Interactions of the β -blocker drug, propranolol, with detergents, β -cyclodextrin and living cells studied using fluorescence spectroscopy and imaging

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Abstract. Interactions of the β -blocker drug, propranolol, with amphipathic systems have been studied using fluorescence spectroscopy. The results show a strong binding of propranolol with micelles of sodium dodecyl sulfate revealed through changes in the fluorescence spectrum and an increase in fluorescence lifetime. Quenching of propranolol fluorescence by iodide is used to demonstrate interaction with β -cyclodextrin. At high concentrations, self-quenching of propranolol fluorescence was also observed with $k_q = 2.5 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$. Two-photon excited (630 nm) fluorescence lifetime imaging of propranolol in cells showed propranolol to be widely distributed in the cell cytoplasm, with fluorescence lifetimes shorter than in solution. The results suggest that intracellular propranolol is mainly confined within the aqueous cytoplasm and rather than membrane associated.

Keywords: Propranolol, fluorescence, lifetime, quenching, micelle, cyclodextrin, imaging, live cell

1. Introduction

Propranolol (I) is a drug used to regulate blood pressure and anxiety levels [7,12]. As a β -blocker it prevents binding of epinephrine and norepinephrine to cell surface receptors and inhibits intracellular signalling cascades. Propranolol is an amphipathic molecule that interacts with cellular membranes [18]. Binding of propranolol to lipid bilayers is stronger association for the neutral species in alkaline solution than for the protonated form at neutral pH [1]. In contrast the evident hydrophilic character of propranolol is demonstrated by its high solubility in neutral aqueous solutions (up to 50 mmol dm^{-3}). Recent studies [10] have shown that propranolol is rapidly taken up by live mammalian cells, but the intracellular location of accumulated propranolol was unknown. With convenient absorption and fluorescent

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properties enabling it to be selectively excited in cellular environments [3,4], intracellular propranolol may be imaged during uptake into live mammalian cells.

2. Materials and methods

($\pm$)-Propranolol hydrochloride and other chemicals were obtained from Sigma-Aldrich. Fluorescence spectra were recorded using a Spex Fluoromax fluorimeter and absorption spectra were measured with a Perkin-Elmer Lambda-25 spectrometer. Fluorescence lifetimes with one-photon excitation were determined by time-correlated single photon counting (TCSPC) apparatus (IBH with pulsed LED excitation at 296 nm).

3. Results and discussion

As previously observed [11,18], the fluorescence spectra of propranolol vary with solvent. In ethanol (Fig. 1), methanol and acetonitrile a structured emission with peaks at 321, 336 and 352 nm is observed. In water, dimethylformamide and dimethyl sulfoxide an additional broad band at longer wavelength at 370–380 nm is observed. The spectrum in neutral aqueous buffer was invariant with concentration in the micromolar region, excluding excimer as the origin of this additional band. There is no obvious correlation of intensities at 336 and 380 nm with solvent dielectric constant and the additional band observed in water, DMF and DMSO is suggested to stem from charge transfer between the naphthalene ring and the side chain depending on strong hydrogen bond accepting character of the solvent.

Fluorescence spectra recorded on titration of propranolol with sodium dodecyl sulfate (SDS) show (Fig. 1) a distinct change at the critical micelle concentration (CMC, ca 1 mmol dm⁻³) although more subtle changes are also apparent pre-micellization. Below the CMC spectra are similar to that in water. Above the CMC spectra display the structure observed in solvents such as ethanol and lack the charge transfer band. This suggests association between propranolol and the micelle with the naphthalene ring residing within the micelle and shielded from the aqueous solvent. Similar experiments with neutral (reduced Triton X-100) and cationic (cetyltrimethyl ammonium chloride) micelles failed to show similar interactions and indicate the important contribution from the anionic head group of SDS in the binding process. Changes in fluorescence spectra (Fig. 1) during titration of propranolol with SDS and the solvent effects are very much more pronounced than for naphthalene itself [14,15]. This provides strong support for the assignment of the long wavelength band in solvents such as water and DMSO to an intramolecular charge transfer state as previously described in related molecules [5,17].

4. Fluorescence lifetimes

Fluorescence lifetimes of propranolol were measured using one-photon excitation at 296 nm. Lifetimes in aqueous solutions measured with two-photon excitation at 630 nm gave essentially identical results. In neutral aqueous solutions the fluorescence decay gave a good single exponential fit with a lifetime of 10.0 ns. At pH 11, above the pK_a of the secondary amine function ($pK_a = 9.5$) the lifetime was 6.55 ns. In non-aqueous solvents the fluorescence decay was biexponential. In deaerated ethanol the dominant longer lifetime component was 14.8 ns, whereas in aerated DMSO the dominant component

Fig. 1. Fluorescence spectra recorded on increasing SDS concentration ($0\text{--}4.5\text{ mmol dm}^{-3}$) in a solution of propranolol ($10\text{ }\mu\text{mol dm}^{-3}$) in phosphate buffer (0.1 mol dm^{-3} at pH 7.0). The spectrum of propranolol in ethanol ($\blacklozenge\text{--}\blacklozenge$) is also shown for comparison. INSET: Changes in fluorescence intensity at 322 nm ($\blacksquare\text{--}\blacksquare$) and 380 nm ($\square\text{--}\square$), and average fluorescence lifetime ($\diamond\text{--}\diamond$) on titration of propranolol with SDS.

was the shorter lifetime of 2.23 ns. In solvents showing the charge transfer band at $\sim 380\text{ nm}$, the lifetimes and pre-exponential weightings were independent of emission wavelength (325–400 nm) showing that equilibrium between emitting states occurs faster than fluorescence decay. Differences in fluorescence lifetimes between air- and nitrogen-saturated solutions were consistent with diffusion controlled quenching by O_2 ($k \approx 2 \times 10^{10}\text{ dm}^3\text{ mol}^{-1}\text{ s}^{-1}$ in ethanol). In SDS solutions the lifetime became biexponential with a dominant (96%) longer lifetime of 17.7 ns and the average lifetime increased abruptly at the CMC (inset to Fig. 1).

5. Fluorescence quenching

Fluorescence quenching by iodide was used to determine the solvent accessibility of propranolol when associated with SDS micelles and β -cyclodextrin. Figure 2 shows the Stern–Volmer plots for quenching of fluorescence intensities by iodide anion. In solutions of propranolol alone these show a slight upwards curvature indicative of a major dynamic quenching component (K_D) with a minor static component (K_S). Evaluation as outlined by Lakowicz [13] gave a value of $K_D = 71\text{ dm}^3\text{ mol}^{-1}$. Combined with the fluorescence lifetime of 10.0 ns, this gives a second-order rate constant for dynamic quenching of $7.1 \times 10^9\text{ dm}^3\text{ mol}^{-1}\text{ s}^{-1}$. In the presence of SDS concentrations above the CMC, the overall Stern–Volmer quenching constant, K_{SV} , reduces to $1.3\text{ dm}^3\text{ mol}^{-1}$. This is consistent with the location of the naphthalene ring of propranolol within the micellar structure that is inaccessible to iodide in the aqueous compartment.

Fig. 2. Stern–Volmer plots for quenching of propranolol by iodide in aqueous solution buffered to pH 7 with phosphate (0.1 mol dm^{-3}). Solutions contained: (a) propranolol only; (b) 1.5 mmol dm^{-3} β -cyclodextrin; (c) 3 mmol dm^{-3} β -cyclodextrin; (d) 6 mmol dm^{-3} β -cyclodextrin; (e) 12 mmol dm^{-3} β -cyclodextrin; (f) 4 mmol dm^{-3} SDS. INSET: Plot of Eq. (1) (see text) for a solution of propranolol ($10 \mu\text{mol dm}^{-3}$) containing NaI (35 mmol dm^{-3}).

A previous investigation of the association between propranolol and β -cyclodextrin by fluorescence spectroscopy [9] showed that there was $<10\%$ increase in propranolol fluorescence on complex formation. This was too small for accurate evaluation of the association constant. The binding of propranolol by β -cyclodextrin is readily detected (Fig. 2) by a decrease in quenching of propranolol fluorescence by iodide. The observed fluorescence intensity (I_{obs}) as a function of β -cyclodextrin and iodide concentrations yields values of the Stern–Volmer constants, K_f , K_b , for diffusional quenching of propranolol free in solution and bound to cyclodextrin respectively, together with the association constant for propranolol and β -cyclodextrin, K_A , as described by Eq. (1), where I_0 is the intensity in the absence of iodide and β -cyclodextrin:

$$\frac{I_{\text{obs}}}{I_0} = \left[\frac{1}{1 + K_A[\beta - CD]} \right] \left[\frac{1}{1 + K_f[Q]} + \frac{K_A[\beta - CD]}{1 + K_b[Q]} \right]. \quad (1)$$

Figure 2 shows the effect of adding β -cyclodextrin to solutions of propranolol and iodide and non-linear fitting gives a value of $K_A \approx 195 \text{ dm}^3 \text{ mol}^{-1}$, in good agreement with a value of $239 \text{ dm}^3 \text{ mol}^{-1}$ from calorimetry [8]. The fitted values of K_f and K_b were 80.7 and $23.3 \text{ dm}^3 \text{ mol}^{-1}$, respectively, and the second-order rate constant for quenching by iodide of bound propranolol was $2.3 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$. K_A for propranolol is similar to that for related molecules such as tryptamine ($160 \text{ dm}^3 \text{ mol}^{-1}$ [16]) and serotonin ($\sim 53 \text{ dm}^3 \text{ mol}^{-1}$ [2]).

A Stern–Volmer plot (not shown) of propranolol fluorescence lifetimes (determined using two-photon excitation at 630 nm [6]) shows that dynamic self-quenching occurs with a second-order rate constant of $2.5 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$. In cells propranolol accumulates to concentrations appreciably greater than millimolar [3,4] and intracellular lifetimes may therefore be shortened by this route.

6. Multiphoton fluorescence lifetime imaging in mammalian cells

Fluorescence lifetime images of propranolol uptake by cells [3,4] indicate rapid accumulation to concentrations up to $\sim 10\text{--}20 \text{ mmol dm}^{-3}$ within the cell cytoplasm and exclusion from the cell nucleus. The lifetime images and distributions show shorter lifetimes than in solution and are shorter than expected on the basis of self-quenching alone. The observation that the lifetime is shorter than that in water, compared with the longer lifetimes in the less polar systems described above, suggests that intracellular propranolol remains in the cell aqueous compartment rather than being solubilised in lipid environments.

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