Biosensors and Bioelectronics 63 (2015) 204–211

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

An azide functionalized oligothiophene ligand – A versatile tool for multimodal detection of disease associated protein aggregates

Leif B.G. Johansson [a], Rozalyn Simon [a,1], Gunnar Bergström [a,1], Mikaela Eriksson [a], Stefan Prokop [b], Carl-Fredrik Mandenius [a], Frank L. Heppner [b], Andreas K.O. Åslund [a,2], K. Peter R. Nilsson [a,*]

[a] Department of Physics, Chemistry and Biology (IFM), Linköping University, Linköping SE-581 83, Sweden
[b] Department of Neuropathology, Charité-Universitätsmedizin Berlin, Charitéplatz 1, Berlin 10117, Germany

ARTICLE INFO

Article history:
Received 16 May 2014
Received in revised form
3 July 2014
Accepted 6 July 2014
Available online 24 July 2014

Keywords:
Protein aggregates
Oligothiophene
Fluorescence
Surface plasmon resonance
Click chemistry

ABSTRACT

Ligands for identifying protein aggregates are of great interest as such deposits are the pathological hallmark of a wide range of severe diseases including Alzheimer's and Parkinson's disease. Here we report the synthesis of an azide functionalized fluorescent pentameric oligothiophene that can be utilized as a ligand for multimodal detection of disease-associated protein aggregates. The azide functionalization allows for attachment of the ligand to a surface by conventional click chemistry without disturbing selective interaction with protein aggregates and the oligothiophene–aggregate interaction can be detected by fluorescence or surface plasmon resonance. In addition, a methodology where the oligothiophene ligand is employed as a capturing molecule selective for aggregated proteins in combination with an antibody detecting a distinct peptide/protein is also presented. We foresee that this methodology will offer the possibility to create a variety of multiplex sensing systems for sensitive and selective detection of protein aggregates, the pathological hallmarks of several neurodegenerative diseases.

1. Introduction

Ligands for visualization, identification and characterization of protein aggregates are of great interest as such deposits are associated with many devastating diseases (Sipe et al., 2012). These protein deposits, termed “amyloid” or inclusion bodies, result from the misfolding and/or partial unfolding of proteins, which results in the formation of protein aggregates. From a biophysical perspective, amyloid deposits consist of fibrils with a diameter around 10 nm and structural studies of amyloid have shown that the protein or peptide molecules are arranged so that the polypeptide chain forms β-strands that run perpendicular to the axis of the fibril (Petkova et al., 2002; Ritter et al., 2005; Makin et al., 2005; Nelson et al., 2005). Since most amyloid fibrils have extensive cross β-pleated sheet conformation and sufficient structural regularity, small hydrophobic amyloid ligands targeting this core structure have been developed. The most common ligands for classification of protein aggregates in tissue or identification of *in vitro* formed amyloid fibrils are derivatives of Congo red or Thioflavins (Bennhold, 1922; Divry, 1927; Naiki et al., 1989; LeVine, 1993). In addition, other chemical scaffolds, such as stilbene derivatives (Kung et al., 2001; Ono et al., 2005), vinylbenzoxazole derivatives (Shimadzu et al., 2004; Kudo et al., 2007) and molecular frameworks, including derivatives of fluorene, thiophene, biphenyl thiophene, and biphenyltriene (Furumoto et al., 2007; Nesterov et al., 2005; Raymond et al., 2008), have also been utilized for the development of amyloid-specific ligands.

Lately, chemically-defined molecular scaffolds consisting of repetitive thiophene moieties, denoted luminescent conjugated oligothiophenes (LCOs), have proven to be a remarkable class of fluorescent molecules for investigating protein deposits associated with protein aggregation diseases, such as Alzheimer’s disease (AD) and Parkinson's disease (PD), as well as the infectious prion diseases (Åslund et al., 2009; Lord et al., 2011; Klingstedt et al., 2011, 2013; Nyström et al., 2013). In comparison with conventional ligands, LCOs identify a broader sub-set of disease-associated protein deposits and heterogenic populations of protein aggregates could also be distinguished due to distinct spectroscopic signatures from these dyes. Hence, LCO can be utilized as fluorescent ligands for sensitive detection and spectral assignment of a plethora of disease-associated protein deposits.

* Corresponding author. Tel.: +46 13 28 27 87.
E-mail address: petni@ifm.liu.se (K.P.R. Nilsson).
[1] These authors contributed equally to this work.
[2] Present address: The Norwegian University of Science and Technology, 7491 Trondheim, Norway.

http://dx.doi.org/10.1016/j.bios.2014.07.042

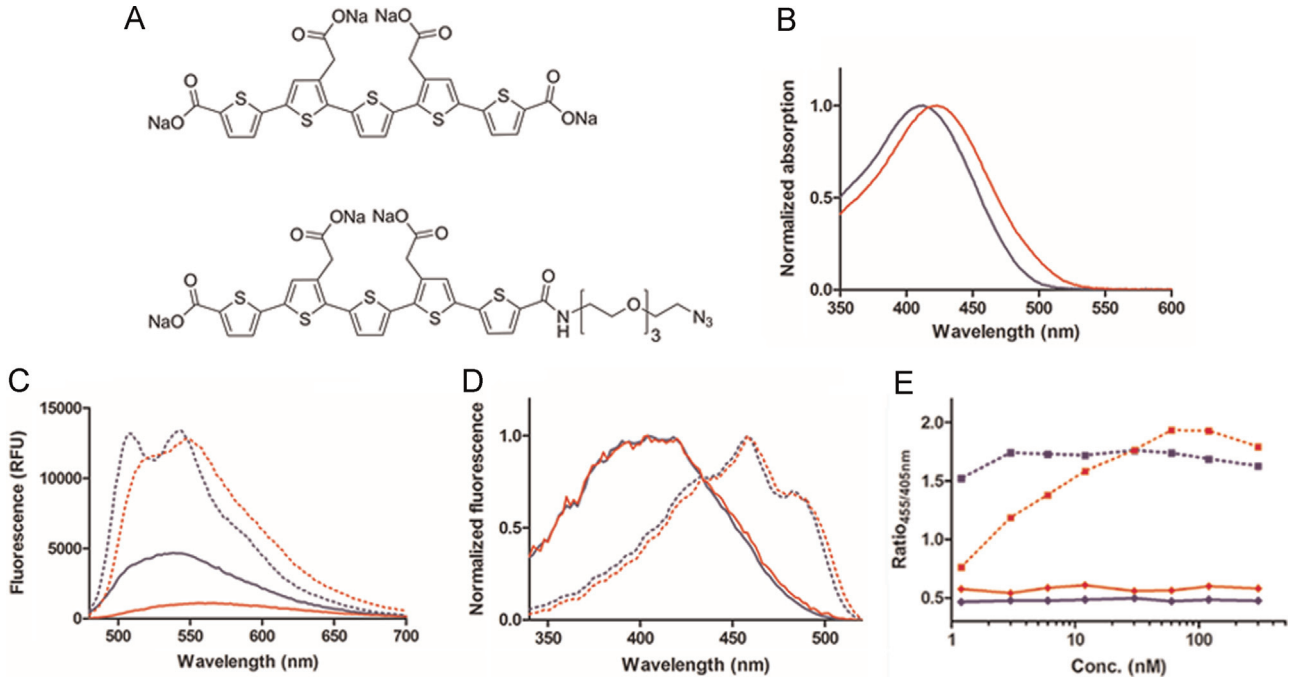

Fig. 1. Chemical structures and optical properties of the oligothiophene ligands. A) Chemical structures of p-FTAA (top) and p-FTAA-azide (bottom). B) Absorption spectra of 3 μM p-FTAA (blue) or 3 μM p-FTAA-azide (red) in PBS. C) Emission spectra of 300 nM p-FTAA (blue) or 300 nM p-FTAA-azide (red) in PBS (lines) or mixed with 10 μM recombinant Aβ amyloid fibrils in PBS (dotted lines). D) Excitation spectra of 300 nM p-FTAA (blue) or 300 nM p-FTAA-azide (red) in PBS (solid lines) or mixed with 10 μM recombinant Aβ amyloid fibrils in PBS (dotted lines). Emission at 515 nm. E) Binding curve showing the ratio, Ratio455/405 nm, of the intensity of the emitted light at the respective excitation maximum, 455 nm (bound dye) and 405 nm (unbound dye) against different dye concentration. Different concentrations of p-FTAA (blue) or p-FTAA-azide (red) in PBS (lines) or mixed with 10 μM recombinant Aβ amyloid fibrils in PBS (dotted lines).

So far, the intrinsic fluorescence of LCOs has been utilized to identify protein aggregates and it would be of great interest to modify this class of compounds for multimodal detection of protein aggregates. Properly functionalized LCOs that can be covalently attached to a surface will also allow the development of versatile LCO-based sensing systems for a variety of protein aggregates. Recently, we introduced a pentameric oligothiophene with a porphyrin moiety linked to the thiophene backbone via a tetra ethylene glycol spacer and this hybrid molecule allowed for improved fluorescent assessment of heterogeneous amyloid morphologies compared to the corresponding oligothiophene dye (Arja et al., 2013). Herein, we introduce an azide-functionalized pentameric oligothiophene ligand that can be utilized for copper-free click chemistry (Agard et al., 2004; Ning et al., 2008) and evaluate the potential of utilizing this molecule as a multimodal ligand towards protein aggregates. The azide-functionalized oligothiophene ligand, p-FTAA-azide (Fig. 1A), exhibited an excellent specificity towards recombinant Aβ (M1-42) amyloid fibrils and Aβ-deposits in tissue sections from transgenic mice with AD pathology. In addition, the azide functionality could be utilized for attaching the molecule to a solid support without losing the amyloid specificity and p-FTAA-azide could be implemented as a tool for detecting protein aggregates by surface plasmon resonance (SPR).

2. Materials and methods

2.1. Synthesis of p-FTAA and p-FTAA-azide

The synthesis of p-FTAA has been reported previously (Åslund et al., 2009) and the synthesis of p-FTAA-azide is described below and in Scheme 1.

2.2. General methods

NMR-spectra were recorded on a Varian 300 MHz instrument, using $CDCl_3$ as solvent. Chemical shifts were assigned with the solvent residual peak as a reference (Gottlieb et al., 1997). TLC was carried out on Merck pre-coated 60 F254 plates using UV-light (λ=254 nm and 366 nm) and charring with ethanol/sulfuric acid/p-anisaldehyde/acetic acid 90:3:2:1 for visualization. Flash column chromatography (FC) was performed using silica gel 60 (0.040–0.063 mm, Merck). Organic phases were dried over anhydrous magnesium sulfate. Solvents were evaporated with a rotary evaporator under reduced pressure (1–2 kPa) at a water bath temperature of 40 °C. HPLC-MS was performed on a Gilson system (Column: Waters X-Bridge C-18 or C-8 5 μ, 250 × 15 mm^2 and Waters X-Bridge C-18 or C-8 2.5 μ, 150 × 4.6 mm^2 for semipreparative and analytical runs respectively; Pump: Gilson gradient pump 322; UV/vis-detector: Gilson 155; MS detector: Thermo Finnigan Surveyor MSQ; Gilson Fraction Collector FC204) using acetonitrile with 0.05% ammonium acetate and deionized water with 0.05% ammonium acetate as mobile phase. MALDI-TOF MS was recorded on a Voyager-DESTR Biochemistry workstation.

2.3. Synthesis of 1

Compound **1** was synthesized as described previously (Chen and Baker, 1999). PEG-Diol (8.9 mL, 51.3 mmol) and p-toluenesulfonyl chloride (29.45 g, 154.5 mmol) were dissolved in THF (60 mL) and stirred for 5 min at 0 °C, then a solution of KOH (18.94 g, 337 mmol) dissolved in H_2O (21 mL) was added drop wise over 20 min. The ice bath was removed and the reaction was stirred overnight. After 16 h ice cold water (50 mL) was added, and the aqueous layer extracted with Et_2O (3* 50 mL). The collected organic phases were washed with Brine (2* 50 mL) dried over $MgSO_4$, filtered and evaporated to give **1** as clear oil. (23.81 g, 92%).