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Cyanine dye–protein interactions: Looking for fluorescent probes for amyloid structures

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Abstract

We ascertained the ability to detect fibrillar β-lactoglobulin (BLG) of a series of mono-, tri-, penta-, and heptamethinecyanines based on benzothiazole and benzimidazole heterocycles, and of benzothiazole squaraine. Fluorescence properties of these cyanine dyes were measured in the unbound state and in the presence of monomeric and fibrillar BLG and compared with those for the commercially available benzothiazole dye Thioflavin T. The correlation between the chemical nature of the dye molecules and the ability of dyes to bind aggregated proteins was established. We found that meso-substituted cyanines with amino substituents in heterocycle in contrast to the corresponding unsubstituted dyes have a binding preference to fibrillar BLG and a noticeable fluorescence response in the presence of the aggregated protein. For the squaraines and benzimidazole penthamethinecyanines studied, fluorescence emission increased both in the presence of native and fibrillar protein. The trimethinecyanines T-49 and SH-516 exhibit specifically increased fluorescence in the presence of fibrillar BLG. These dyes demonstrated the same or higher emission intensity and selectivity to aggregated BLG as Thioflavin T, and are proposed for application in selective fluorescent detection of aggregated proteins.

Keywords: Cyanine dyes; Amyloid proteins; Fluorescent detection

1. Introduction

The deposition of insoluble protein aggregates known as amyloid fibrils in different tissues and organs is associated with a number of neurodegenerative diseases, including Alzheimer's and Parkinson's diseases, senile systemic amyloidosis and spongiform encephalopathies [1–4]. Fibrillar deposits with characteristics of amyloid are also formed by several other proteins unrelated to disease, including the whey protein beta-lactoglobulin (BLG). Despite the lack of amino acid sequence homology among the amyloidogenic proteins, the common morphology of amyloid fibrils was revealed using X-ray fiber diffraction analysis and electron microscopy. In particular, the fibrils usually have a diameter ranging from 5 to 20 nm [5–7], a cross-β structure consisting of a core of β-strands [8], and an affinity to bind a range of dyes such as Thioflavin T [9,10] and Congo Red [11,12]. In the past, fluorescent dyes were used to stain amyloidogenic material in histology, while insights into the prerequisites and kinetics of amyloid formation were obtained by the *in vitro* analysis of this process using similar dyes.

The fluorescent molecules Thioflavin T and Congo Red are the most frequently used dyes to detect the presence of amyloid deposits [13,14]. Both the benzothiazole dye Thioflavin T and the symmetrical sulfonated azo dye Congo Red have been adapted to study the formation of amyloid fibrils in solution using the fluorescence properties of these molecules [15]. Besides these dyes very few new compounds have been used

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extensively to detect amyloid inclusions. Most of these new dyes, including Chrysamine-G [16], X-34 [17] and K114 [18], are derived from the structure of Congo Red.

Fibril detection assays have revealed several drawbacks of Thioflavin T, Congo Red and their derivatives. The binding of dyes can influence the stability of amyloid aggregates, and the interplay with other components (for example, during testing of potential amyloid inhibitors) is unpredictable. Additionally there exists a great variability among the different amyloids in the binding of Congo Red and Thioflavin T [19]. Another drawback of the Thioflavin T assay is that it is not always quantitative because Thioflavin T fluorescence can vary depending on the structure and morphology of the fibrils [19]. Furthermore, it has been shown by Khurana et al. that Congo Red can bind to native α-proteins such as citrate synthase and interleukin-2 [20]. As a consequence of its poor optical properties, the Congo Red derivative Chrysamine-G only weakly stains neuritic plaques and cerebrovascular amyloid in postmortem tissue [21]. Thus the design of new dyes which can selectively interact with fibrillar amyloidogenic proteins is of substantial importance for basic research, and has a crucial practical significance for biotechnology and medicine.

In this paper we report the ability of a series of mono-, tri-, penta-, and heptamethinecyanines based on benzothiazole and benzimidazole heterocycle end groups, as well as squaraine based on benzothiazole end groups (Fig. 1) to selectively recognize fibrillar β-lactoglobulin (BLG). We present fluorescence properties of these cyanine dyes in the unbound state and in the presence of native and fibrillar BLG and compare these properties with those for the commercially available benzothiazole dye Thioflavin T. The correlation between chemical nature of the dye molecules and the ability of dye to interact selectively with aggregated proteins is presented.

2. Materials and methods

2.1. Reagents

10 mM HEPES buffer (pH 7.4) and 10 mM HCl, containing 0.02% NaN_3 were used as solvents. HEPES and native beta-lactoglobulin were purchased from “Sigma” (USA). The L-34, T-49 and SH-441 dyes were synthesized as described in [22–24]. Cyanine dyes Sl-1964 and T-207 were synthesized according to [25] and [26] respectively. The L-43 and T-284 dyes were obtained as described in [27]. The Sl-663 cyanine dye was synthesized as described in [24]. Squaraine Sl-2751 and cyanine dye Sl-899 were obtained according to [28]. The SH-516, SH-1082 and 2904y dyes were kindly provided by Dr. A. Bogolyubskyi (ENAMINE, Kyiv, Ukraine).

2.2. Preparation and fibrillogenesis of BLG

Monomeric and fibrillar BLG samples were prepared according to published protocols [29]. Briefly, BLG was dissolved in deionized water and the pH was adjusted to 2.0 by addition of small amounts of 1 M HCl. The solution was

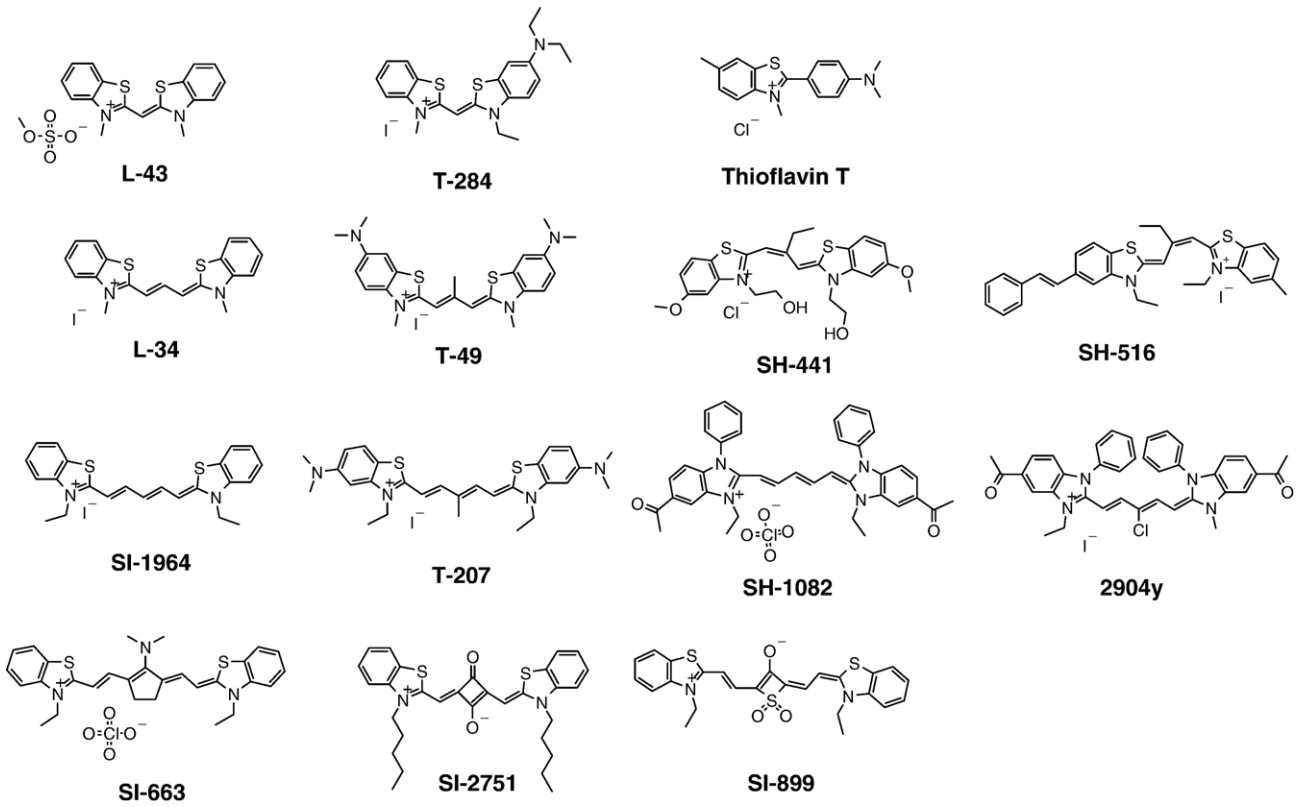

Fig. 1. Structures of studied dyes.