) and CDRF (0.46) (red color). These products have previously been identified in the photodegradation of RF under similar conditions and arise from the photoreduction and photoaddition pathways [9,17,27,38]. The formation (FMF, LC, LF, CMF, CDRF) and loss (RF, FMF, CMF) of these compounds was monitored by changes in the intensity of their spots. The formation of LF in the reaction takes place at pH 7.0–8.0.

3.2. Spectral characteristics of photodegraded solutions

The spectral variations in the photodegraded solutions of RF in the presence of CF [27] and the phosphate buffer [9,11,14] have been studied. In the presence of CF, RF shows a loss of absorbance at 374 and 445 nm and a slight increase around 385 nm indicating the formation of FMF with time in the aqueous phase (pH 2.0) after chloroform extraction to remove LC and LF. The mixture of these products in the aqueous solution (pH 4.5) exhibits a gradual increase in absorbance around 356 and 445 nm, the respective absorption maxima of LC and LF. The absorption spectrum of the photodegraded solutions of RF in the presence of phosphate buffer