High efficiency amplified spontaneous emission from a fluorescent anticancer drug–dye complex

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ABSTRACT

External amplified spontaneous emission (ASE) efficiency around 30% is reported for an optically active molecule, which at the same time shows antitumor activity. The complex is formed by the covalent binding of an anticancer drug, tamoxifen, commonly applied in breast cancer therapy, and nitro-2-1,3-benzoxadiazol-4-yl (NBD) dye, which is frequently used as a biomarker in hydrophobic environments, such as lipid membranes. A laser-like pump threshold around 100 kW/cm^2 was found in solutions of the fluorescent drug diluted in acetone or in oil. Agreement with an ASE spatial propagation model, as well as the lack of optical feedback in the walls of the dilution cuvette confirms that ASE is the physical mechanism that explains the high efficiency observed. The waveguide character and the polarization dependence of ASE are also studied. Highly efficient optical gain in such systems suggests new biophotonic applications.

1. Introduction

Current medical practice could not be envisaged without the use of external lasers both for diagnosis and therapy [1,2]. As an example, in photodynamic therapy (PDT) laser light is used to activate photosensitizer drugs, and generate cytotoxic photodynamic reactions [3–5]. However, some recent reports have extended the concept of laser applications by replacing the traditional gain media by fluorescent proteins in living cells [6], dye labeled DNA molecules [7], or random lasers in organic materials embedded in biological platforms [8,9]. In spite of these examples of bio-inspired lasers and applications, to our knowledge, there are no reports of active drugs themselves used as optical gain media. In this sense, we have designed,

and synthesized a fluorescent anticancer drug formed by the covalent binding of a drug moiety and a conventional dye [10]. More precisely, we have synthesized a composite molecule (Fig. 1a), FLTX1, formed by the covalent binding of tamoxifen (Tx), which is the most widely used drug for breast cancer therapy, and nitro-2-1,3-benzoxadiazol-4-yl (NBD) dye, a common biomarker for lipids and hydrophobic environments, such as lipid membranes [11]. Most breast cancer cells exhibit estrogen-dependent proliferation and growth rates, upon binding of estrogen molecules to their cognate intracellular receptors (mainly estrogen receptor alpha, ERα), which behave as transcription factors. Tx acts as a competitive antagonist because it binds ERα at their estrogen-binding pocket, and inhibits receptor activation and dimerization, thereby preventing activation of proliferation and growth [12]. FLTX1 was designed so that NBD motif would not interfere with the ability of the triphenyl moiety of the tamoxifen molecule to bind the active site of the estrogen receptor protein (see Supplementary Materials, Fig. S1). In this way, FLTX1 exhibits

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nearly identical pharmacological properties to Tx [13]. Moreover, we have recently reported laser emission of FLTX1 in a whispering gallery mode configuration using a high Q-value cylindrical microresonator, which demonstrates that the fluorescent drug can act as an optical gain medium [10]. However, the efficiency of the laser emission could not be determined in that laser configuration because it is difficult to collect all the energy emitted by the laser.

Amplified spontaneous emission (ASE) is a very convenient technique for researchers to explore the laser properties of organic materials [14–17]. ASE is a cooperative effect that occurs by stimulated emission of radiation in a gain medium in which optical feedback is not necessary. In fact, ASE has been described as a kind of "mirrorless laser" [18,19]. In this work, we demonstrate efficient output laser-like ASE in solutions of FLTX1 in acetone and in vegetal oil, under nanosecond pulsed excitation, although neither Tx nor NBD show optical gain alone. Moreover, the external efficiency of the ASE pulses in FLTX1 is about 30%, which is similar or even higher than commercial dyes, such as Rh6G. The emission characteristics and the spatial confinement of ASE have been studied and agree with the ASE model. The physical foundation of the predictions of the model is based on gain saturation. In addition to this, unusual polarization state of ASE has been detected. Although no ASE is expected when the polarization of the pump beam is parallel to the amplification direction [20,21], we have observed ASE in those circumstances. This anomalous behavior has been explained in terms of rotational diffusion of the dye molecules, and allows control of optical gain by selection of the pump and detection polarizations. The results suggest that fluorescent drugs can be synthesized to obtain efficient optical gain medium for biophotonic applications.

2. Experimental section

For the ASE experiments the solutions of FLTX1 were placed in a quartz cuvette (10 mm front length × 4 mm side length). The excitation source was an Optical Parametric Oscillator (OPO) laser tuned at 470 nm (pulse duration about 8 ns, and repetition rate 10 Hz). A pinhole was used to select a homogenous excitation laser beam. Since the OPO laser output has horizontal polarization, a λ/2 plate was used to change to vertical polarization. Two linear polarizers were used to modulate the intensity of the pump beam. A cylindrical lens focused the pump beam on the cuvette front face to form a horizontal line which had a length of 12 mm (completely covering the front length of the cuvette) and width of about 210 μm. A 400 μm diameter fiber coupled CCD spectrometer (Andor) was used to record the emission spectra from the sides of the cuvette. The pulse energies from the OPO laser and from ASE were measured using an energy meter from Newport connected to a digital oscilloscope.

3. Results and discussion

To characterize the FLTX1 molecule as a gain material we have prepared solutions in acetone that range from 30 mM down to 100 μM. It is known that NBD dye has a poor fluorescence. However it can form efficient fluorescent products when it reacts upon aliphatic amines. FLTX1 compounds show a vivid orange color due to its ground state absorption, which is centred at about 470 nm, Fig. 1b, while pure Tx or NBD solutions look transparent at the same concentrations owing to their low absorption coefficients in the visible range of the spectrum. The photoluminescence (PL) was measured when the excitation was tuned at 470 nm and the pump beam was focused on a stripe line on the front face of the cuvette. The results obtained for the 10 mM FLTX1/acetone solution are the following. At low pump density a broad PL band is recorded with a full width at half maximum (FWHM) of about 45 nm. At a given pump threshold FWHM dramatically drops, reaching a value of about 8 nm, Fig. 1b. Together with the narrowing process, a sudden increase of the emission intensity is observed at the same pump threshold (0.75 mJ/cm^2, i.e. about 100 kW/cm^2). These are the characteristic signatures of ASE [14–19].

Additionally, the energy of the output ASE beams has been plotted as a function of the pump pulse energy in Fig. 1c, where a linear dependence is observed. The slope of the linear fit yields an external laser slope efficiency of 29%. In fact the ASE efficiency was high enough to allow the observation of bright and directional output laser beams at both sides of the cuvette by the naked eye (see Supplementary Materials, video). In addition to this, the ASE intensity was stable, showing a constant value during operation time of more than 30 min. Some researchers have reported on the transition from ASE to lasing with coherent feedback in different systems [22–24]. In the case of solutions of organic molecules, laser with optical feedback has been observed due to Fresnel reflections at the cuvette–solution interfaces [25,26]. Consequently, it is crucial to distinguish between ASE and lasing with optical feedback. However, in our experiments there are several evidences that indicate that ASE is responsible of the observed emission. First, the emission band around 555 nm has a bandwidth about 10 nm above the pump threshold (Fig. 1b). Such a bandwidth is characteristic of ASE, while typical laser lines show further spectral narrowing [15,25]. Second, there was no cavity effect from back reflections at the cuvette sidewalls, as the output beams remained unchanged when the cuvette was tilted to the sides (see Supplementary Materials, video). And third, agreement between the experimental data and the predictions of an ASE model has been obtained. The model is based on a set of coupled rate equations for the excited state population and the ASE photon flux [27,28]. Basically, the propagation of the amplified spontaneous emission along the length (z-direction) of the amplifier is simulated. The model consists of a z-dependent population of dye molecules which are homogeneously excited. It considers a four-level dye pumped under stationary conditions, as the pulse duration is 8 ns, which is much longer than the decay time (about 0.3 ns, see below). The model is similar to the ones in Refs. [27,28], with the main difference that the signal self-absorption term has been neglected (four-level regime), as in this particular dye, the absorption at the maximum emission is negligible as shown in Fig. 1b. Under these conditions, the dye excitation rate is given by: