PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY

Chemistry and Biology 2015, № 3, p. 39–46

Biology

PLANT PREPARATIONS SUPPRESS THE AGGREGATION OF AMYLOID BETA PEPTIDES AND PROMOTE THEIR DISAGGREGATION

S. G. SHAROYAN[1], A. A. ANTONYAN [1], H. A. HARUTYUNYAN [1,2], S. S. MARDANYAN [1*]

[1] *H.Ch. Buniatyan Institute of Biochemistry, NAS of Armenia*
[2] *Yerevan State Medical University After M. Heratsi, Armenia*

Fibrillation of amyloid Aβ (1-42) and Aβ (1-40) peptides (Aβs) is considered as one of causes of Alzheimer's disease. In the present work we studied the influence of some plant preparations on the aggregation state of these peptides, evaluated by fluorescence of thioflavin T-containing samples. The abilities of some preparations to inhibit the self-aggregation of Aβs and to disaggregate their preformed aggregates were demonstrated. For somecases, IC_{50} values in these processes were evaluated. Based on the obtained data we recommend: a) frequent use of these plants by persons at risk group; b) clinical evaluating their extracts as sources of neurodegeneration preventing agents.

Keywords: Aβ (1-40) and Aβ (1-42) peptides, aggregation, disaggregation, plant extracts, plant constituents.

Introduction. Alzheimer's disease (AD) is the most common form of dementia, accounting for 80% of all dementia in elderly patients [1, 2]. Worldwide, the prevalence of dementia was more than 35 mln in 2010, and expected to exceed 65 mln by 2030 and 115 mln by 2050. No drugs preventing or slowing the progression of AD are currently approved. The development of effective AD therapeutics is a medical challenge and should be one of top medical priorities of society. A characteristic pathologic feature in the brain of patients with AD is diffusely distributed on the surface of cells depositions of fibrillar structures and neurofibrillar coils in the cells.

The accumulation of Aβ (1-40) and Aβ (1-42) peptides (Aβs) is shown as main reason of fibrillation and neurotoxicity in AD [3]. The therapeutic approaches usually have targeted reducing the levels of Aβ monomers and/or their deposits in the brain. Soluble Aβ oligomers also constitute an immunotherapeutic target [4]. Some differences were noted concerning the fibrillation and toxicity of these two amyloid peptides. Although Aβ (1-42) is produced *in vivo* at about 10-fold lower levels than Aβ (1-40), senile plaque in AD brains is composed primarily of Aβ (1-42). One of the reasons might be the increase of Aβ (1-42) production relative to Aβ (1-40) as a result of AD causing mutations [5]. However, it was also observed that *in vitro* Aβ (1-42) forms fibrils more rapidly, than Aβ (1-40). The biophysical

[*] E-mail: biochem@ipia.sci.am

studies revealed that the specific properties of residues Ile 41 and Ala 42 cause Aβ (1-42) to aggregate more readily than Aβ (1-40) but not the length *per se* [6]. The environment, in which Aβ aggregation occurs *in vivo* is complex, and the question is how biophysical parameters influence this process and affect the distribution of toxic and non-toxic Aβ conformations. Nonetheless, the analysis of dynamic features of aggregation in simplified and controlled conditions *in vitro* can contribute to the knowledge on this process *in vivo*.

One of the main approaches in the treatment of AD includes inhibiting the aggregation of Aβs and increasing the decay and destruction of the formed aggregates. The bioactive compounds extracted from the medical plants, showing neuroprotective properties, have become of interest in brain neurodegeneration [7]. Below are some examples. Methyl caffeate present in different plants and human diet has been identified as a good ligand of Aβ (1-42) oligomers showing anti-amyloidogenic activity [8]. *Vitis vinifera* grape vine shoots appeared potentially interesting as a source of new bioactive stilbenes exerting a significant activity against amyloid-β aggregation [9]. 2 flavonoids from the roots of *Iris tenuifolia* inhibited Aβ aggregation and promoted neural stem cells proliferation [10]. Radix Notoginseng flavonol glycoside (RNFG), quercetin 3-O-beta-D-xylopyranosyl-beta--D-galactopyranoside from roots of Panax notoginseng possessed a strong activity in preventing Aβ-induced cell death [11].

It is worth to remind, that Armenia has a rich history in the field of phytotherapy. Some medical plants have been disseminated from here throughout Europe since the V century BC. Herbal medicine developed thanks to medieval Armenian scholars Mkhitar Heratsi, Amirdovlat Amasiatsi, Grigor Magistros and many others [12, 13]. Several edible plants widespread in Armenia are applied in folk medicine. The extracts and constituents of various plants show different pharmacological effects and are used in traditional and current medicine for treatment of various health disorders. Having this in mind, we directed our attention to the study of possible medical properties of some Armenian plants, neuroprotective properties of which are not yet known. Recently [14–16], we described the *in vitro* inhibition of aggregation and disaggregation by several plant preparations (PPs) of amylin, a 37-residue pancreatic islet peptide hormone, amyloidosis of which concern the development of another amyloid disease, type 2 diabetes mellitus [17].

Earlier we have shown the truncation of Aβ (1-40) and Aβ (1-42) peptides and slowing their aggregation/fibrillation by dipeptidyl peptidase IV [18, 19]. In the present work the results of our *in vitro* study of the influence of some plant extracts and their fractions on the aggregation of these peptides are presented. The ability of PPs to disaggregate the preformed aggregates of the peptides was observed also.

Materials and Methods.

Reagents and Equipment. Thioflavin T (ThT) was purchased from "Sigma Ltd" (USA); Aβs were purchased from "China peptide" (China) and gifted by Prof. H-U. Demuth (Germany). All other chemicals were of the highest purity. Spectral measurements were performed on spectrophotometer Specord M-40 (Germany) and spectrofluorometer Perkin-Elmer MPF-44A (USA).

The plants were collected, dried, extracted, fractionated and characterized by thin layer chromatography, spectral and chemical analyses as we described earlier [20].

Peptides Preparation. To prepare stock solution of Aβ (1-40), 0.8 *mL* of bidistilled water was added to 1 *mg* peptide and centrifuged. Because of low

solubility of Aβ (1-42), it was preliminary dissolved in 0.1 *M* NaOH, immediately neutralized with dilute HCl and 20 *mM* phosphate buffer, pH 7.4, and centrifuged. The absorption spectra of the solutions were recorded and the molar concentrations of the peptides were evaluated using the extinction coefficient of tyrosine at 276 *nm*, 1.39 $mM^{-1}cm^{-1}$. The aggregates of Aβs were formed by incubating the proteins for 7 days at 37°*C* in 40 *mM* phosphate buffer, pH 7.4, containing 0.02% Na azide (w/v).To study the effect of a herbal preparation, it was added into the incubation medium at a concentration of 2.5–25.0 *μg*/*mL*. The disaggregation of preliminary aggregated peptides was researched upon their incubation for 3 days at 37°*C* in the presence of PPs at the same conditions.

Determination of Aggregation State of the Peptides. ThT staining was used as described elsewhere [18, 19, 21]. The fluorescence assay mixture in 290 *mL* of 10 *mM* phosphate buffer, pH 7.4, contained 0.14 *M* KCl, 8.6 *μM* ThT and 8 *μM* of the pre-incubated peptide. The fluorescence was measured at λ_{ex}=430 *nm* and λ_{em}=485 *nm* (430/485). The excitation and emission wavelength slits were 10 *nm*. The aggregation state of a peptide sample in the presence of PPs was evaluated as the intensity of its fluorescence expressed as percentage of the fluorescence of control sample without PPs.

Statistical Analysis. The calculation of IC_{50} values was done using GraFit Version 5 software (Leatherbarrow, 2001, Erithacus Software Ltd., Horley, U.K). The data, obtained at least in three independent experiments, were analyzed using the software InStat, version 3 for Windows (GraphPad Software, Inc., San Diego, CA, USA). The results were expressed as means ± stand. error.

Results and Discussion.

Inhibition of Aβ (1-42) Peptide Aggregation by Plant Preparations. The ability of plant extracts and their fractions to influence on the *in vitro* aggregation of amyloid Aβ (1-42) peptide were evaluated. In these experiments, the identical solutions of Aβ (1-42) in concentration of 100 *μM* were incubated for 7 days at 37°*C* in the presence and absence of PPs. The aliquots of these solutions were added to the ThT-containing mixture, as described in Materials and Methods, and 430/485 fluorescence was registered.

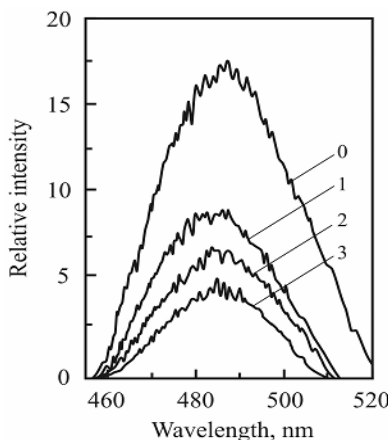

Fig. 1. ThT fluorescence spectra of Aβ (1-42) samples after incubation: without any additions, control sample (0), and in the presence of 25 *μg/mL* ethanol extracts of SL (1), GL (2); coumarin fraction of SL (3). The spectra were registered at λ_{ex} = 430 *nm* and $\lambda_{em.}$ = 485 *nm*.

In Fig. 1 typical spectra of fluorescence of ThT complexes with Aβ (1-42), incubated in the absence (control, curve 0) and in the presence of three herbal preparations are shown. The decrease of ThT fluorescence intensities of peptide samples incubated in the presence of PPs compared with the intensity of control evidences that these preparations inhibited the aggregation of the peptide. The emission intensities show, that the extracts of sorrel leaves (SL, curve 1) and grape leaves (GL, curve 2) inhibited the formation of the peptide aggregation by 50 and 60% respectively. The coumarin of sorrel (Cm, curve 3) suppressed the peptide aggregation by 75%.

In the following study the aggregation state of the peptide sample in the presence of PPs is shown by fluorescence intensity of its ThT complex expressed as percentage of the fluorescence of control sample. Unfortunately, like the case of our investigations on the amylin aggregation [15], we failed to use more PP and wider interval of concentrations in characterizing of their influence on the amyloidosis of Aβs. This study was strongly hindered by two features of PPs: 1) some of them were provided with their own fluorescence, interfering ThT 430/485 fluorescence; 2) several PPs quenched the ThT complex fluorescence. Both these circumstances limited the application of the well-known ThT fluorescence technique in estimation of the peptides aggregation state in the presence of PPs. Therefore, preliminary the PPs under study were screened for their own fluorescence and for quenching the ThT-fluorescence. Then, in the following study, the weakly affecting preparations were used under preferentially low concentrations.

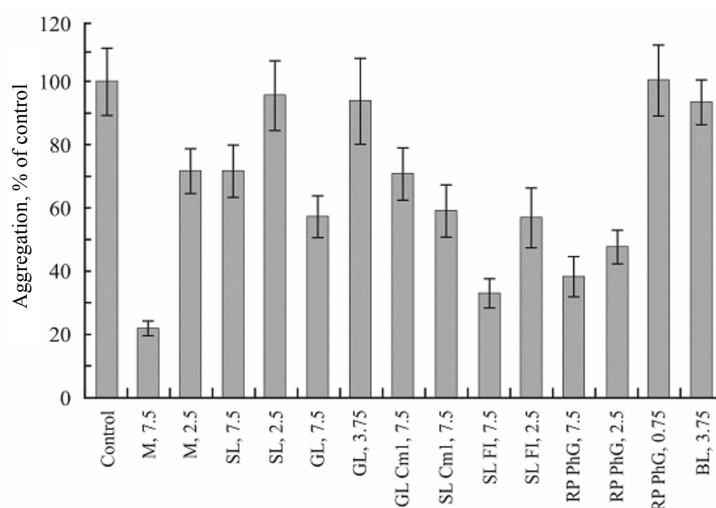

Fig. 2. Aggregation state of 30 *μM* Aβ (1-42) incubated in the absence (control) and in presence of PPs. The concentrations of PPs are indicated in *μg/mL*.

In Fig. 2 the aggregation states of Aβ (1-42) samples are shown after 7-day incubation, as described in the Materials and Methods, in the presence of ethanol extracts of melilot (M), leaves of blackberry (BL), sorrel and grape, and of the isolated fractions: phenol glycoside from rose petals (RP PhG), flavonoid (Fl) from SL and Cm1 from GL. Near the PPs their concentrations in *μg/mL* are indicated.

The data evidence the significant inhibition of the peptide aggregation by PPs in general ($p < 0.05$), excluding SL, 2.5; GL, 3.75 and RP PhG in the both used concentrations. It is seen that the inhibition of Aβ (1-42) aggregation positively correlated with the concentration of PPs: more the concentration of the PPs, more the degree of the aggregation inhibition (lower fluorescence intensity).

Disaggregation of Pre-aggregated Aβ (1-42) in the Presence of Plant Preparations. In the next series of experiments, the ability of PPs to destabilize (disaggregate) the preformed aggregates of Aβ (1-42) was studied.

The aliquots of preliminary aggregated during 7 days peptide suspension were incubated for 3 days in the presence of several PPs. A control sample without plant additions was incubated also. The aggregation states of the PP-containing samples were expressed as percentage of the control (Fig. 3).

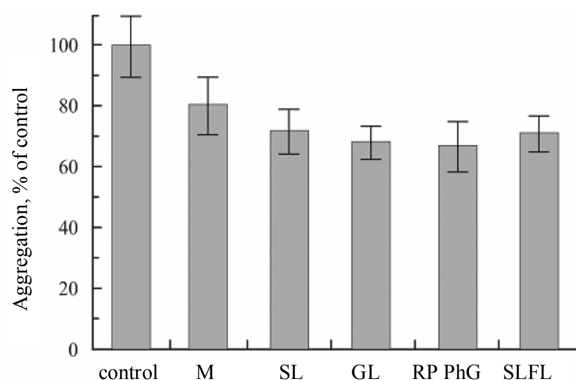

Fig. 3. The aggregation states of 25 μM preliminary aggregated Aβ(1-42) after 3-day incubation in the absence (control) and in the presence of PPs (7.5 $\mu g/mL$).

The data evidence the partial disaggregation of pre-formed aggregates of Aβ (1-42) in the presence of PPs. The extent of disaggregation in the presence of melilot is not significant ($p > 0.05$). The disaggregation in the presence of other used preparations is significant ($p < 0.05$ or 0.01).

Plant Preparations Lower the Aggregation Degree of Aβ (1-40). The ability of PPs to prevent the formation of Aβ (1-40) aggregates was studied also. The experiments were implemented identical to those described for Aβ (1-42). In Fig. 4 the aggregation states of samples incubated in the absence (control) and in the presence of ethanol extracts of RP, M, SL and GL, and of five isolated fractions: RP PhG, Fl of SL, Cm of RP, SL and GL are shown. The concentrations of all preparations were of 7.5 $\mu g/mL$. Like the case of Aβ (1-42), these data also evidence significant inhibition of Aβ (1-40) aggregation by nearly all PPs ($p < 0.01$ besides RP Cm1).

To study the ability of PPs to destabilize (disaggregate) the preformed aggregates of Aβ (1-40), incubated for 3 days in the presence (or absence, control) of PPs. In the case of Aβ (1-40) we have some success during study of dependences of disaggregation on the PPs concentrations: the obtained results were more consistent. In Fig. 5 the aggregation states of the 5 $\mu g/mL$ PPs-containing samples, expressed as percentage of the control were shown.

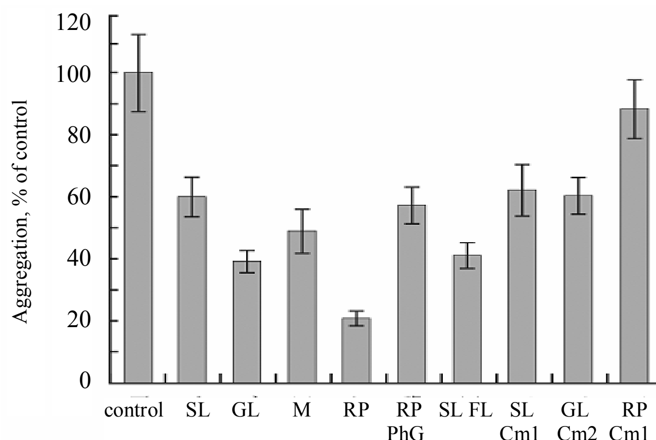

Fig. 4. Aggregation states of 50 μM Aβ (1-40) incubated during 7 days at 37°*C* in the absence (control) and presence of indicated PPs (all of 7.5 $\mu g/mL$).

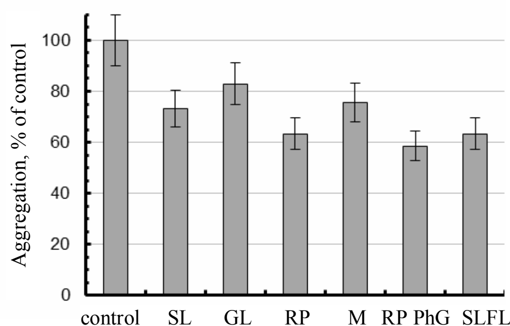

Fig. 5. Aggregation states of 50 μM preliminary aggregated Aβ (1-40) after a 3-day incubation in the absence (control) and in the presence of 5 $\mu g/ml$ PPs.

The IC_{50} values of PPs in disaggregation of Aβ (1-40) aggregates were evaluated from the dependences of the aggregation state on their concentration. In Table IC_{50} values for four plant extracts and two fractions in disaggregation of 50 μM Aβ (1-40) are listed. Let's note that these values are rather low, meaning that the PPs are enough effective in the disaggregation of Aβ (1-40) aggregates.

IC_{50} values of PPs in disaggregation of preformed aggregates of Aβ (1-40)

Plant preparation		IC_{50} ± stand. error, $\mu g/mL$
Extracts	RP	11.6 ± 1.0
	GL	12.4 ± 3.5
	SL	19.1 ± 3.1
	M	22.3 ± 6.7
Fractions	RP PhG	7.1 ±1.7
	SL Fl	22.6 ± 4.4

It is worthy to note that the fact of more concordant experimental results in our experiments with Aβ (1-40) compared with Aβ (1-42) may be a reflectance of

differences in the molecular peculiarities of the peptides, studied in the work [6]. The character of Aβ (1-40) interaction with PPs appeared more predictable and more suitable for processing.

Conclusion. The obtained results permit to conclude that the extracts of rose petals and melilot, leaves of grape and sorrel, as well as several their fractions *in vitro* inhibited the process of aggregation of amyloid peptides Aβ (1-40) and Aβ (1-42) significantly. Moreover, some of them partially disaggregate preformed aggregates of the studied peptides. Hence, the studied plants, some of which are used in the Armenian (oriental) cuisine, can be recommended to persons at risk group, and for testing as sources of the agents for prevention and/or cure neurodegeneration.

Authors appreciate Prof. H-U. Demuth (Germany) for gifted Aβ peptides.

This work was realized at H. Buniatyan Institute of Biochemistry of Armenian NAS by financial support of the State Committee of Science MES RA in frame of the research project № SCS 13-1F186; of a research grant awarded in 2015 to H. Harutyunyan from the Armenian National Science and Education Fund (ANSEF, # biochem-3787).

Received 17.09.2015

REFERENCES

1. World Alzheimer Report, 2010. The Global Econ. Impact of Dementia. Alzheimer's Disease Int.
2. **Bleiler T.W.** Alzheimer's Disease Facts and Figures. // Alzheimer's Dement, 2013, v. 9, p. 208–245.
3. **Selkoe D.J.** Alzheimer's Disease Results From the Cerebral Accumulation and Cytotoxicity of Amyloid β-Protein. // J. Alzheimer's Dis., 2001, v. 3, p. 75–80.
4. **Goure W.F., Krafft G.A., Jerecic J., Hefti F.** Targeting the Proper Amyloid-Beta Neuronal Toxins: A Path Forward for Alzheimer's Disease Immunotherapeutics. // Alzheimer's Research & Therapy, 2014, v. 6, p. 42.
5. **Kuperstein I., Broersen K., Benilova I., Rozenski J., Jonckheere W., Debulpaep M., Vandersteen A., Segers-Nolten I., Van Der Werf K., Subramaniam V., Braeken D., Callewaert G., Bartic C., D'Hooge R., Martins I.C., Rousseau F., Schymkowitz J., De Strooper B.** Neurotoxicity of Alzheimer's Disease Aβ Peptides is Induced by Small Changes in the Aβ 42 to Aβ 40 Ratio. // The EMBO Journal, 2010, v. 29, p. 3408–3420.
6. **Kim W., Hecht M.H.** Sequence Determinants of Enhanced Amyloidogenicity of Alzheimer Aβ 42 Peptide Relative to Aβ 40. // J. Biol. Chem., 2005, v. 280, p. 35069–35076.
7. **Clardy J., Walsh C.** Lessons from Natural Molecules. // Nature, 2004, v. 432, p. 829–837.
8. **Airoldi C., Sironi E., Dias C., Marcelo F., Martins A., Rauter A.P., Nicotra F., Jimenez-Barbero J.** Natural Compounds Against Alzheimer's Disease: Molecular Recognition of Aβ (1-42) Peptide by Salvia Sclareoides Extract and Its Major Component, Rosmarinic Acid, As Investigated by NMR. // Chem. Asian J., 2013, v. 8, p. 596–602.
9. **Chaher N., Arraki K., Dillinseger E., Temsamani H., Bernillon S., Pedrot E., Delaunay J.C., Mérillon J.M., Monti J.P., Izard J.C., Atmani D., Richard T.** Bioactive Stilbenes from Vitisvinifera Grapevine Shoots Extracts. // J. Sci. Food Agric., 2013.
10. **Cui Y.M., Wang H., Liu Q.R., Han M., Lu Y., Zhao C.Q.** Flavans From Iris Tenuifolia and Their Effects on β-Amyloid Aggregation and Neural Stem Cells Proliferation *in vitro*. // Bioorg. Med. Chem. Lett., 2011, v. 21, p. 4400–4403.
11. **Choi R.C., Zhu J.T., Leung K.W., Chu G.K., Xie H.Q., Chen V.P., Zheng K.Y., Lau D.T., Dong T.T., Chow P.C., Han Y.F., Wang Z.T., Tsim K.W.** A Flavonol Glycoside, Isolated From Roots of Panaxnotoginseng, Reduces Amyloid-Beta-Induced Neurotoxicity in Cultured Neurons: Signaling Transduction and Drug Development for Alzheimer's Disease. // J. Alzheimers Dis., 2010, v. 19, p. 795–811.

12. **Torosian A.** The Armenian Medical Herbs. Armenia, 2006.
13. **Vardanian S.A.** Histiore de Lamédicine en Arménie. Del'Antiquitéá Nosjours. ImpriméparL' Europenned' Edition, Paris, 1999.
14. **Sharoyan S.G., Antonyan A.A., Mardanyan S.S., Harutyunyan H.A.** Inhibition of Fibrilogenesis of Amylin by Extracts of Medicinal Plant and Their Components. // Biol. J. Arm., 2014, v. 66, p. 13–19.
15. **Sharoyan S., Antonyan A., Harutyunyan H., Mardanyan S.** Inhibition of Amylin Fibril Formation and Protection of Islet β-Cells by Medicinal Plants. // Int. J. Pharmacognosy, 2015, v. 2, p. 234–241.
16. **Sharoyan S., Antonyan A., Harutyunyan H., Mardanyan S.** Medicinal Plants Support the Amylin-Suppressed Viability of Islet β-Cells. // Int. J. Pharmacognosy, 2015, № 5, p. 448–453.
17. **Westermark P., Andersson A., Westermark G.T.** Islet Amyloid Polypeptide, Islet Amyloid, and Diabetes Mellitus. // Physiological Reviews, 2011, v. 91, p. 795–826.
18. **Movsisyan N.M., Sharoyan S.G., Antonyan A.A., Mardanyan S.S.** Breakdown of Some Neuronal Peptides with Dipeptidyl Peptidase IV. // Proceedings of YSU. Chemistry and Biology, 2013, № 1, p. 36–39.
19. **Sharoyan S., Antonyan A., Mardanyan S., Harutyunyan H., Movsisyan N., Hovnanyan N., Hovnanyan K.** Interaction of Dipeptydil Peptidase IV with Amyloid Peptides. // Neurochemistry International, 2013, v. 62, p. 1048–1054.
20. **Antonyan A., Sharoyan S., Harutyunyan H., Movsisyan N., Sargisova Y., Stepanyan H., Mardanyan S.** Cytotoxicity of Some Edible Plants Toward Ehrlich Ascites Carcinoma Cells. // Research Journal of Medicinal Plant, 2014, v. 8, p. 20–31.
21. **LeVine H.** Thioflavine T Interaction with Synthetic Alzheimer's Disease Beta-Amyloid Peptides: Detection of Amyloid Aggregation in Solution. // Protein Science, 1993, v. 2, p. 404–410.