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Inhibition of Amyloid Fibril Formation by Polyphenols: Structural Similarity and Aromatic Interactions as a Common Inhibition Mechanism

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The formation of well-ordered fibrillar protein deposits is common to a large group of amyloid-associated disorders. This group consists of several major human diseases such as Alzheimer's disease, Parkinson's disease, prion diseases, and type II diabetes. Currently, there is no approved therapeutic agent directed towards the formation of fibrillar assemblies, which have been recently shown to have a key role in the cytotoxic nature of amyloidogenic proteins. One important approach in the development of therapeutic agents is the use of small molecules that specifically and efficiently inhibit the aggregation process. Several small polyphenol molecules have been demonstrated to remarkably inhibit the formation of fibrillar assemblies *in vitro* and their associated cytotoxicity. Yet, the inhibition mechanism was mostly attributed to the antioxidative properties of these polyphenol compounds. Based on several observations demonstrating that polyphenols are capable of inhibiting amyloid fibril formation *in vitro*, regardless of oxidative conditions, and in view of their structural similarities we suggest an additional mechanism of action. This mechanism is assuming structural constraints and specific aromatic interactions, which direct polyphenol inhibitors to the amyloidogenic core. This proposed mechanism is highly relevant for future *de novo* inhibitors' design as therapeutic agents for the treatment of amyloid-associated diseases.

Key words: Aromatic interactions, pi-pi interactions, polyphenols, protein misfolding, self-assembly.

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Amyloidogenic diseases are characterized by conformational changes that are followed by aggregation of proteins inside or outside cells. This self-assembly into well-ordered supramolecular assemblies and eventually deposits (also termed plaques in neurodegenerative diseases) is associated with major human diseases and has key medical

importance. A partial list of more than 20 amyloid-related diseases includes Alzheimer's disease, Parkinson's disease, Huntington's disease, prion diseases, familial amyloidosis, and type II diabetes (1–13).

In spite of the fact that various amyloid-forming proteins and polypeptides do not reveal any simple sequence homology, all amyloid assemblies share similar ultrastructural and physicochemical properties. All amyloid fibrils have a characteristic, elongated fibrillar morphology with a diameter of 5–15 nm, a β -sheet-rich structure (14–16), and typical X-ray fiber diffraction with a reflection of 4.6–4.8 Å in the meridian (17,18). Another well-known characteristic of all amyloid fibrils is their interaction with specific dyes such as Congo red and thioflavin T, which results in definite optical behavior such as birefringence and fluorescence, respectively (19,20).

In this article, we will describe the up-to-date knowledge on the molecular interactions that lead to amyloid fibril formation and the therapeutic approaches to amyloidogenic diseases, and will focus on a small molecular family of polyphenols that were described to specifically inhibit various amyloid proteins *in vitro*. We then suggest a new approach to the inhibition mechanism that is relevant to future *de novo* design of amyloid inhibitors.

Interactions that promote amyloid fibril formation

The ability of polypeptide chains to form amyloid structures is not restricted to the relatively small number of proteins that are associated with recognized clinical disorders, but appears to represent a generic feature of aggregated polypeptide chains (21). The core structure of all amyloid fibrils seems to be primarily stabilized by hydrogen bonds, involving the polypeptide main chain. As the same chemical structure of the main chain is common to all polypeptides, this observation explains why fibrils formed from polypeptides of very different sequences seem to be so similar (4). Even though the ability to form amyloid fibrils appears to be generic, the propensity to do so under given conditions can vary markedly between different protein and peptide fragments. The relative aggregation rates for a wide range of peptides and proteins correlates with their physicochemical features, such as hydrophobicity, secondary-structure propensities, charge, and aromatic interactions (5,22–24).

Aromatic interactions and amyloid fibril formation

The initial hypothesis about the role of aromatic interactions in amyloid fibril formation was based on the remarkable occurrence of

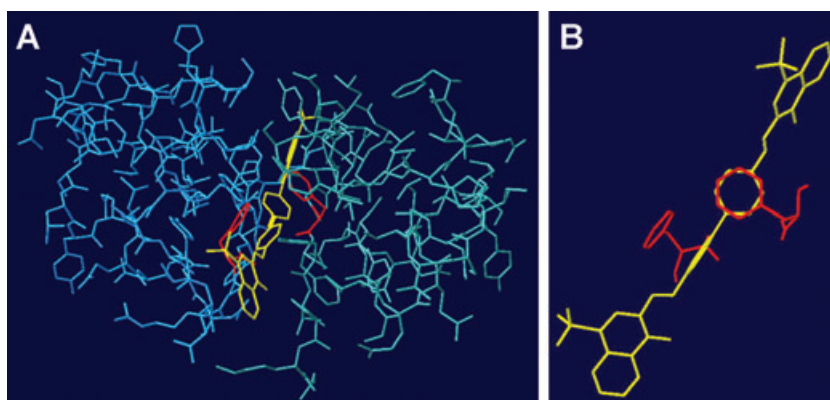


Figure 1: Crystal structure of amyloidogenic insulin dimer (blue and cyan) and Congo red. A) Insulin Amyloidogenic dimer (blue and cyan) interact with Congo red (yellow), a dye commonly used to strain amyloid fibrils. Aromatic interactions between phenylalanine₂₄ (red) in both monomers and the aromatic rings of Congo red are evident. B) Top view of the aromatic interaction. Figures were done using SPDV viewer based on coordinates from Turnell and Finch (32).

aromatic residues in many amyloid-related proteins and short peptide fragments (24,25), and the well-known role of aromatic stacking in processes of self-assembly in chemistry and biochemistry (23,26–28). This hypothesis led to the suggestion that stacking of aromatic residues may play a role in acceleration of the assembly process in many cases of amyloid fibril formation. Stacking interactions may provide an energetic contribution as well as directionality and orientation that are facilitated by the restricted geometry of planar aromatic stacking (29–31). Structural analysis, based on co-crystallization of insulin and Congo red under amyloid-promoting conditions, showed that Congo red interacts with the aromatic moiety of phenylalanine₂₄ in an amyloidogenic insulin dimer, by specific aromatic interactions (Figure 1). It was also shown that Congo red inhibits insulin fibril formation (32). This theory was further supported by the observation that short aromatic dipeptides contain all the molecular information needed to self-assemble into well-ordered nanostructures that are structurally related to amyloid fibrils (33). The role of aromatic interactions was recently shown to have a strong effect on amyloidogenic propensity (23) and, furthermore, recent X-ray diffraction of a short, crystallized amyloidogenic sequence revealed that aromatic interactions stabilized the anti-parallel β -sheet structure (34).

Amyloid cytotoxicity effect

Several possible mechanisms for the observed cytotoxicity of amyloidogenic assemblies have been suggested. The current models could be divided into two major categories. The first category suggests a direct effect of amyloidogenic assemblies on cell membrane stabilization. The suggested direct effects are: (a) pore formation in cellular membranes (35,36); (b) permeation effect of amyloidogenic bundles, which lead to membranal instability (37,38). The second model suggests an indirect effect of these protein assemblies on cellular mechanisms, which eventually lead to cell death. Indirect effects include: (a) increased formation of reactive oxygen and nitrogen species; (b) abnormalities in cell redox systems; (c) protein loss of function after aggregation; and (d) hyper-phosphorylation of proteins that accumulates in aggregative protein deposits (39).

Therapeutic approaches toward amyloidogenic diseases

Several therapeutic approaches have been suggested so far for treatment of amyloidogenic diseases. These approaches include

reduction in the production of the amyloidogenic form of proteins, increase in the clearance rate of misfolded or aggregated proteins, increase of the native state stability in amyloidogenic proteins, and direct inhibition of the self-assembly process (3).

The small molecule inhibitors approach was initially based on early findings that demonstrated that small aromatic molecules such as Congo red and thioflavin T interact specifically with amyloid fibrils and inhibit their formation (40–42). In the past few years, there is an accumulation of reports that describe small molecule inhibitors of amyloid fibril formation.

A partial list of these reports shows that aromatic-rich compounds, and especially certain polyphenolic small molecular compounds, dramatically inhibit cell death in cell culture amyloidogenic cytotoxicity assays. Several of these compounds were also described as efficient *in vitro* inhibitors of amyloid fibril formation.

Polyphenol classification and antioxidative functions

Polyphenols are a large group of natural and synthetic small molecules that are composed of one or more aromatic phenolic rings. Natural polyphenols are a class of phytochemicals found in high concentrations in wine, tea, nuts, berries, cocoa, and a wide variety of other plants. More than 8000 polyphenolic compounds have already been identified, and their natural function is correlated to protection of plants from diseases and ultraviolet light and prevention of damage to seeds until they germinate.

Natural polyphenols are grouped into several categories: vitamins (e.g., β -carotene and α -tocopherol), phenolic acids (e.g., benzoic acid and phenylacetic acid), flavonoids (e.g., flavanone and isoflavone), and other miscellaneous polyphenols (ellagic acid, sesamol, eugenol, thymol, etc.). Synthetic polyphenols are commonly used as pH indicators in cell culture media and synthetic food additives [e.g., phenolsulfonphthaleine, Butylated hydroxyanisole (BHA), and Butylated hydroxytoluene (BHT)].

Antioxidants are compounds that can delay, inhibit, or prevent the oxidation of materials that can be oxidized by scavenging free radicals and diminishing oxidative stress. Oxidative stress is an imbalanced state, where excessive quantities of reactive oxygen species

(e.g., superoxide radical anion, hydrogen peroxide, and hydroxyl radical) are present at higher levels than those required for normal cell function and overwhelm endogenous antioxidant capacity and repair. Oxidation in biological systems may lead to cellular membrane dysfunction and DNA damage. Polyphenols, which include phenolic acids and flavonoids, act as free radical scavengers and have shown beneficial health-promoting effects in chronic and degenerative diseases (43).

Polyphenols act as *in vitro* inhibitors of various amyloidogenic protein fibril assemblies

In the past few years various *in vitro* assays were performed that directly linked certain polyphenols with the inhibitory effect of amyloid fibril formation.

Apomorphine inhibits α -synuclein and β -amyloid

Lansbury and coworkers (44) screened 169 compounds for *in vitro* inhibition of α -synuclein, an amyloidogenic protein that is found to accumulate as Lewy bodies in the brain of Parkinson's disease patients. Using thioflavin T assay, the researchers had identified 15 inhibitory catecholamine compounds, among them the polyphenol compounds dobutamine and apomorphine (Table 1) (44). Apomorphine was later reported by Callaway and coworkers (45) to inhibit β -amyloid_{1–40}, whose aggregates are considered to be one of the main pathological hallmarks of Alzheimer's disease. After screening of several apomorphine derivatives, these authors suggested two structural principles regarding this kind of inhibitor: (a) requirement of two hydroxy groups on the D-aromatic ring of apomorphine for the inhibitory effectiveness of apomorphines, as methylation of these hydroxyl groups reduces their inhibitory potency; (b) relative to the low inhibition potency of catechol, apomorphine has an additional ring structure that distinguishes it from naturally occurring catechols, thus making it more hydrophobic, and it could increase its affinity for binding especially with β -amyloid, which has a hydrophobic core (45).

Resveratrol and catechin inhibit β -amyloid

Tagliazucchi and coworkers (39) showed that resveratrol and catechin have protective synergistic effects against the cytotoxicity of β -amyloid_{1–41}. On the other hand, while utilizing oxidative stress induced by peroxide alone, no synergistic effect was obtained. The authors suggested that these polyphenols act not only by their antioxidant activity, but also that structural differences appear to be relevant when cell toxicity is due to hydrogen peroxide (39). Similar results were shown by other groups (46,47).

Tannic acid inhibits β -amyloid and prion PrP^{Sc}

Yamada and coworkers (48) characterized tannic acid as a potential inhibitor of β -amyloid fibrillization and showed a dose-response inhibition of β -amyloid assembly from monomeric β -amyloid into well-ordered fibrils. Tannic acid is a water-soluble polymeric polyphenol composed of phenolcarboxylic and gallic acids. Tannic acid did not extend the length of the lag phase, but decreased the rate of polymerization (48). Tannic acid was also described as the most

potent polyphenol inhibitor of prion PrP^{Sc} (the infectious form of prion protein) by Caughey and coworkers (49). By the use of high-throughput screening in cell culture system and solid-phase cell free PrP^{Sc} conversion assay, this group had identified several polyphenol inhibitors with IC₅₀ values smaller than 1 μ M, and tannic acid was the most potent inhibitor with an IC₅₀ value of 0.1 μ M (49).

Wine-related polyphenols inhibit β -amyloid

Another direct *in vitro* observation of the anti-amyloidogenic effect of polyphenols was described by Yamada and coworkers (50). This group had used thioflavin T assay and electron microscopy to examine the effect of wine-related polyphenols on the formation, extension, and destabilization of β -amyloid_{1–40} and β -amyloid_{1–42}. The examined polyphenols dose-dependently inhibited the formation of fibrils, with activity in the following order: myricetin = morin = quercetin > kaempferol > catechin = epicatechin (Table 2), and IC₅₀ values that are in the range of 0.1–1 μ M (detailed in Table 1). The authors concluded that "it may be reasonable to speculate that red wine derived polyphenols could prevent the development of Alzheimer disease, not only through scavenging of reactive oxygen species, but also through directly inhibiting the deposition of fibrillar β -amyloid in the brain" (50).

Curcumin and rosmarinic acid inhibit β -amyloid

Curcumin and rosmarinic acid as efficient *in vitro* amyloid inhibitor polyphenols were suggested primarily by Yamada and coworkers (51). In this paper, they proposed that the compact and symmetric structure of curcumin and rosmarinic acid might be suitable for specific binding of free β -amyloid and inhibition of its polymerization into the fibrillar form (specific IC₅₀ values are detailed in Table 1) (51).

These findings were re-evaluated by Cole and coworkers (52), who studied the effect of curcumin on β -amyloid, and implied that curcumin inhibits the formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid *in vivo*. In this study, the structural similarity of curcumin to Congo red is mentioned as a possible factor for its inhibition characteristics (52). Furthermore, the structural similarity of curcumin and chrysin G, a brain permeable compound, is mentioned as a possible factor for blood-brain barrier permeability. It is also shown that curcumin has structural similarity to a β -sheet breaker, *N,N'*-bis(3-hydroxyphenyl)pyridazine-3,6-diamine (named RS-0406), which was found using high-throughput screening of approximately 113,000 compounds. Compound RS-0406 was described to significantly inhibit 25 μ M β -amyloid_{1–42} fibrillogenesis (53). Cole and coworkers have used a special enzyme-linked immunosorbent assay and TEM to monitor β -amyloid_{1–40} assembly, and used a specific oligomer antibody to show that curcumin effectively blocks the formation of soluble β -amyloid oligomers (52).

Furthermore, they had demonstrated that curcumin stains hippocampus sections, thus concluding that like Congo red and thioflavin T, curcumin binding is not sequence-dependent but rather conformation-dependent. One of the overall conclusions of this work was that "it is possible that a subset of other phenolic antioxidants

Polyphenol	Studied protein	IC ₅₀ <i>in vitro</i> , μM (ref)
NDGA	β-Amyloid ₁₋₄₀	0.14 (50), 0.16 (51)
	β-Amyloid ₁₋₄₂	0.86 (50), 0.74 (51)
Curcumin	β-Amyloid ₁₋₄₀	0.81 (52) 0.19 (51)
	β-Amyloid ₁₋₄₂	0.63 (51)
Rosmarinic acid	β-Amyloid ₁₋₄₀	0.29 (51)
	β-Amyloid ₁₋₄₂	1.1 (51)
Resveratrol	β-Amyloid _{25-35,1-40,1-42}	n.d. <i>in vitro</i> (46,47,68)
Dobutamine	α-Synuclein	n.d. <i>in vitro</i> (44)
Myricetin	β-Amyloid ₁₋₄₀	0.29 (50), 0.9 (54) 0.2 (51)
	β-Amyloid ₁₋₄₂	0.40 (50), 0.34 (51)
Morin	β-Amyloid ₁₋₄₀	0.24 (50)
	β-Amyloid ₁₋₄₂	0.67 (50)
Quercetin	β-Amyloid ₁₋₄₀	0.24 (50)
	β-Amyloid ₁₋₄₂	0.72 (50)
Exifone	β-Amyloid ₁₋₄₀	0.7 (54)
	Tau ₄₁₂	3.3 (54)
Kaempferol	β-Amyloid ₁₋₄₀	1.7 (50)
	β-Amyloid ₁₋₄₂	3.2 (50)
2,3,4,2',4'-Pentahydroxybenzophenone	β-Amyloid ₁₋₄₀	2.8 (54)
	Tau ₄₁₂	2.4 (54)
Gossypetin	β-Amyloid ₁₋₄₀	1.3 (54)
	Tau ₄₁₂	2.0 (54)
Purpurogallin	β-Amyloid ₁₋₄₀	0.5 (54)
	Tau ₄₁₂	5.6 (54)
THBP (2,3,4-Trihydroxybenzophenone)	β-Amyloid ₁₋₄₀	3.1 (54)
	Tau ₄₁₂	12.2 (54)
Baicalein	α-Synuclein	4.0 (57)
Catechin	β-Amyloid ₁₋₄₀	2.9 (50)
	β-Amyloid ₁₋₄₂	5.3 (50)
Epicatechin	β-Amyloid ₁₋₄₀	2.8 (50)
	β-Amyloid ₁₋₄₂	5.6 (50)
	Tau ₄₁₂	>200 (55)
Phenolsulfonphthaleine	IAPP	1.0 (56)
	Insulin	1.5 (Levi, M. et al., unpublished data)
	β-Amyloid ₁₋₄₀	17 (Levi, M. et al., unpublished data)
Epicatechin gallate	β-Amyloid ₁₋₄₀	3.0 (54)
	Tau ₄₁₂	1.8 (54)
Epigallocatechin gallate	β-Amyloid ₁₋₄₀	0.18 (Levi, M. et al., unpublished data)
	Insulin	17.0 (Levi, M. et al., unpublished data)
	Calcitonin	4.9 (Levi, M. et al., unpublished data)
	Prion PrP ^{Sc}	n.d. <i>in vitro</i> (49)
Hypericin	β-Amyloid ₁₋₄₀	0.9 (54)
	Tau ₄₁₂	26.8 (54)
Tannic acid	Prion PrP ^{Sc}	0.1 (49)
	β-Amyloid ₁₋₄₀	0.012 (48)
	β-Amyloid ₁₋₄₂	0.022 (48)

n.d., not determined.

found in plant extracts, including ginkgo biloba and resveratrol from red wine, might be similarly protective and potentially contribute to AD risk reduction because of direct effect on β-amyloid aggregation" (52).

Green tea polyphenol epigallocatechin gallate inhibits prion PrP^{Sc} and β-amyloid

Since its early days, Chinese culture has made sacred the role of green tea in slowing the aging process. In modern science,

Table 1: IC₅₀ values of various polyphenol inhibitors *in vitro*

Caughey and coworkers (49) have shown that epigallocatechin gallate is an effective inhibitor of prion PrP^{Sc} fibril formation, and have emphasized the obligatory role of the third gallate group for the inhibition process. They also reported that radio-labeled epigallocatechin gallate was detected in mouse brain after oral administration (49). We have recently found that epigallocatechin gallate inhibits various amyloidogenic proteins *in vitro*, including β-amyloid, insulin, and calcitonin (Levi, M., Porat, Y. and Gazit, E., unpublished data).

Table 2: Structural similarities among various polyphenol inhibitors

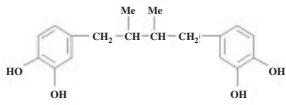
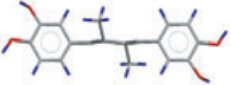
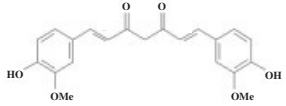
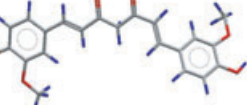
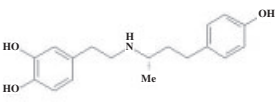
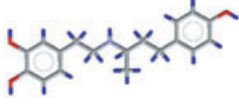
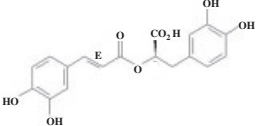
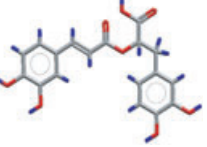
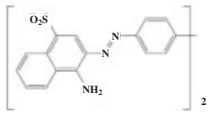
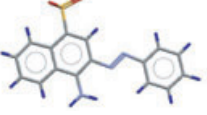
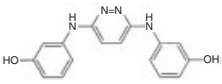
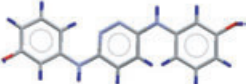
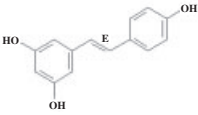
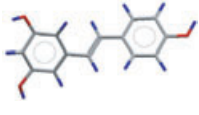
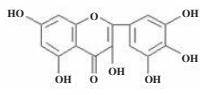
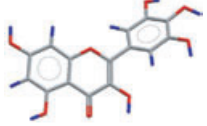
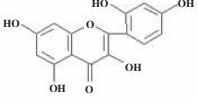
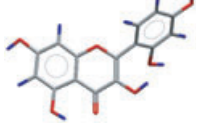
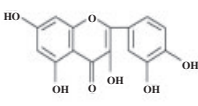
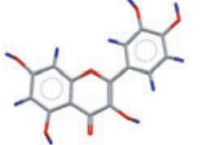
Polyphenol (ref)	2D structure	3D structure
NDGA ^a (50, 51)		
Curcumin ^a (51, 52)		
Dobutamine ^b (44)		
Rosmarinic acid ^b (51)		
Congo red ^a (19, 20, 32)		
RS-0406 ^b 3,3'-bis(3-hydroxyphenyl)pyridazine-3,6-diamine (53)		
Resveratrol ^a (39, 46, 47, 68, 73, 74)		
Myricetin ^a (50, 54)		
Morin ^a (50)		
Quercetin ^a (50)		

Table 2: Continued

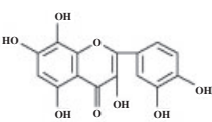
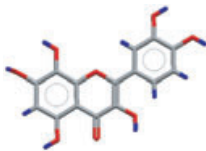
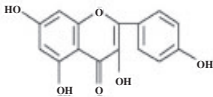
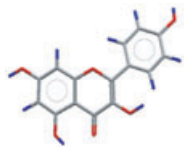
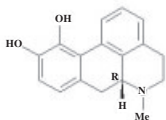
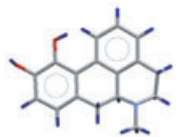
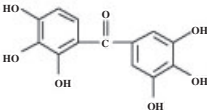
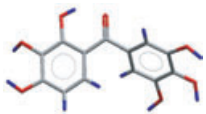
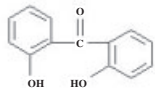
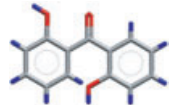
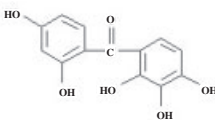
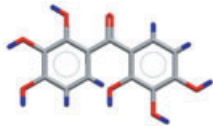
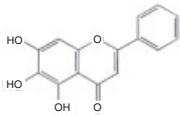

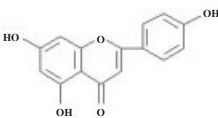
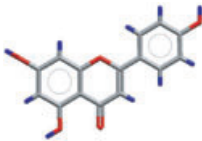
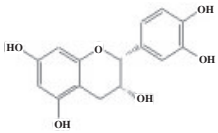
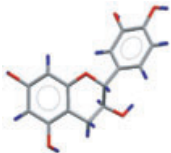
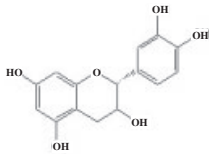
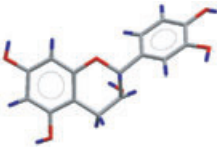
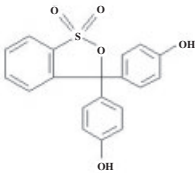

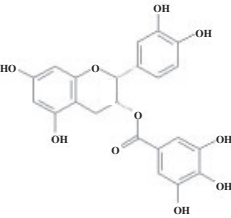
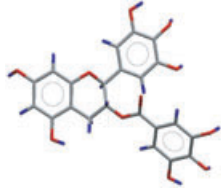
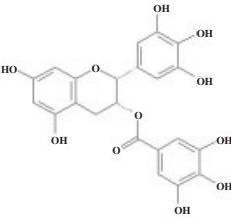
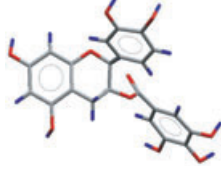
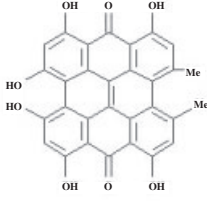
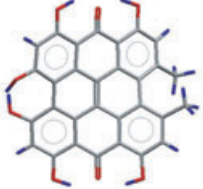
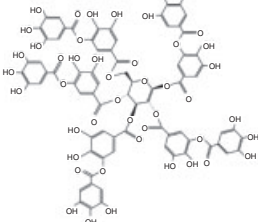
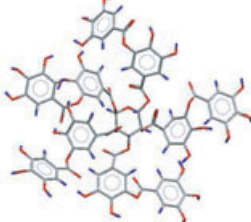
Gossypetin ^b (54)		
Kaempferol ^a (50)		
Apomorphine ^a (45)		
Exifone ^b (55)		
2,2'-Dihydroxybenzophenon ^b (54)		
2,3,4,2',4'-Pentahydroxybenzophenone ^b (54)		
Baicalein ^a (57)		
Apigenin ^b (54)		
Catechin ^b (50, 54, 71)		
Epicatechin ^a (50, 54, 71)		

Table 2: Continued

Phenolsulphonphthalein ^a (56)		
Epicatechin gallate ^b (49, Levi, M. et al., unpublished data)		
Epigallocatechin gallate ^b (49, 61, 62, 64, 65)		
Hypericin ^a (54)		
Tannic acid ^b (48, 49)		

^aX-ray coordinates

^b3D Model minimization

Purpurogallin, exifon, hypericin, myricetin, gossypetin, pentahydroxybenzophenon, epicatechin gallate, and 2,3,4-trihydroxybenzophenone (THBP) polyphenols inhibit Tau protein and β -amyloid

Hasegawa and coworkers (54) have studied the inhibitory effect of several small molecules, among them 12 polyphenol compounds, for their ability to inhibit the assembly of heparin-induced Tau protein and β -amyloid₁₋₄₀ into filaments. Tau protein inclusions are found in various neurodegenerative disorders, among them corticobasal

degeneration, frontotemporal dementia, Alzheimer's disease, and Parkinsonism (54). Good correlation was observed between the relative potencies of polyphenols in inhibiting the assembly of Tau protein and β -amyloid₁₋₄₀. The following polyphenols, purpurogallin, exifon, hypericin, myricetin, gossypetin, pentahydroxybenzophenon, epicatechin gallate, and THBP, were all found to inhibit both Tau protein and β -amyloid₁₋₄₀, with IC₅₀ in the low micromolar concentration range, with significant lower IC₅₀ values toward β -amyloid₁₋₄₀ inhibition (see Table 1). Using separation of monomeric and filamentous pro-

teins by ultracentrifugation and SDS gel electrophoresis, the authors demonstrated that myricetin was bound more strongly to oligomeric and filamentous Tau than to the monomeric form. They also showed that the polyphenol inhibitory compound does not influence the ability of Tau proteins' functionality to interact with microtubules. In summary, they concluded that "similar mechanisms may thus underlie β -amyloid and Tau assembly, and a given compound might be effective against both pathologies ..." (54).

Phensulfonphthaleine inhibits islet amyloid polypeptide, insulin, and β -amyloid

Phensulfonphthaleine is a synthetic polyphenol commonly used as a pH indicator in cell culture media. Islet amyloid polypeptide (IAPP) is a 37-residue hormone peptide that is co-secreted with insulin in the pancreas and found in large deposits in type 2 diabetes patients; it was recently recognized as one possible reason for the failure of β -cells in type 2 diabetes (55). Gazit and coworkers (56) have shown that phensulfonphthaleine dose-dependently inhibited fibril formation by IAPP *in vitro*, using thioflavin T fluorescence, circular dichroism, and electron microscopy with an IC_{50} value of 1 μM . Furthermore phensulfonphthaleine inhibited IAPP cytotoxicity toward pancreatic rodent β -cells, and increased cell viability after external addition of IAPP to the cell growth medium by 30% (56). Recent results from our group show that IAPP inhibits insulin, and β -amyloid with IC_{50} in the low micromolar values as well (Levi, M., Porat, Y. and Gazit, E., unpublished data).

Baicalein inhibits α -synuclein

Fink and coworkers (57) have identified baicalein, a flavonoid compound from Chinese herbal medicine, as an inhibitor of α -synuclein, which was mentioned earlier in the context of Parkinson's disease. Baicalein was described to mostly inhibit the elongation phase, although delayed addition of baicalein to seeded α -synuclein without a lag phase did not alter the elongation rate. It was also claimed that oxidation of baicalein to its quinone form increased its inhibition capacity with IC_{50} in the low micromolar range (Table 1). The authors conclude that the antioxidation potential of baicalein is not directly involved in the inhibition of fibrillation, as other effective antioxidants did not have any inhibition of fibrillation (57,58).

Polyphenols that inhibit amyloid cytotoxicity

Various studies have shown the protective effect of polyphenols against amyloid cytotoxicity in cell culture and primary culture systems; for recent review, see Ref. (57). Most of the studies have demonstrated the antioxidative features of polyphenols as the primary mechanism of cell rescue, and the reported values of effective polyphenols concentration in the range of 5–50 μM (39,46,49,53,59–71).

Discussion

Several mechanisms were previously suggested to explain polyphenol-induced protection against amyloid assemblies' cytotoxicity [for a recent review, see Ref. (72)]. For example, resveratrol has been

shown to possess significant free radical scavenging properties in a variety of cellular types (73). Other polyphenols were reported to act as antioxidants against nitric oxide induced toxicity (50). Besides its antioxidant properties, various intracellular signaling mechanisms have been suggested to be involved, at least partly, in the neuroprotective effects of polyphenols (50). For example, resveratrol was shown to induce the activation of MAP kinase in cells (74).

Nevertheless, in some of the data reported here, several authors have shown that *in vitro* inhibition of amyloid formation is not dependent on oxidative conditions. Furthermore, several authors have reported that structural properties of the polyphenol compounds should be considered as affecting their inhibitory effect. We therefore propose that antioxidative properties of polyphenols cannot provide a central mechanistic basis for the inhibitory process using *in vitro* conditions. No correlation was found between *in vitro* inhibitory IC_{50} values and antioxidative features of the reported polyphenols. For example, epicatechin is an efficient antioxidant [2.54 times higher than vitamin C (43)], but not an efficient inhibitor of amyloid formation (Table 1). On the other hand, morin has relatively low antioxidative features [1.65 higher than vitamin C (43)] and high efficiency for amyloid inhibition. Furthermore, several studies have shown that polyphenols do not inhibit cell death by hydrogen peroxide or other oxidative stress factors (39,46,47).

Therefore, we suggest a new mechanistic approach based on structural similarities between various highly efficient polyphenol inhibitors, and relative to the well-known amyloidogenic dye Congo red. All the efficient polyphenol inhibitors are composed of at least two phenolic rings with two to six atom linkers, and a minimum number of three OH groups on the aromatic rings. We suggest that these structural similarities imply for three-dimensional conformations that are essential for the non-covalent interaction with β -sheet structures, which are common to all amyloidogenic structures. This interaction may occur only when the native conformation of the amyloidogenic proteins transforms to the "assembly" conformation and cannot therefore occur with the folded native protein.

Several studies have reported that certain polyphenols mostly inhibited the elongation phase of fibril assembly or the assembly of large oligomers, and did not interfere with early nucleation events. This observation is in agreement with the reports that polyphenols do not interact with the amyloidogenic monomer but rather interact with amyloidogenic structures, and that inhibitor binding is not sequence-dependent but rather conformation-dependent (52). These observations are also in agreement with results by Markwell and coworkers (75), who described a number of common structural features that seem to be associated with the inhibitory properties of β -amyloid. These authors suggested, using molecular modeling data, that several polyphenol compounds have in common the ability to adopt a specific three-dimensional pharmacophore conformation that might be essential for binding to β -amyloid and preventing it from forming typical amyloid fibrils (75).

We have previously suggested that aromatic residues, and especially phenylalanine and tryptophan, are relatively abundant in amyloidogenic sequences (24). These data were recently confirmed by Dobson

and coworkers (22), who evaluated the amyloidogenic propensity of all amino acids. We also suggested that aromatic interactions facilitate molecular recognition of amyloidogenic sequences and enhance directionality and orientation needed for the ordered self-assembly process, by enhanced fibril assembly kinetics (25,29,56). We imply that, as previously shown (21), using the right experimental conditions, almost any protein sequence can form amyloid fibrils and the presence of aromatic residues can enhance the fibrillation process.

Furthermore, we suggested that phenol rings have a different stacking mode as compared to benzene rings, and that this difference allows phenol rings to interact with amyloidogenic aromatic residues; on the other hand, it does not allow efficient fibril assembly (56). However, the phenolic ring by itself does not efficiently inhibit amyloid formation (54) and further structural elements are necessary for the specific interaction with the amyloidogenic β -sheet conformation, creating hydrogen bonds that enhance the stability of the inhibitor–protein complex.

Information regarding the inhibitory effect of polyphenols toward amyloidogenic proteins that lack aromatic compounds in their amyloidogenic core (e.g., huntingtin) is so far incomplete. Nevertheless, an initial illustration of the importance of aromatic residues in the core of amyloidogenic proteins is demonstrated in a comparative study between β -amyloid_{1–40}, which includes two consecutive phenylalanine residues in the amyloidogenic core, and Tau protein, which lacks aromatic residues in R(1–4) repeats of the amyloidogenic core (50). These authors have shown a significant difference in IC₅₀ values of various polyphenol inhibitors with significant higher concentration of the inhibitor required for efficient inhibition of Tau protein relative to β -amyloid_{1–40}. This result may be explained by the fact that Tau polyphenol inhibition is affected only by structural conformation interaction, but is not influenced by the aromatic interaction that targets the inhibitor to the amyloidogenic core, leading to lower inhibitor concentrations needed for inhibition.

In summary, the data reviewed in this article imply two general assumptions regarding the inhibition mechanism of amyloid protein fibril formation by small polyphenolic compounds: (a) specific structural conformation is necessary for β -sheet interaction and stabilization of the inhibition–protein complex; (b) aromatic interaction between the phenolic compound in the inhibitor molecule and aromatic residues in the amyloidogenic sequence may direct the inhibitor to the amyloidogenic core and facilitate interaction, but interfere with fibril assembly.

These assumptions should be highly relevant for the future *de novo* design of small molecule inhibitors for the treatment of amyloidogenic diseases.

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