FEBS 30142 FEBS Letters 579 (2005) 6601–6603

Chiral bias of amyloid fibrils revealed by the twisted conformation of Thioflavin T: An induced circular dichroism/DFT study

Wojciech Dzwolak[a,*], Magdalena Pecul[b]

[a] *Institute of High Pressure Physics, Polish Academy of Sciences, Sokolowska 29/37, 01-142 Warsaw, Poland*
[b] *Department of Chemistry, Warsaw University, Pasteura 1, 02-093 Warsaw, Poland*

Received 5 October 2005; accepted 25 October 2005

Available online 9 November 2005

Edited by Jesus Avila

Abstract **Since it was implicated in a number of neurodegenerative conditions, such as Alzheimer disease, formation of β-sheet-rich protein fibrils (amyloids) has been drawing a lot of attention. One of elusive aspects of amyloidogenesis concerns the mechanisms of specific binding of molecules such as Congo red, or Thioflavin T by amyloid fibrils. A comprehensive understanding of these docking interactions is needed, however, for the sake of furthering biochemical studies and developing molecular, pharmacological strategies preventing proliferation of amyloids in vivo. Through the application of circular dichroism, here we show that upon binding to insulin fibrils, a twisted conformation is enforced in molecules of Thioflavin T, manifested in a strong negative Cotton effect around 450 nm, which is supported by density functional theory–based calculations. This finding may lead to circular dichroism of Thioflavin T becoming a new diagnostic technique for protein fibrils, complementary to fluorescence spectroscopy.**

Keywords: Amyloid; Handness; Aggregation; Ab initio calculations; Stains

1. Introduction

Thioflavin T (ThT) is an optically-inactive fluorophore possessing high affinity toward amyloid fibrils [1,2]. A ThT molecule consists of a pair of benzothiazole and benzaminic rings freely rotating around a shared C–C bond. Should the free rotation of ThT be hindered, e.g., by viscosity, the molecule exhibits strong fluorescence at 482 nm, when excited at 450 nm [3]. This is particularly the case of ThT-amyloid complexes, wherein the rotation is effectively blocked and quantum yield of the fluorescence is at its highest [2,3]. While studies employing ThT fluorescence have become routine, conformation and optical properties of the amyloid-trapped fluorophore are unexplored. In this study, we used insulin fibrils as a model amyloid scaffold and a target for ThT.

2. Experimental

The fibrils were prepared by vortexing (60 °C, 750 rpm) 1 mM bovine insulin (from Sigma, USA) in 0.1 M NaCl, pH 1.8, in the presence of varying concentrations of ThT. After 40 h, fluorescence, UV-absorption and CD-measurements of mature aggregates followed. The fluorescence spectra were collected at room temperature on AMINCO Bowman Series 2 luminescence spectrometer (excitation at 450 nm, 3 repetitions, 10 mm cuvette), whereas circular dichroism (CD) spectra were acquired with a Jasco 715 CD System equipped with 1 mm pathlength cuvettes.

For calculations of ECD spectra of the resulting structures, B3LYP functional and 6-311++G** basis set were used (Gaussian 03 program package [4]). The theory underlying ECD calculations has been described elsewhere [5].

3. Results and discussion

The VIS-CD spectra in Fig. 1A reveal a previously unknown feature of amyloid-bound ThT: a profoundly negative Cotton effect centered around 450 nm, which coincides with UV absorption maximum of the complex [3]. In the studied range of ThT:fibrils (per monomers) molar ratios (1:200 to 1:1), the extrinsic CD signal grows linearly with the ThT share. Even in the most diluted ThT samples (1:100 and 1:200), a proportional CD is detectable. The fluorescence intensity increases proportionally at the low, but levers off at the higher dye's concentration (Fig. 1B). This is paralleled by an initially single, then split absorption band, as seen in the second derivative UV-absorption spectra (inset in Fig. 1B). Fig. 2 quantifies ellipticity and fluorescence intensity of ThT-insulin amyloid samples.

An induced optical activity in an otherwise achiral system, such as ThT, may either result from enforcing a chiral conformation in single molecules of the dye, or, from a chiral assembly of achiral molecules. In the latter scenario, proximity and proper orientation of interacting chromophores are crucial for a measurable ECD signal to occur. The presented data show that "dilution" of ThT and subsequent spatial separation of the dye molecules has no significant effect on CD signal. The linearly increasing (with ThT concentration) "ellipticity [deg]" (Fig. 2) follows from the fact that "molar ellipticity [deg cm^2 $dmol^{-1}$]" and therefore rotationary strength of the amyloid-bound dye molecules is constant. This observation may be only reconciled with a twisted, optically-active conformation assumed by ThT molecules upon binding to the fibrils.

Interestingly, the wide linear range of the CD vs. [ThT], compared to the fluorescence intensity plot (Fig. 2) and UV-absorption data (Fig. 1B, inset) implies a large number of docking spots for the twisted ThT molecules. At the high concentration, incoming ThT molecules, while still binding to fibrils are likely to interact, which may quench fluorescence (Fig. 2) and affect the UV absorption spectrum. Strong absorption of concentrated ThT may per se decrease fluorescence intensity.

*Corresponding author. Fax: +48 22 632 42 18.
E-mail address: wdzwolak@unipress.waw.pl (W. Dzwolak).

0014-5793/$30.00
doi:10.1016/j.febslet.2005.10.048

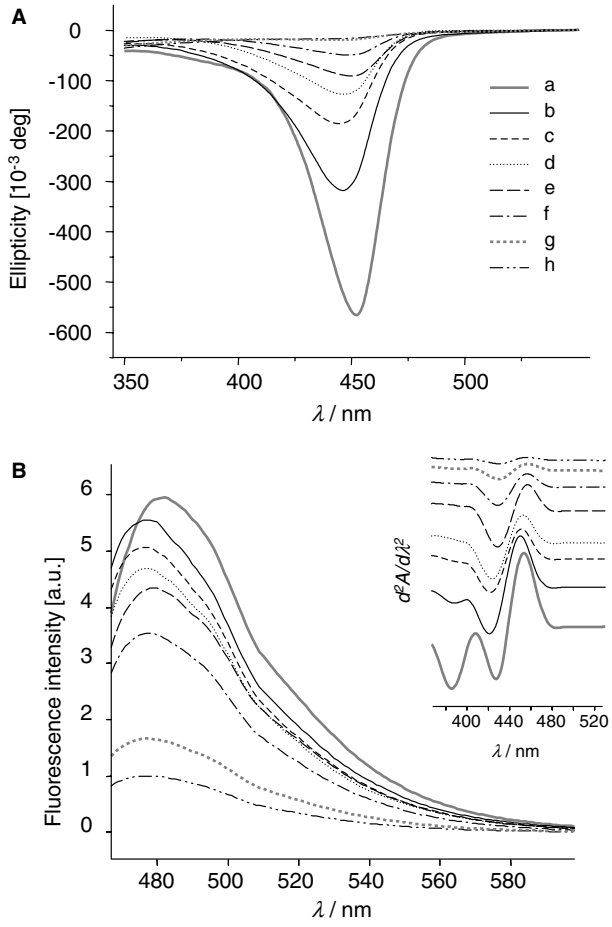

Fig. 1. Circular dichroism (A), fluorescence emission $\lambda_{excit} = 450$ nm (B), and second derivative UV-absorption spectra (B-inset) of insulin fibrils doped with ThT at various ThT:amyloid molar ratios – 1:1 (a), 1:2 (b), 1:5 (c), 1:10 (d), 1:20 (e), 1:50 (f), 1:100 (g), 1:200 (h); at 25 °C, in 0.1 M NaCl, pH 1.8. For CD and UV-absorption samples with uniform 1 mM insulin in 1 mm cuvettes were used. For fluorescence measurements, samples were diluted 11-times with 0.1 M NaCl, pH 1.8. Control experiments with native insulin showed no CD signals of ThT.

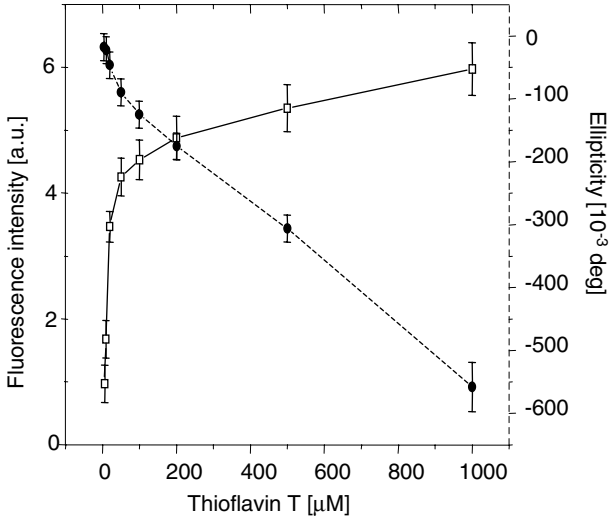

Fig. 2. Dependence of ellipticity (filled circles) and fluorescence intensity at 482 nm (empty squares, $\lambda_{excit} = 450$ nm) of 1 mM insulin amyloid (per monomer) on ThT concentration.

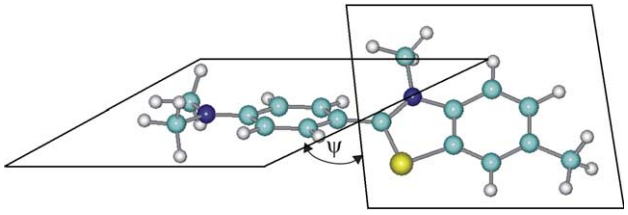

Fig. 3. A twisted, optically-active conformer of Thioflavin T binds to insulin amyloid giving rise to the strong negative Cotton effect. According to the DFT data, the dihedral angle ψ between the planes of the benzothiazole and benzaminic rings is +34.6° for an isolated molecule.

Seeking to determine the particular "twisted" conformation binding to fibrils with the negative Cotton effect, geometry of an isolated ThT cation has been optimized using density functional theory (DFT) with B3LYP exchange-correlation functional and the 6-311G** basis set. The structure resulting from the DFT calculations has a dihedral angle ψ (see Fig. 3) of 34.6°, and it is separated from its mirror image by a barrier of 13.2 kJ/mol (the "ambient" flat conformation is a transition state between the two minima). This low barrier explains the lack of optical activity in free ThT. As the conversion between the two enantiomeric minima is very rapid, the system is, on the average, of C_s symmetry. If a chiral environment (such as right-handed amyloid fibrils) traps ThT molecules, the conversion is hindered, and the sterically-favored chiral conformer prevails. According to the theoretical calculations, the structures with positive dihedral angle ψ exhibit strong negative circular dichroism for the HOMO–LUMO transition. Therefore, we propose that the experimentally observed negative extrinsic circular dichroism of the amyloid-bound ThT may originate from the dye acquiring a twisted conformation, like the one shown in Fig. 3.

According to Krebs et al. [6], flat ThT molecules bind amyloids in a parallel alignment to the fibril axis. Our study suggests that the locking of the dye molecule in the matrix of perpendicular β-strands and side-chain residues accommodates a significantly twisted, rather than flat molecule. Extrinsic CD of small molecules binding to proteins is not exceptional (e.g. [7,8]). Showing this effect for ThT, a selective ligand for amyloid fibrils, is advantageous, because chiral bias, or more precisely: handness (fibrils' supramolecular twist) of fibrils can be rationalized to play a key role in determining the sign of the induced CD. In this context, interactions of Congo red with amyloids should be also revisited [8,9]. The ubiquitous left-handness of amyloids can be explained as a hierarchical consequence of the left-handness of L-amino acids in biological proteins, from which fibrils are formed [10]. Because insulin fibrils are no exception in this regard, and alternative "right-handed" protein enantiomers do not exist in nature, we used a pair of fibrillogenic [11] sequenceless polypeptides – poly(L-lysine) and poly(D-lysine) to show that binding to ThT actually induces: a negative and a positive Cotton effect, respectively (Supplementary Information).

4. Conclusion

Upon binding to Thioflavin T, insulin amyloid induces a twisted conformation in the dye, likely to follow from the chiral

bias of the fibrils, and therefore adding a complementary to fluorescence spectroscopy diagnostic approach to protein fibrils.

Acknowledgments: W.D. acknowledges support from 2P04A 011 28(2005–2007). M.P. acknowledges support from 120000-501/68-BW-1681/12/05.

Appendix A. Supplementary data

CD spectra of ThT with poly(L/D-lysine) fibrils, output of the DFT calculations. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2005.10.048.

References

[1] Naiki, H., Higuchi, K., Hosokawa, M. and Takeda, T. (1989) Fluorometric determination of amyloid fibrils in vitro using the fluorescent dye, thioflavin T1. Anal. Biochem. 177, 244–249.
[2] LeVine, H. (1999) Quantification of beta-sheet amyloid fibril structures with thioflavin T. Meth. Enzymol. 309, 274–284.
[3] Voropai, E.S., Samtsov, M.P., Kaplevskii, K.N., Maskevich, A.A., Stepuro, V.I., Povarova, O.I., Kuznetsova, I.M., Toroverov, K.K., Fink, A.L. and Uverskii, V.N. (2003) Spectral properties of Thioflavin T and its complexes with amyloid fibrils. J. Appl. Spectrosc. 70, 868–874.
[4] Frisch, M.J. et al. (2004) Gaussian 03, Revision B.04, p. 2004, Gaussian Inc., Wallingford, CT.
[5] Pecul, M., Ruud, K. and Helgaker, T. (2004) Density functional theory calculation of electronic circular dichroism using London orbitals. Chem. Phys. Lett. 388, 110–119.
[6] Krebs, M.R.H., Bromley, E.H.C. and Donald, A.M. (2002) The binding of thioflavin-T to amyloid fibrils: localisation and implications. J. Struct. Biol. 149, 30–37.
[7] Lighter, D.A., Wijekoon, W.M.D. and Zhang, M.H. (1988) Understanding bilirubin conformation and binding. J. Biol. Chem. 263, 16669–16676.
[8] Benditt, E.P., Eriksen, N. and Berglund, C. (1970) Congo red dichroism with dispersed amyloid fibrils, an extrinsic Cotton effect. Proc. Natl. Acad. Sci. USA 66, 1044–1051.
[9] Khurana, R., Uversky, V.N., Nielsen, L. and Fink, A.L. (2001) Is Congo red an amyloid-specific dye?. J. Biol. Chem. 276, 22715–22721.
[10] Jimenez, J.L., Nettleton, E.J., Bouchard, M., Robinson, C.V., Dobson, C.M. and Saibil, H.R. (2002) The protofilament structure of insulin amyloid fibrils. Proc. Natl. Acad. Sci. USA 99, 9196–9201.
[11] Fandrich, M. and Dobson, C.M. (2002) The behaviour of polyamino acids reveals an inverse side chain effect in amyloid structure formation. EMBO J. 21, 5682–5690.