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Fluorescence quenching of flavins by reductive agents

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Abstract

The fluorescence behaviour of the flavins riboflavin, flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), and lumiflavin in aqueous solution at pH 8 in the presence of the reducing agents β-mercaptoethanol (β-ME), dithiothreitol (DTT), and sodium nitrite ($NaNO_2$) is studied under aerobic conditions. The fluorescence quantum yields and fluorescence lifetimes are determined as a function of the reducing agent concentration. For all three reducing agents diffusion controlled dynamic fluorescence quenching is observed which is thought to be due to photo-induced reductive electron transfer. For DTT additionally static fluorescence quenching occurs.

Keywords: Flavins; Riboflavin; Lumiflavin; FMN; FAD; β-Mercaptoethanol; Dithiothreitol; Sodium nitrite; Dynamic fluorescence quenching; Static fluorescence quenching; Reductive electron transfer

1. Introduction

Flavins are blue-light absorbing dyes [1] with rich redox chemistry and photochemistry. Riboflavin (vitamin B_2) is a precursor co-enzyme of flavoproteins, and as such is needed for animal and human well being [2]. Flavin mononucleotide (FMN) and flavin dinucleotide (FAD) are cofactors in flavoproteins with biological activity in enzymes [2] and photoreceptors [3–6]. Lumiflavin is the parent molecule of many flavins.

In flavin-cofactor-based blue-light photoreceptors [3,4] (phototropins [7], cryptochromes [8], BLUF domains [9]) efficient flavin fluorescence quenching is observed in the dark-adapted (receptor) state and in the light-adapted (signalling) state (for phototropins see [10,11], for cryptochromes see [12], for BLUF proteins see [13–16]). This fluorescence quenching is thought to be caused by photo-induced reductive electron transfer from an adjacent amino acid residue (tyrosine, tryptophan, phenylalanine, cysteine, and histidine are known as electron donors for photo-excited flavins [17]) to the excited flavin moiety. In order to add information to the fluorescence quenching of flavin-based blue-light photoreceptors, here some fluorescence quenching studies on flavins in aqueous solution at pH 8 (flavins are in the oxidized redox-state, i.e., in the quinone state) by some reducing agents under aerobic conditions have been undertaken. The photo-reduction of flavins from the oxidized form (quinone state) to the fully reduced form (hydroqinone state) by some reducing agents was studied previously [18] (see also [19]).

The fluorescence behaviour of flavins is discussed in [1,20–27]. The fluorescence quenching of flavins by various substances was studied in [28–31].

In this paper, the fluorescence quenching of the flavins riboflavin, FMN, FAD, and lumiflavin by the reducing agents dithiothreitol (DTT) [32,33], β-mercaptoethanol (β-ME) [34], and sodium nitrite ($NaNO_2$) [35] is studied. In neutral aqueous solution the fluorescence quantum

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yields of riboflavin ($\phi_F \approx 0.26$ [36]), FMN ($\phi_F \approx 0.25$ [37] and this work), and lumiflavin ($\phi_F \approx 0.235$ [37]) are determined by intersystem-crossing and internal conversion. The fluorescence quantum yield of FAD ($\phi_F \approx 0.033$ [38]) is additionally reduced by internal photo-induced electron-transfer from the adenine part to the isoalloxazine part [38–46]. The reducing agents β-ME, $NaNO_2$, and DTT are found to act as dynamic fluorescence quenchers where the quenching rate is diffusion controlled (excited flavin and quenching molecule come together by diffusion, and fluorescence is quenched by electron transfer from the HOMO level of the reducing agent to the HOMO level of the excited flavin; HOMO = highest occupied molecular orbital). The bimolecular quenching rate constants, k_q, of dynamic fluorescence quenching are determined. DTT is found to act additionally as static fluorescence quencher (DTT – flavin complexes or closely spaced pairs are present in ground-state, excitation of the flavin moiety causes fluorescence quenching by electron transfer from the DTT part to the flavin part).

2. Experimental

The flavins riboflavin, FMN, FAD, lumiflavin, and the reducing agents DTT, β-ME, and $NaNO_2$, were purchased from Sigma–Aldrich. Their structural formulae are shown in Fig. 1. They were used without further purification. The flavins were dissolved in aqueous sodium phosphate buffer, pH 8, consisting of 10 mM Na_2HPO_4, 10 mM NaH_2PO_4, and 10 mM NaCl. The experiments were carried out under aerobic conditions.

The experimental setup for fluorescence quantum distribution measurement is depicted in Fig. 2a [47,48]. The samples were excited with a high-pressure mercury lamp through an interference filter at 365 nm. A dichroitic polarizer was used for vertical polarization of the excitation light. The fluorescence emission was collected in front-face arrangement with a lens. Then it was passed through a second dichroitic polarizer under magic-angle orientation (angle of 54.7° to vertical direction) [49] and focused to a grating spectrometer equipped with a diode-array detection system. The absolute intrinsic fluorescence quantum distributions [50], $E_F(\lambda)$, and the absolute intrinsic fluorescence quantum yields, $\phi_F = \int E_F(\lambda)d\lambda$, were determined by using the dye quinine sulphate dihydrate (from Aldrich) dissolved in 1 normal aqueous H_2SO_4 as reference standard ($\phi_F(C) = 0.546/[1 + 14.5C]$ where C is the dye concentration in mol dm^{-3} [51], note that units M and mol dm^{-3} are used synonymously, i.e., 1 M = 1 mol dm^{-3}). The fluorescence quantum distributions are corrected for the spectral sensitivity of the detection system (spectrometer and silicon diode-array detector, see [47,48]).

The experimental setup for fluorescence life-time measurement is shown in Fig. 2b. The samples were excited with second harmonic pulses of a femtosecond Ti:sapphire laser (system Hurricane from Spectra-Physics, second harmonic

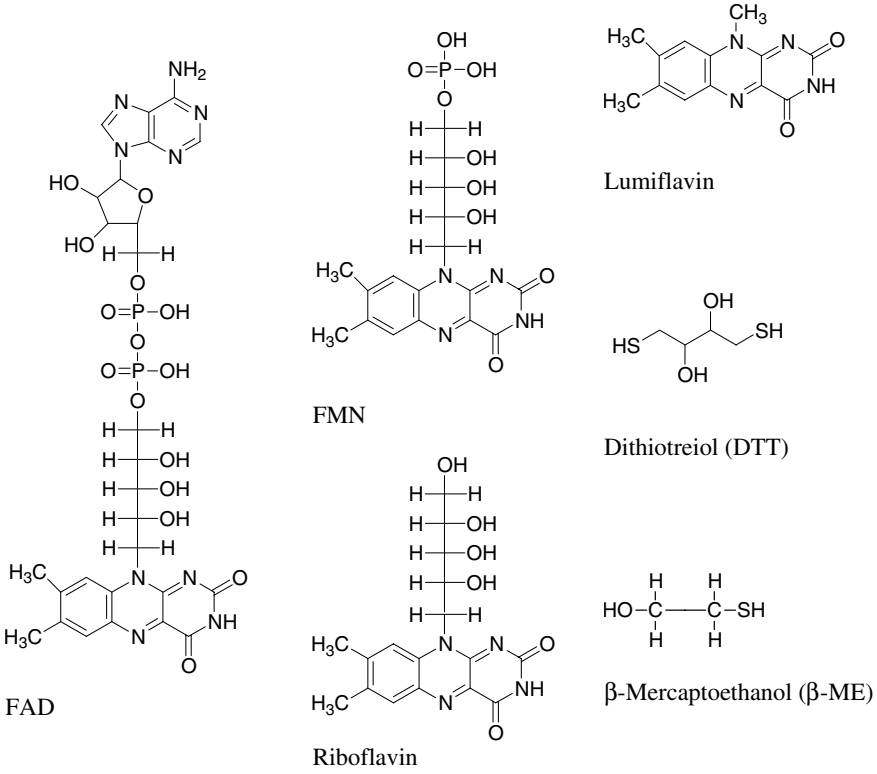

Fig. 1. Structural formulae of flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), riboflavin, lumiflavin, dithiotreiol (DTT), and β-mercaptoethanol (β-ME).