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Identification of an aspidospermine derivative from borage extract as an anti-amyloid compound: A possible link between protein aggregation and antimalarial drugs

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ABSTRACT

A number of human diseases, including Alzheimer's and Parkinson's have been linked to amyloid formation. To search for an anti-amyloidogenic product, alkaloid enriched extract from borage leaves was examined for anti-amyloidogenic activity using Hen Egg White Lysozyme (HEWL) as a model protein. After isolation of the plant extract using rHPLC, only one fraction indicated a significant bioactivity. TEM analysis confirmed a remarkable reduction of amyloid fibrils in the presence of the bioactive fraction. To identify the effective substance in the fraction, mass spectrometry, FTIR, and NMR were performed. Our analyses determined that the bioactive compound as 1-acetyl-19,21-epoxy-15,16-dimethoxyaspidospermidine-17-ol, a derivative of aspidospermine. To investigate the mechanism of the inhibition, ANS binding, intrinsic fluorescence, and amide I content were performed in the presence of the bioactive compound. All the results confirmed the role of the compound in assisting the proper folding of the protein. In addition, molecular docking indicated the aspidospermine derivative binds the amyloidogenic region of the protein. Our results show that the alkaloid extracted from borage leaves reduces protein aggregation mediating through structural elements of the protein, promoting the correct folding of lysozyme. Since a number of aspidospermine compounds have been shown to possess potent antimalarial activities, the action of compound identified in the present study suggests a possible link between protein aggregation and aspidospermine drugs.

1. Introduction

Life is dependent on the functions of proteins. In order for proteins to attain their functions, they need to fold into their proper tertiary structures. Without the proper folding, protein is nonfunctional. Protein folding is known to be a spontaneous process as long as the proper chemical and physiological conditions such as pH and temperature are met (Ohnishi and Takano, 2004; Amani and Naeem, 2013; Mahmudi et al., 2013). Inside the cell and in the extracellular matrix, there exist a number of proteins called molecular chaperones that help proteins achieve their proper folding. However, in certain conditions such as stress, molecular crowding, mutations, and diseased conditions, proteins tend to misfold and form an insoluble fibril called amyloid. There have been a number of human debilitating diseases in which one or two proteins or polypeptides misfold, leading to amyloid formation. Alzheimer, Parkinson, and Huntington represent some of the conformational diseases that have been linked to amyloid formation (Eisenberg and Jucker, 2012; Brodsky, 2014; Siddiqi et al., 2017). Although there have been a number of reports using synthetic compounds to inhibit protein fibrillation (Razavi et al., 2003; Bobylev et al., 2011; Siddiqi et al., 2017), one effective approach has been to look for inhibitors in natural products. Since plants are enriched in numerous small molecules, a number of plant extracts have shown promising medicinal remedies for several diseases, including neurodegenerative diseases such as Alzheimer (Stephen et al., 2009; Cragg and Newman, 2013; Taizong et al., 2015; Xian-Le et al., 2015). In addition, there has not been much amyloid formation reported in the plants; only reports of amyloid-like in transgenic plants have been documented (Villar-Piqué et al., 2010). The lack of high frequency amyloid formation in plants compared to humans may suggest that plants may possess special mechanisms to combat protein misfolding.

One of the remarkable plants that has been used for its

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medicinal and pharmacological applications throughout the world is *Borago officinalis* (Boraginaceae). The plant is native to India and Iran and its leaves have been used as tea for years in the Middle East. Some of the reported medical effects of the plant include hyperactive gastrointestinal, respiratory, and cardiovascular disorders. In addition, borage is known as a source of alkaloids, demonstrating antibacterial and antioxidant activities mainly from its stems (Gilani et al., 2007; Asadi-Samania et al., 2014). One of our goals has been to look into the extract of these medicinal plants for anti-amyloid bioactive natural products. Hen Egg White Lysozyme (HEWL) was used in the present investigation as a model protein for examining the effects of the plant extract; the amyloid formation of HEWL has been well studied, and a number of investigations have used the protein to examine anti-amyloid activities of variety of bioactive compounds (Morshedi et al., 2007; Holmes and Thompson, 2008; Jing et al., 2009; Jianwei et al., 2014). HEWL is a relatively small (14.5 kD) protein that forms amyloid fibril at high temperature and low pH (Frarel et al., 2004). The kinetic of amyloid formation consists of three phases: a lag phase where nucleus formation takes place, an exponential phase where soluble oligomers are made, and plateau in which maximum protein fibrils, 2–10 nm in diameters, are formed (Frarel et al., 2004; Kalhor et al., 2009).

Seeking a natural product having anti-amyloid activity, initially we used the total extract enriched in alkaloids from borage leaves. This showed a reduction of HEWL amyloid formation. In order to pinpoint the chemical identity of anti-amyloid compound in the extract, the extract was fractionated using rHPLC. One fraction showed the highest anti-amyloid activity. Using several analytical and spectroscopic approaches, including GC-MS, NMR, and FTIR, the bioactive material was identified to be an aspidospermine derivative: 1- acetyl-19,21-epoxy-15,16-dimethoxyaspidospermidine-17-ol To further understand the biological effects of the bioactive compound, the conformational change, the protein core hydrophobicity and protein intrinsic fluorescence were measured in the presence of this compound. Using molecular docking, the alkaloid was shown to interact with the amyloidogenic region of the protein. To the best of our knowledge, this is the first report on the anti-amyloiodogenic effect of an aspidospermine derivative. In addition, since a number of aspidospermines, including aspidoscarpine, are known as potent antimalarial drugs, a possible link between the aspidospermine compounds and protein amyloid formation can be suggested.

2. Results

2.1. Borage leaf extract reduces protein amyloid formation

The initial approach was to examine the effect of total extract from borage leaves, enriched in alkaloids, for inhibition of amyloid formation. One of the effective methods to analyze the intermediates formed in amyloid fibrillation has been agarose gel electrophoresis of protein fibrillation (Kalhor et al., 2009). In the gel electrophoresis method, the three phases in the process of amyloid formation are detectable; that is, the lysozyme monomer at its specific size of 14.5 kD, soluble oligomers as a heterogeneous smear-like appearance, and fibrils as large fragments that are entrapped within wells of the agarose gel due to their large mass. As shown in Fig. 1, panel A, the amyloid formation assay (see Experimental for details) was performed in the presence and absence of the total alkaloid extract and further examined by agarose gel electrophoresis. Based on the results, as the incubation time increased, the control reaction containing only the lysozyme protein showed a steady increase in amyloid formation, conversion of monomer to oligomers (smear-looking) to large insoluble fragments that are entrapped in the wells of the gel (lanes 1, 3, 5, 7, 9 in panel A). However, the reaction containing the alkaloid extract showed retarded progression into fibril formation. Indeed, the samples that were examined at 196 h and 220 h (panel A, lanes 8 and 10 respectively) showed significant reductions of oligomers. To obtain a quantitative measurement of the effect of the total extract on the protein amyloid formation, Thioflavin-T (ThT) binding was performed on the same samples that were examined by gel electrophoresis. The results of ThT binding indicated that in the presence of borage total alkaloid extract, the rate of the amyloid formation was significantly reduced (Fig. 1, panel B). Moreover, when the alkaloid-enriched extract of borage leaves from a different region of Iran (Qazvin) was examined for comparison, there was not much difference in its anti-amyloid inhibition (Fig. S1).

2.2. Isolation of the fraction possessing anti-amyloid bioactivity

In order to determine the chemical identity in the total extract responsible for the amyloid inhibition, the extract was fractionated using rHPLC; eighteen well-separated fractions were obtained after the rHPLC runs (Fig. S2). These were tested for anti-amyloid activity by ThT binding assay. Fraction eleven indicated a substantial anti-amyloid inhibitory activity as indicated by the reduction of emitted fluorescence upon ThT binding (Fig. 2). The lag times were calculated 91.3 h for the kinetics of protein fibrillation in the absence of the fraction (control), and 122.7 h in the presence of fraction 11. These results represent a significant extension of the lag time in the presence of the fraction. The maximum percent yield of fibril reduction in the presence of the bioactive fraction was near 40%. To further analyze the inhibitory effects of fraction eleven, the protein aggregation reactions at various time points in the presence or absence of fraction eleven were examined by agarose gel electrophoresis. The sample containing fraction 11 as compared to control, showed a substantial inhibition of oligomers and fibrils formation (Fig. 3, panel A, lanes 8, and 10 as compared to lanes 7 and 9). The results of gel electrophoresis along with ThT binding assay agreed remarkably well together, confirming the inhibitory effect of fraction 11.

2.3. TEM analysis of the fraction containing bioactive material showed significant amyloid fibril reduction

To further characterize the anti-amyloid activity of the compound in the fraction eleven, the protein samples (from the late stage of amyloid formation) in the presence of fraction 11 and in the absence of the fraction, were examined by Transmission Electron Microscopy (TEM). In the absence of the fraction, there existed numerous fibrils with a diameter ranging from 5-13 nm (Fig. 3, panel B). However, in the presence of fraction 11, the amount of fibril formation was markedly reduced (Fig. 3, panel B, numbers 3 and 4).

2.4. Identification of the bioactive compound as an aspidospermine derivative

In order to determine the identity of compound present in fraction eleven, which displayed anti-amyloid activity, the fraction was initially subjected to GC-MS (Fig. S3). The mass analysis of the fraction predicted a compound with a mass of 414 *m/z*. The mass data confirmed the identity of an alkaloid compound with 90% similarity to the aspidospermine derivative 1-acetyl-19,21-epoxy-15,16-dimethoxyaspidospermidine-17-ol (Fig. S4, inset). To further characterize the compound in fraction eleven, after purification using preparative TLC, FTIR analysis was performed. Various functional groups present in the compound were identified, including