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Conference Paper

The Inhibitory Effect of Resveratrol on Elastin Amyloidogenesis

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The role of polyphenols in the prevention of degenerative diseases is emerging in the last years. In this report, we will investigate *in vitro* the inhibitory effect of resveratrol on elastin amyloidogenesis. The effect of resveratrol on molecular structure was investigated by circular dichroism spectroscopy, while the inhibitory effect on self-assembly was evaluated by turbidimetry as a function of temperature and by atomic force microscopy.

1. Introduction

Research on the effects of dietary polyphenols on human health has developed considerably in the past ten years. It strongly supports a role for polyphenols in the prevention of degenerative diseases, particularly cardiovascular diseases and cancers [1]. Polyphenols are secondary metabolites produced by plants in order to defend against herbivores, attract insects by colors for insemination, and protect themselves from UV irradiations. The important presence of plants containing polyphenols in human diet is correlated to the intake as food of the producing plants. The main dietary sources of polyphenols are fruits and plant-derived beverages such as fruit juices, tea, coffee, and red wine [2]. Vegetables, cereals, chocolate, and dry legumes also contribute to the total polyphenol intake. Current evidence strongly supports a contribution of polyphenols in the prevention of cardiovascular diseases, cancers, and osteoporosis and suggests a role in the prevention of neurodegenerative diseases and diabetes mellitus [3].

Resveratrol is a nonflavonoid polyphenolic compound found in a large number of plant species (at least 72), a number of which are components of the human diet, including mulberries, peanuts, grapes, and red wines. Resveratrol exists as cis- and trans-isomeric forms, with trans to cis isomerization facilitated by UV exposure. Two phenol rings are linked by a styrene double bond to generate 3,4′,5-trihydroxystilbene (Figure 1). The trans-isomer of resveratrol displays *in vitro*, *ex vivo*, and/or *in vivo* a number of pharmacological effects. As a matter of fact, resveratrol has been the focus of a number of studies investigating its beneficial effects on neurological, hepatic, and cardiovascular systems [4].

Some of the effects are due to the widely studied antioxidant properties of polyphenols, even if recent studies have shown that the mechanisms of action of polyphenols go beyond the modulation of oxidative stress. Important biological activities involve downregulation of the inflammatory response through inhibition of synthesis and release of proinflammatory mediators, modification of eicosanoid synthesis, inhibition of activated immune cells, or inhibiting, such as iNOS and COX-2, via its inhibitory effects on NF-kB or the AP-1 [5, 6].

Further studies have shown the beneficial effect of resveratrol in the AD. Some studies have shown that resveratrol markedly lowers the levels of secreted and intracellular amyloid-β (Aβ) peptides produced from different cell lines. Resveratrol does not inhibit Aβ production, because it has no effect on the Aβ-producing enzymes β- and γ-secretases but promotes, instead, intracellular degradation of Aβ via a mechanism that involves the proteasome. These findings

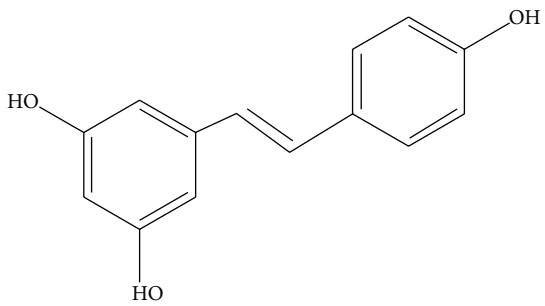

Figure 1: Resveratrol (trans-3,5,4′-trihydroxystilbene).

demonstrate a proteasome-dependent antiamyloidogenic activity of resveratrol and suggest that this natural compound has a therapeutic potential in Alzheimer's disease [7]. However, the exact molecular mechanisms involved in the beneficial effects of wine intake on the neurodegenerative process in Alzheimer's disease brain remain to be clearly defined.

Elastin is the extracellular protein responsible for elasticity in vertebrates. Its production occurs during the early neonatal period and then drops dramatically and is nearly completely repressed at maturity [8]. However, elastin degradation by proteolytic enzymes is observed in aging and in pathological processes. The resulting fragments are involved in physiological as well as in pathological tissue remodeling [9]. Recent studies have shown that elastin fragments resulting from cleavages by elastases in humans are able to give rise to amyloid-like fibrils, *in vitro* [10].

In this report, we will investigate the inhibitory effect of resveratrol on the elastin amyloidogenesis. The effect of resveratrol on molecular structure was investigated by circular dichroism (CD) spectroscopy, while the inhibitory effect on self-assembly was evaluated by turbidimetry as a function of temperature and by atomic force microscopy (AFM).

2. Methods

2.1. Peptide Synthesis and Purification. The S4 peptide was synthesized by solid-phase methodology and purified by RP-HPLC as previously described [10].

2.2. CD Spectroscopy. CD spectra of S4 peptide (0.1 mg/mL) were acquired at different temperatures with a Jasco J-815 Spectropolarimeter equipped with a HAAKE thermostat as temperature controller by using 0.1 cm path length quartz cell. Samples were equilibrated at the temperature for 5 min before acquisition. Spectra were acquired by taking points every 0.1 nm, with 100 nm/min scan rate, 16 scans, an integration time of 2 s, and a 1 nm bandwidth. The data are expressed in terms of $[\theta]$, molar ellipticity as deg cm^2 dmol^{-1}.

2.3. Turbidimetry Experiments. 1.5 mL of 2 mM solutions of the S4 peptide in 15% ethanol (EtOH) in TBS buffer [Tris (50 mM), NaCl (1.5 M), and $CaCl_2$ (1.0 mM) (pH 7.0)] was analyzed by turbidimetry at 440 nm as function of temperature on a Cary 50 UV spectrophotometer equipped with a Peltier temperature controller using quartz cells of 1 cm path length and reported as TAA (turbidimetry on apparent absorbance). The solution temperature was increased from 10 to 90°C with 5°C every 5 minutes and then decreased back to 10°C, monitoring the absorbance under stirring after 5 minutes to reach the equilibrium temperature. Resveratrol (1 mM) was added to the peptide solution. The effect of resveratrol was evaluated in the same experimental conditions.

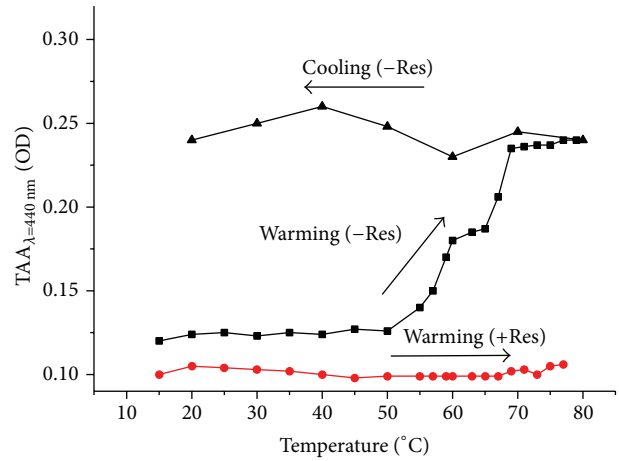

Figure 2: Turbidimetry as a function of temperature. Aggregation studies by turbidimetry of S4 peptide in 15% EtOH in TBS showing the warming cycle (■) and cooling (▲) cycle. The warming cycle of S4 peptide in 15% EtOH in TBS in the presence of resveratrol is shown (●).

2.4. Atomic Force Microscopy (AFM). After turbidimetry experiments, 10 μL of the suspension/solution of S4 peptide in the presence and absence of resveratrol was deposited on silicon (100) wafer substrate (Aldrich, Saint Louis, MO, USA). The samples were air-dried and repeatedly rinsed with ultrapure water in order to remove salts. After water evaporation, the AFM images were carried out by using the XE-120 microscope (Park Systems) in air and at room temperature. Data acquisition was carried out in intermittent contact mode at scan rates between 0.4 and 3 Hz, using rectangular Si cantilevers (NCHR, Park Systems) having the radius of curvature less than 10 nm and with the nominal resonance frequency and force constant of 330 kHz and 42 N m^{-1}, respectively, or diamond tips (MikroMasch) with typical spike curvature radius less than 7 nm, nominal resonance frequency of 325 kHz, and typical force constant 46 N m^{-1}.

3. Results

3.1. Amyloid Aggregation. The propensity of S4 peptide to self-assemble in aggregates was monitored by turbidimetry (Figure 2). The aggregation process is experimentally measured by spectrophotometry as an increase in the apparent absorbance value concomitant with heating to a critical temperature. To perform the aggregation studies in the

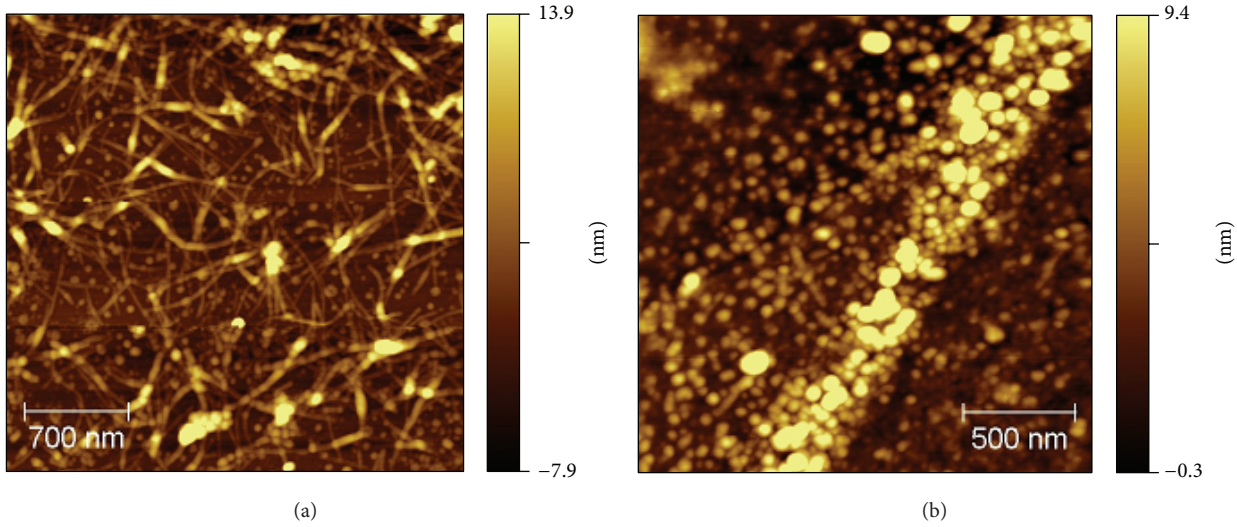

Figure 3: AFM images of S4 peptide deposited on Si (100) wafers after turbidimetry in the absence (a) and presence (b) of resveratrol.

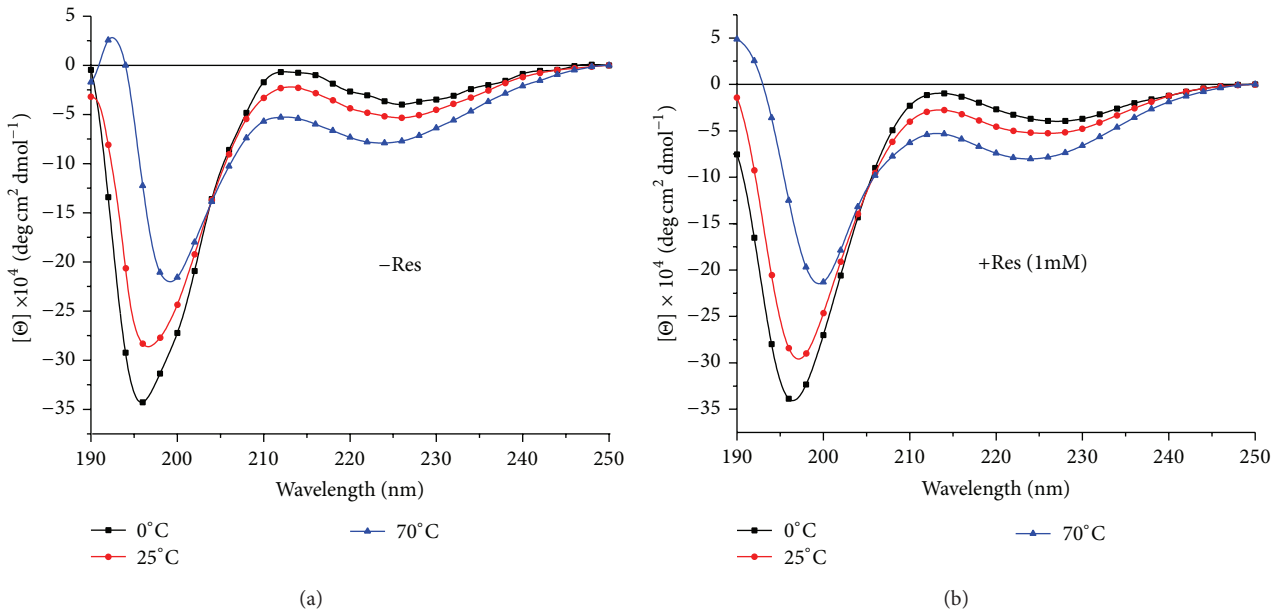

Figure 4: CD spectra of S4 peptide recorded in 15% EtOH in TBS at different temperatures in the absence (a) and presence (b) of 1 mM resveratrol.

presence of Res, the addition of an organic cosolvent (15% EtOH) was necessary, due to the low solubility of Res in TBS solution. As a consequence, we determined that the S4 peptide is able to form aggregates also in the presence of EtOH. The S4 peptide starts to aggregate at 55°C as observed by the gradual increase of the apparent absorbance until 70°C. The development of aggregates determines the formation of a cloudy suspension that scattering the light reduces its passage through the sample. In the presence of Res, the solution remains clear, without increase in the turbidity even after increasing the temperature to 80°C. Usually the temperature triggered the formation of amyloids because hydrophobic interactions together with H-bonds have a predominant role in stabilization of the aggregates. The samples after turbidimetry were investigated by atomic force microscopy, to assess the morphology of the formed aggregates (Figure 3). The sample without Res shows the formation of long and flexible fiber. Some globular structures in the background are observed, which were not present when we analyzed S4 peptide in TBS, suggesting that the cosolvent EtOH has a slight effect in disfavoring amyloid fiber. However, the AFM images acquired on the sample with Res showed only highly aggregated globular structures, confirming that Res has an inhibitory effect on the amyloid formation of the S4 peptide.

Previous studies have investigated the capacity of resveratrol to recognize and remodel different conformers

(monomers, soluble oligomers, nontoxic oligomers, fibrillar intermediates, and amyloid fibrils) of the Aβ1-42 peptide associated with Alzheimer disease and have found that resveratrol selectively remodels soluble oligomers, fibrillar intermediates, and amyloid fibrils into an alternative high molecular weight aggregated species that is nontoxic and unstructured [7].

3.2. CD Spectroscopy. To define the effect of the Res on the conformation of the peptide, CD spectra were recorded. CD spectroscopy is a useful tool able to define the peptide secondary structures, as well as the effect of ligands on the conformation of the peptide/protein. The conformational analysis of S4 peptides was previously performed and revealed the presence of significant amount of PPII conformation, whose content is reduced on increasing the temperature favoring the β-strand structure [10]. The spectra recorded in TBS with the presence of 15% EtOH show similar features. The CD spectrum of S4 peptide at 0°C was characterized by a strong negative band at 195 nm and by a small positive band at 215 nm (Figure 4(a)). The increase of the temperature to 25, 37, and 60°C induced a slight reduction of the positive band and a strong reduction of the negative one. The strong negative π-π^* band at 195 nm is usually attributed either to unordered or to the left-handed polyproline II conformation (PPII). However, its reduction in intensity on increasing the temperature, together with the presence of the π-π^* band at 215 nm, is diagnostic of the PPII conformation [11]. The spectra of S4 peptide recorded at different temperatures in the presence of resveratrol (1 mM) in 15% EtOH solution showed similar spectra, suggesting that resveratrol did not interact with the monomers in solution, or probably the interaction did not change the conformation of the peptide.

4. Conclusions

Recently, great effort was made in defining the protective properties of polyphenols, such as resveratrol. Many different effects were discovered that are working at various levels. In this work, we have shown a possible inhibitory effect of resveratrol on elastin amyloid deposition acting at molecular levels, which could be added among others to the possible mechanisms involved in the beneficial effects of resveratrol.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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