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ORIGINAL PAPER

Acridine derivatives inhibit lysozyme aggregation

Zuzana Gazova · Andrea Bellova · Zuzana Daxnerova · Jan Imrich · Pavol Kristian · Jana Tomascikova · Jaroslava Bagelova · Diana Fedunova · Marian Antalik

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Abstract We have screened a library of structurally distinct acridine derivatives (19 compounds) for their ability to inhibit lysozyme amyloid aggregation in vitro. Studied acridines were divided into three structurally different groups depending on the molecule planarity and type of the side chain—planar acridines, spiroacridines and tetrahydroacridines. Thioflavine T fluorescence assay and transmission electron microscopy were used for monitoring the inhibiting activity of acridines. We have found that both the structure of the acridine side chains and molecule planarity influence their antiamyloidogenic activity. The planar acridines inhibited lysozyme aggregation effectively. Spiroacridines and tetrahydroacridines had no significant effect on the prevention of lysozyme fibrillization, probably resulting from the presence of the heterocyclic 5-membered ring and non-planarity of molecule. Moreover, in the presence of some tetrahydroacridines the enhanced extent of aggregation was detected. We identified the most active acridine derivates from studied compound library characterized by low micromolar IC_{50} values, which indicate their possible application for therapeutic purpose.

Keywords Protein aggregation · Amyloid · Lysozyme · Acridine

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Z. Gazova (✉) · J. Bagelova · D. Fedunova · M. Antalik
Department of Biophysics, Institute of Experimental Physics, Watsonova 47, 040 01 Kosice, Slovakia
e-mail: gazova@saske.sk

A. Bellova · Z. Daxnerova · J. Imrich · P. Kristian · J. Tomascikova · M. Antalik
Faculty of Sciences, P. J. Safarik University, Moyzesova 11, 041 54 Kosice, Slovakia

Introduction

Protein amyloid aggregation has been recognized as a hallmark of more than 20 human diseases, including Alzheimer's, Parkinson's and Huntington's diseases, type II diabetes, prion-related transmissible spongiform encephalopathies, and hereditary amyloidosis (Dobson 1999; Koo et al. 1999; Sipe 2005). The amyloid diseases are, in terms of incidence, one of the most important groups of pathologies in the developed world. The conversion of a specific protein or protein fragment from soluble native state into insoluble amyloid fibrils results in the formation of protein deposits in a variety of organs and tissues with a single predominant protein component that is characteristic of each disease (Lansbury 1999; Merlini and Bellotti 2003; Stefani and Dobson 2003; Sipe 2005).

Numerous proteins have been identified as forming amyloid in vivo (Bennett 2005; Lee et al. 2001; Goedert and Spillantini 2006; Nguyen et al. 1995). Although the proteins differ in their primary and tertiary structures, as well as their size and function, the highly ordered amyloid fibrils formed from these proteins share common morphological and histochemical staining properties (Cooper 1974; Chamberlain et al. 2000; Serpell et al. 2000). The amyloid fibrils possess a common cross-β structural motif, having β-strands oriented perpendicular to the fibril axis, and they bind selectively the aromatic dyes Congo red (Klunk et al. 1989) and Thioflavin T (LeVine 1993). Due to the morphological similarities between many fibrils from different protein building blocks, it has been hypothesized that different proteins follow similar fibril formation pathways (Dobson 1999). However, the precise mechanism of the amyloid aggregation is still not clear.

It is generally accepted that protein aggregation has toxic consequence to different cell types suggesting its

key role in cell impairment and death (Dobson 2001; Khlistunova et al. 2006; Baglioni et al. 2006). Recently, there is growing evidence that soluble oligomers rather than mature amyloid fibrils may be the main toxic species in amyloid-related disorders (Ferreira et al. 2007; Haass and Selkoe 2007; Walsh and Selkoe 2004). Although this concept was originally introduced in the investigation of the neurotoxicity of Aβ oligomers and their role in the pathogenesis of Alzheimer's disease (Lambert et al. 1998; Walsh et al. 2002; Lue et al. 1999; Wang et al. 1999), this notion has now been considerably expanded to include several other proteins involved in amyloid diseases (Conway et al. 2000; Reixach at al. 2004). The reason why early aggregates are more toxic than mature amyloid fibrils is not yet clear. In isolated cells, such toxicity has been shown to result from increased membrane permeability with disruption of membrane integrity and formation of ion channels (Hou et al. 2000; Demuro et al. 2005; Canale et al. 2006), oxidative stress (Moreira et al. 2005; De Felice et al. 2007) and deregulation of cell homeostasis by accumulation of intracellular amyloid (Gouras et al. 2005).

More recently, it has been found that amyloid polymerization is not only possible with disease-associated proteins, but also with proteins that are not associated with any known amyloid disease under certain conditions in vitro (Chiti et al. 1999; Fandrich et al. 2001). Amyloid fibrils and prefibrillar assemblies formed from non-disease related proteins have similar morphological features and cytotoxicity as those detected for disease-associated proteins (Vieira et al. 2007; Guijarro et al. 1998). This has led to the suggestion that ability to form amyloid aggregates is a generic property of polypeptide chains, and that most or indeed all peptides and proteins have the potential to form such structures in vitro under appropriate conditions (Chiti et al. 1999; Bucciantini et al. 2002). Therefore, the study of the amyloid aggregation of non-disease associated proteins can add to our understanding of possible inhibition of amyloid aggregation.

Hereditary systemic amyloidosis is associated with one of the best known of all proteins-lysozyme. This disease results from single point mutations in the gene giving rise to variant proteins which form massive amyloid deposits in the liver and kidney of individuals affected by this disease (Valleix et al. 2002; Yazaki et al. 2003). Studies of these proteins have shown that amyloid formation of the variants is due to a tendency to favour partially denaturated structures (Funahashi et al. 1996; Canet et al. 1999; Booth et al. 1997). The ability to form amyloid aggregates in vitro has been found for the single point mutants and wild-type human lysozymes (Pepys et al. 1993; Morozova-Roche et al. 2000) and also for hen egg white lysozyme (Cao et al. 2004; Vernaglia et al. 2004).

Currently, there are no effective cures for amyloid diseases, but experiments from various cell and animal models suggest that the reduction of amyloid aggregation is beneficial (Khlistunova et al. 2006; Roberson et al. 2007). The antiaggregating activity has been identified for a range of substances including the antibodies, synthetic peptides, heat shock proteins, and chemical compounds. A great number of diverse small molecule compounds have been found to inhibit or reduce the aggregation of various proteins, particularly in relation to Aβ deposition, aggregation of lysozyme and transthyretin (DeFelice et al. 2001; Vieira et al. 2006; Raghu et al. 2002) and the formation of protease-resistant forms of the prion protein (Caughey et al. 1998). Recently, it has been detected that anthraquinones are able to inhibit tau aggregate formation in vitro (Pickhardt et al. 2005) and in cells (Khlistunova et al. 2006). Similar effect was observed also for other low molecular weight compounds as phenothiazines (Wischik et al. 1996), N-Phenylamine derivatives (Pickhardt et al. 2007), polyphenols and porphyrins (Taniguchi et al. 2005). Acridine-based compounds were identified as potent inhibitors of protease-resistant forms of the prion protein (Korth et al. 2001; May et al. 2003). Thus, small molecules could provide a basis for the development of tools for the treatment of amyloid pathology.

In this paper, we studied anti-amyloidogenic ability of low molecular weight compounds, acridines, as it has been shown that some acridine and bis-acridine derivatives are able to reduce scrapie prion concentration in infected cells (May et al. 2003). We screened a library of structurally distinct acridine derivatives (19 compounds) for their ability to inhibit lysozyme amyloid aggregation in vitro.

Materials and methods

Chemicals and proteins

Lysozyme from chicken egg white (CEW lysozyme) (lyophilized powder, lot number L 6876, −50,000 units mg^{-1} protein), thioflavinT (ThT) and Congo red (CR) were obtained from the Sigma Chemical Company (St Louis, MO). Guanidine hydrochloride (GdnHCl) was purchased from Fluka. The protein concentrations were determined spectrophotometrically (Specord S100, Analytik Jena), using extinction coefficient (at $\lambda = 280$ nm) of 2.63 Lg^{-1} cm^{-1} (Vernaglia et al. 2004). Acridine derivatives investigated here were synthesized at the Department of Organic Chemistry, Faculty of Science at P. J. Safarik University (P1, P2, P5–7, P9, P10–Tomaščiková et al. 2007; P4–Tomaščiková et al. 2008; T1–Kristian et al. 1998; P3, P8, S1–S5, T2–T4, unpublished results). The stock solutions of 10 mM acridines in DMSO were freshly prepared. The

volume of DMSO in measuring samples was lower than 2%. All other chemicals were of analytical reagent grade and were purchased from Fisher or Sigma. All solutions were prepared with deionized water.

Lysozyme aggregation

Solution of lysozyme (10 μM) was prepared in 20 mM potassium phosphate in the presence of 3M GdnHCl, pH 6.3 ± 0.1 as described elsewhere (Vernaglia et al. 2004). The solution was adjusted to 50°C and stirred constantly for 2 h with a Teflon-coated magnetic stirring rod. Lysozyme aggregation was followed by ThT fluorescence assay, Congo red assay and by transmission electron microscopy.

Thioflavin T (ThT) fluorescence assay

Lysozyme fibril formation was monitored by characteristic changes in ThT fluorescence intensity. Thioflavin was added to the lysozyme samples (10 μM) to a final concentration of 20 μM and the fluorescence intensity was measured using a fluorimeter (type RF-5000 Schimadzu). The excitation was set at 440 nm and the emission recorded at 485 nm. Fluorescence measurements were performed in semimicro-quartz cuvettes with a 1-cm excitation light path; slits were adjusted to 1.5 and 3.0 nm for the excitation and emission accordingly.

Congo red (CR) assay

The lysozyme amyloid aggregates were examined by measuring the CR absorbances of 10 μM lysozyme sample solutions and the free dye controls (CR was added to final concentration of 5 μM) in 10 mM phosphate buffer, pH 7.4. Specific binding of CR to amyloid aggregates resulted in the absorbance maximum red shift of CR as it was described by Klunk et al. (1989). Specifically, a large shoulder peak should appear around 540 nm. The spectrum was recorded by UV-visible spectrometer (Specord S100, Analytik Jena) from 400 to 700 nm. CR was freshly prepared and incubated with lysozyme solutions and control solutions at room temperature for at least 30 min before recording the absorption spectrum.

Transmission electron microscopy

Protein solutions diluted to 10–50 μM were placed on 300-mesh formvar-coated copper grid. After adsorption for 45 s, the samples were washed with distilled water. The grids were then stained with 2% uranyl acetate for 45 s. The excess of stain was removed, and the samples were allowed to air-dry. The samples were analyzed utilizing a Tesla BS 500 operating at 60 kV.

Screening of lysozyme aggregation inhibitors

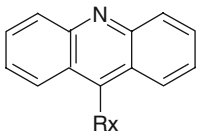
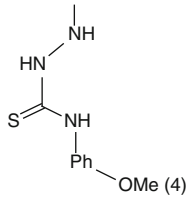
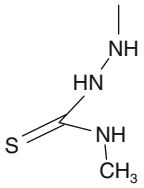
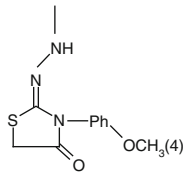
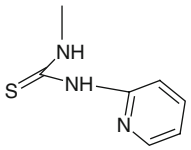
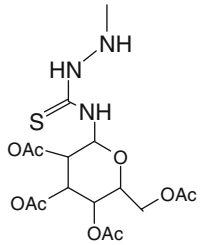
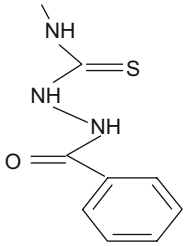
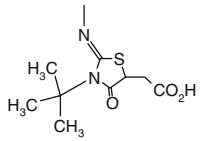
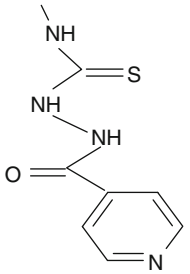
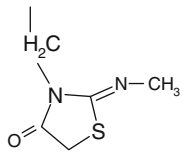
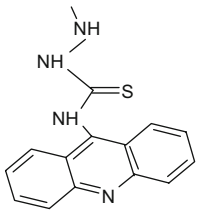
Inhibiting activity of acridine derivatives were detected by ThT assay. Acridine derivative (200 μM, final concentration) was added to lysozyme solution (10 μM) prepared in 20 mM potassium phosphate, 3 M GdnHCl, pH 6.3 and stirred constantly for 2 h at 50°C. After incubation, ThT was added to a final concentration of 20 μM, and signal was measured by spectrofluorimeter (Schimadzu, type RF-5000) at excitation of 440 nm and emission of 485 nm. As a control the protein was replaced with water to measure the fluorescence of the acridine. For acridine derivatives showing substantial inhibiting ability (50% decreasing of the fluorescence intensity observed for lysozyme aggregates alone) we measured the inhibition of lysozyme fibrillization for compound concentrations of 1 mM down to 10 pM at a 10 μM concentration of lysozyme. The single experiment was performed in triplicates and final value is average of measured values.

Results and discussion

The primary cause of protein aggregation processes is not well understood; however, there is a strong interest to identify compounds that inhibit aggregation and might be developed into drugs. Therefore, we screened a library of structurally distinct acridine derivatives (19 compounds), which were synthesized in our laboratory for their ability to inhibit formation of CEW lysozyme amyloid aggregates in vitro. According to the structural similarity, we divided acridine derivatives into three groups—planar acridines, spiroacridines and tetrahydroacridines. The planar acridines are characterized by planar tricyclic core and aliphatic side chain with various lengths and terminal groups in C-9 position of acridine skeleton. In the case of the spiroacridines the aliphatic side chain is substituted by heterocyclic 5-membered ring. Tetrahydroacridines, unlike the acridines belonging to the first class, are characterized by non-planar heterocyclic core. The chemical structures are shown in Table 1. The lysozyme fibrillization was confirmed by ThT fluorescence assay as increase of ThT fluorescence intensity (Fig. 1a), CR assay as the red shift of the CR absorbance maximum (Fig. 1b) and by transmission electron microscopy (Fig. 4a).

To make primary screening we tested the ability of acridine derivatives to inhibit formation of lysozyme amyloid aggregates in presence of 200 μM of acridine compounds by ThT fluorescence assay, which is sensitive to the interaction between the dye and the assembled

Table 1 Acridine derivatives

1. Planar acridines		R_x	I_{rel} (%)[a]	IC_{50} (μM)		R_x	I_{rel} (%)	IC_{50} (μM)
	P1		52.97 ± 1.98	89.10	P6		9.18 ± 1.50	9.99
	P2		23.58 ± 1.05	57.79	P7		14.87 ± 1.42	48.57
	P3		31.80 ± 2.61	75.57	P8		xx	xx
	P4		xx	xx	P9		55.88 ± 4.06	182.10
	P5		88.11 ± 0.93	N/A	P10		5.55 ± 0.91	6.45

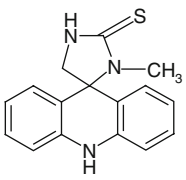
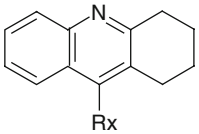
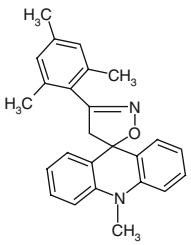
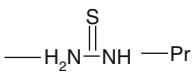
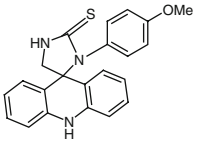
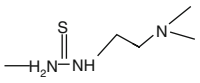
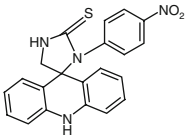
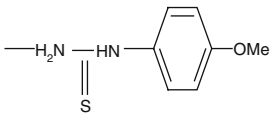
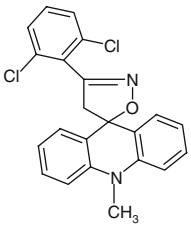
2. Spiroacridines		I_{rel} (%)	3. Tetrahydroacridines	R_x	I_{rel} (%)
S1		83.97 ± 7.72		T1 –NCS	113.93 ± 8.81
S2		88.16 ± 6.65		T2 	133.6 ± 2.48

Table 1 continued

2. Spiroacridines		I_{rel} (%)	3. Tetrahydroacridines	R_x	I_{rel} (%)
S3		76.57 ± 6.22	T3		180.33 ± 4.55
S4		60.16 ± 12.66	T4		177.77 ± 2.28
S5		86.54 ± 10.51			

[a] The lysozyme aggregation investigated by ThT assay. The fluorescence signal of ThT detected for lysozyme polymerization in presence of 200 μM acridine derivative was normalized to fluorescence observed for fibrillization without the compound (control, 100%)

xx high fluorescence signal of acridine

N/A not available due to very weak inhibiting activity

β-structured fibrils or oligomers. The inhibiting activity was quantified as percentage of maximal ThT fluorescence observed for lysozyme fibrillization without acridine (control sample, taken as 100%). The extent of the reduction of fluorescence intensity characterizes the inhibiting potential of acridines (lower fluorescence value indicates more effective inhibitor). Representative results of the primary screening characterizing each structural class of acridines are shown in Fig. 1c. Normalized fluorescence intensities obtained for all studied acridine derivatives are given in Table 1. The planar acridines (P1-P10) caused extensive decline of ThT fluorescence (to values lower than 50–95% of the control sample) indicating their significant ability to inhibit lysozyme amyloid aggregation. The exception was observed only for planar acridine P5, whose inhibiting capability was minimal (about 10% decrease of fluorescence intensity). From the screening the derivatives P4 and P8 were excluded on account of their very intensive fluorescence signal detected in studied wavelength range. The effect of spiroacridines on the inhibition of lysozyme polymerization was very weak. It follows from measured fluorescence intensities, which were about 80% of that observed for the control sample. By this method, we also found that tetrahydroacridines had no influence on the prevention of lysozyme fibrillization. Moreover, T3 and T4 derivatives promoted lysozyme aggregation significantly. The presented results from ThT experiments could be confirmed by CR assay. However, the monitoring of the acridine inhibiting activities by CR binding was excluded as the compounds possess intensive absorption peaks in the same region as CR.

We were interested to investigate effect of acridine structure in more details; therefore, we studied the kinetics of the fibril formation in presence of acridine derivatives belonging to each structural group. By ThT fluorescence assay we characterized the time dependencies of the extent of lysozyme aggregation in the presence of acridine derivatives P10, T4 and S1 (Fig. 2). The data suggest that acridines altered the shape of the curve detected for lysozyme fibrillization, which can be characterized by sigmoidal profile and lag phase taking about 1 h and the steady-state value achieved at about 2 h. The presence of planar acridine P10 caused intensive inhibition of fibril formation. During the studied time interval the fluorescence intensities were very low and the steady-state value indicating the final proportion of the lysozyme aggregation is markedly reduced. Similar results were observed for all planar acridine derivatives (except for P5) suggesting the high-inhibiting effectivity of these compounds. Spiroacridine S1 had no significant effect on the lysozyme aggregation as the time course and steady-state value of ThT fluorescence are similar to those detected for lysozyme fibrillization in absence of compounds. This curve, representing a typical dependence detected for all spiroacridines, supports the fact that spirocaridines are very weak inhibitors. Unlike the planar acridines, the tetrahydroacridines favour lysozyme aggregation as it is shown for tetrahydroacridine T4 in Fig. 2. The time dependence of

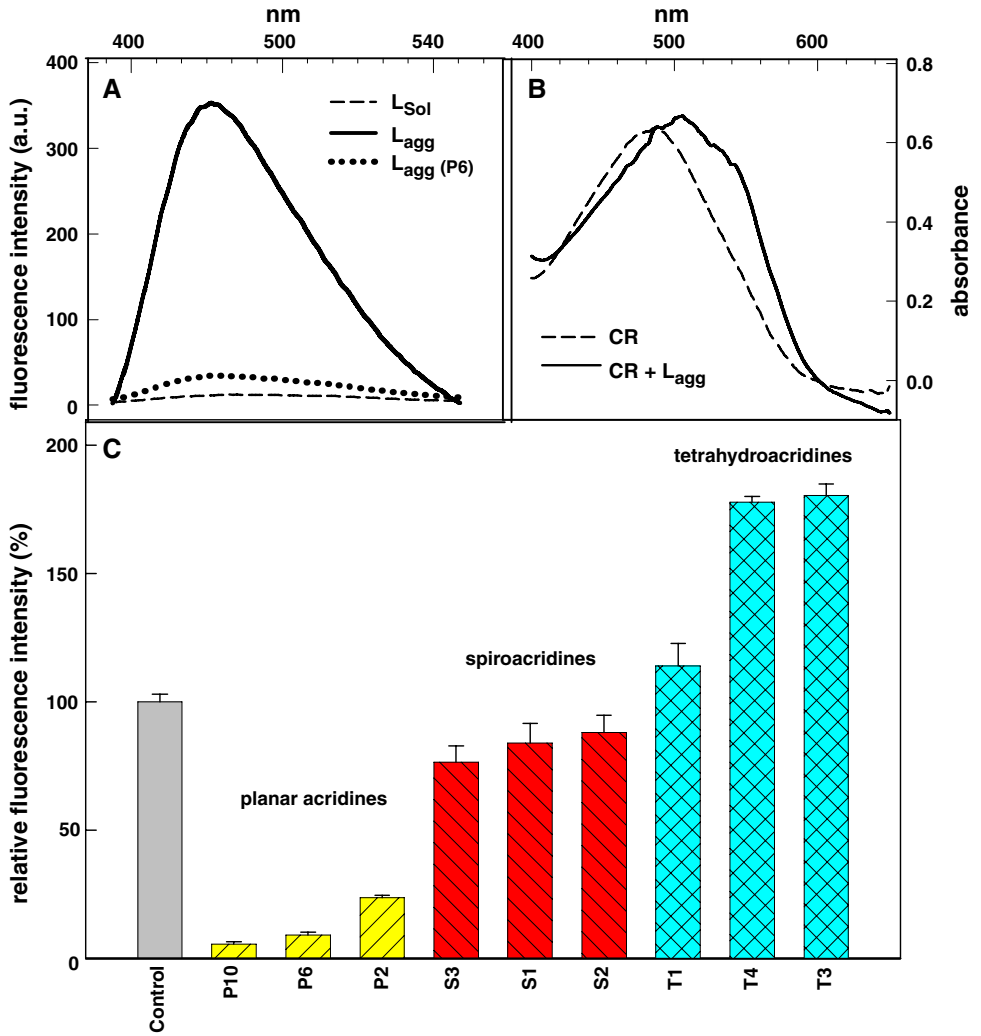


Fig. 1 (**a**) Extent of lysozyme aggregation observed by ThT assay. Fluorescence signal detected for 10 μM lysozyme solution before (*dashed line* L_{sol}), and after process inducing protein fibrillization (3 M GnHCl, 2 h constantly stirred at 50°C) in absence (*solid line* L_{agg}) and presence of 200 μM acridine derivative P6 [*dotted line* L_{agg}(P6)] in 20 mM potassium phosphate buffer, pH 6.3. (**b**) Lysozyme polymerization examined by Congo red assay. Absorbance spectrum detected for free Congo red (*dashed line* CR) and in presence of lysozyme aggregates (*solid line* CR + L_{agg}) in 10 mM phosphate buffer, pH 7.4; molar ratio CR:protein is 1:2. (**c**) Results of ThT assay of the inhibition of lysozyme aggregates by acridine derivatives (200 μM). The extent of lysozyme aggregation was normalized to the control representing the fluorescence signal of the protein solution (10 μM lysozyme, derivative in 20 mM potassium phosphate buffer, 3 M GdnHCl, pH 6.3) detected after 2 h incubation at 50°C and intensive solution stirring in absence of acridine compound (100%). Chemical structures of individual derivates marked on *x*-axis are listed in Table 1. The single experiment was performed in triplicates. The *error bars* represent the average deviation for repeated measurements of three separate samples

ThT fluorescence is characterized by short lag phase and markedly higher steady-state value of fluorescence. This suggests the fact that presence of tetrahydroacridines promotes fibril formation as well as increases the proportion of protein in the aggregate state.

The compounds belonging to the structural group characterized by significant ability to inhibit aggregation, e.g. the planar acridines, were further investigated. Using fixed protein concentration of lysozyme at 10 μM, we tested the ability of these compounds to inhibit lysozyme fibrillization in a concentration range from 10 pM to 1 mM by ThT fluorescence assay. Acridine derivatives inhibited the aggregation of lysozyme in a concentration-dependent manner as it can be seen in Fig. 3 for selected planar acridines (P7, P9 and P10). From the dose-dependencies showing the fairly steeply decay over a compound concentration range of about two orders of magnitude, we determined the values of half-maximal inhibition IC_{50}. The majority of IC_{50} values are in micromolar range as it can be seen in Table 1. Especially, the acridines P6 and P10 were characterized by very low micromolar IC_{50} values (9.99 and 6.45 μM). It means that these compounds interfere with aggregation of lysozyme, already at substoichiometric concentrations.

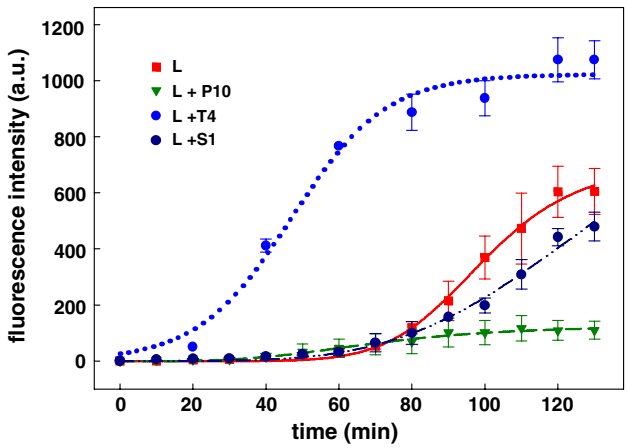


Fig. 2 Time-dependence of lysozyme fibril formation determined in the absence (*filled square*) and in the presence of acridine derivates T4 (*light filled circle*), P10 (*inverted triangle*) and S1 (*dark filled circle*). Each sample contains 10 μM lysozyme, 200 μM acridine derivative, 3 M GdnHCl in 20 mM potassium phosphate buffer, pH 6.3. The solution was incubated with intensive stirring at 50°C. The aliquots were selected at given time intervals and extent of lysozyme aggregation was monitored by ThT fluorescence assay (20 μM–ThT). The *error bars* represent the average deviation for repeated measurements of three separate samples. The curves were obtained by fitting of the average values by non-linear least-square method

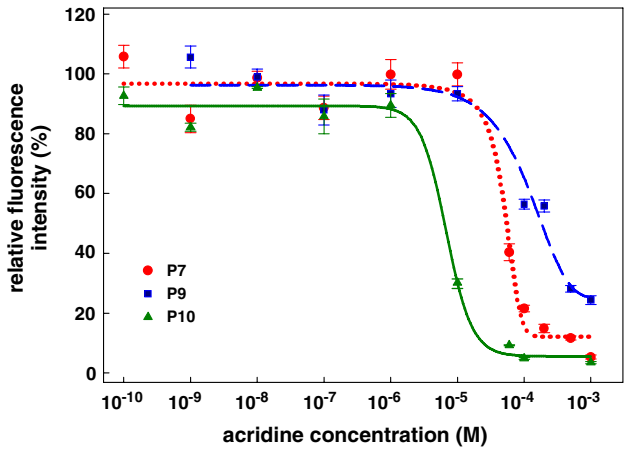


Fig. 3 Aggregation of lysozyme observed at increasing acridine concentration by ThT assay. The inhibiting ability of acridines was quantified by fluorescence intensity, which was normalized to the control in the absence of acridine compound (100%). Each sample contains 10 μM lysozyme, acridine derivatives at increased concentrations, 3 M GdnHCl in 20 mM potassium phosphate buffer, pH 6.3. The solution was intensively stirred at 50°C during 2 h. The single experiment was performed in triplicates. The *error bars* represent the average deviation for repeated measurements of three separate samples. The curves were obtained by fitting of the average values by non-linear least-square method

The ability of the most effective acridine derivatives to inhibit formation of lysozyme fibrils was confirmed by electron microscopy. The electron microscope images of amyloid fibrils formed from CEW lysozyme in the absence and presence of 100 μM acridines P6 and P10 are shown in Fig. 4. In the absence of acridines (Fig. 4a), long needle-like fibrils were observed (the thicker fibrils appeared to arise from interaction of the thinner ones). In the presence of P6 (Fig. 4b) and P10 (Fig. 4c) acridines the amount of fibrils was reduced. Moreover, the fibrils that were produced in the presence of these most effective acridines appear thinner and shorter than those formed in the absence of compounds.

The different activities of studied acridine derivatives indicate that structure of the acridine molecule is one of the key factors, which can interfere with lysozyme fibrillization. In accordance with the findings that many of compounds having the ring structure were identified as effective inhibitors (Wischik et al. 1996; Taniguchi et al. 2005; Pickhardt et al. 2005, 2007; Ono et al. 2006), we presumed that heterocyclic skeleton of acridine is important for inhibiting activity. Analysis of our data indicate that planarity of the acridine cyclic core can be the crucial element determining the extent of lysozyme aggregation. This assumption is supported by results obtained for planar acridines showing the intensive prevention of lysozyme polymerization unlike the non-planar tetrahydroacridines, whose interaction with protein led to expressive enhancement of aggregation. One of the explanations of presented evidences is that only planar skeleton can intercalate between the hydrophobic residues and thus interrupt the interface between two neighboring β-sheets.

The structure of the side chain in C-9 position of acridine skeleton of derivatives seems to be another element effecting the inhibition of lysozyme fibril formation (Table 1). In the case of planar acridines, the side chain is an aliphatic one with various length and terminal groups. Termination of the side chain by aryl or heteroaryl rings caused decrease of the inhibiting activity. The most positive effect, in this sense, was found for derivative P6, where the side chain is terminated by methyl group. The highest inhibiting activity among screened compounds at all was detected for dimeric acridine P10. This fact supports the observations given by May et al. (2003) indicating that potency of acridine compounds can be improved by forming covalent acridine dimers. The simplest interpretation of this intensive inhibiting activity is that duplication of heterocycles in dimeric acridine increases the capacity of compound to interact with protein leading to more effective blockage of the β-structure formation.

The substitution of the aliphatic chain by heterocyclic 5-membered ring caused intensive decline of inhibiting activity as it was observed for spiroacridines. We proposed that it could be induced by enlargement of the bulkiness in the close vicinity of the acridine skeleton, which can result in the decreasing of the ability to intercalate between the forming β-sheets. However, this substitution also leads to

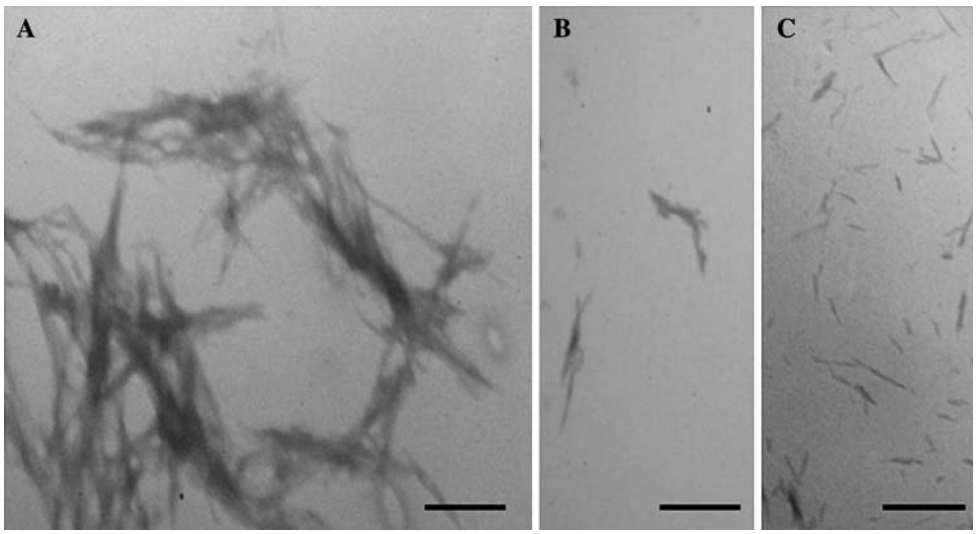


Fig. 4 TEM images of lysozyme solution after process inducing protein fibrillization (3 M GnHCl, 2 h constantly stirred at 50°C in 20 mM potassium phosphate buffer, pH 6.3) in absence (**a**) and in presence of 100 μM acridine derivatives P6 (**b**) and P10 (**c**). The *bars* represent 500 nm

another important modification of the molecule. The amino group moiety with sp^2 nitrogen atom in the middle ring of the planar acridines is a hydrogen bond acceptor contrary to spiroacridines, where sp^3 nitrogen atom amine moiety, due to the loss of the unsaturation in the ring, makes it a hydrogen bond donor. This modification can be also important in interfering with the interaction between the compounds and the protein.

In summary, work reported here is concerned with the identification of compounds with anti-amyloid effect. We found that synthesized acridine derivatives are able to prevent formation of CEW lysozyme fibrillization depending on the structure of acridine molecules. We supposed that planarity of the core ring structure as well as the behaviours of the side chain binding to cyclic skeleton intensively influence the extent of the lysozyme aggregation. We determined very effective inhibitors of CEW lysozyme fibrillization, namely planar acridine derivatives P6 and P10, characterized by inhibiting activity at low micromolar concentrations. This fact is important for a potential therapeutic use of these compounds in the prevention of the human lysozyme amyloidoses. It is interesting that other type of acridine derivatives had capability to inhibit amyloid aggregation of different, unrelated proteins, namely Quinacrine and Quinacrine mustard inhibited the formation of amyloid fibrils of tau and Aβ peptide (Taniguchi et al. 2005). The anti-scrapie activity probably through inhibition of the formation of protease-resistant prion protein has been found also for some other acridine derivatives (Caughey et al. 1998; Priola et al. 2000; May et al. 2003). These evidences could mean that anti-amyloid acridine may have relevance not only to lysozyme-related hereditary amyloidosis but also to amyloid diseases in general.

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