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Research paper

Identification of plant extracts that inhibit the formation of diabetes-linked IAPP amyloid

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ABSTRACT

The extracts of 27 vegetables, spices and herbs were screened for their functional ability to inhibit the aggregation of islet amyloid polypeptide (IAPP, amylin) into toxic amyloid aggregates. The aggregation of IAPP has been directly linked to the death of pancreatic β-islet cells in type 2 diabetes. Inhibiting the aggregation of IAPP is believed to have the potential to slow, if not prevent entirely, the progression of this disease. As vegetables, spices and herbs are known to possess many different positive health effects, the extracts of 27 plants (abundant within the United States and Europe and spanning several plant families) were screened for their ability to inhibit the formation of toxic IAPP aggregates. Their anti-amyloid activities were assessed through (1) thioflavin T binding assays, (2) visualization of amyloid fibers using atomic force microscopy and (3) cell rescue studies. From this research, mint, peppermint, red bell pepper and thyme emerged as possessing the greatest anti-amyloid activity.

1. Introduction

The natural molecular components of plants are known to possess a wide range of functional and medicinally beneficial activities. Extracts of vegetables, spices and herbs have been identified as having beneficial health activities. The major organic compounds in herbs, spices and vegetables have been studied for their antioxidant, anti-tumour and anti-aging properties (Bai et al., 2011; Deng et al., 2013; Manchali et al., 2012). The American Diabetes Association lists many non-starchy vegetables, spices and herbs on their list of best choices for foods for people with diabetes. The authors screened and assessed extracts of edible plants and herbs for their ability to inhibit the formation of toxic aggregates of the diabetes-linked protein islet amyloid polypeptide (IAPP). To the best of our knowledge, this is the first study to directly assess the ability of these plant products to inhibit IAPP aggregation.

Type 2 diabetes afflicts approximately 25.8 million Americans (approximately 8.3% of the population (Bai et al., 2011)), with nearly 2 million new cases each year. While the underlying causes of type 2 diabetes remain unclear, one of the factors contributing to the progression of this disease is the formation of protein aggregates of the amyloid protein IAPP. These aggregates have been shown to be highly toxic to mammalian cells, especially the insulin-producing β-cells of the pancreas (Montane et al., 2012). It is believed that preventing the formation of IAPP amyloid could slow, if not prevent, the progression of type 2 diabetes (Bram et al., 2014).

The exact structure of the IAPP aggregates is not fully understood. It is known that IAPP can aggregate into a variety of amyloidogenic states (Abedini and Schmidt, 2013; Apostolidou et al., 2008; Hull et al., 2004; Kahn et al., 1999). It is apparent that some of these aggregates, termed small soluble oligomers, are highly toxic to mammalian cells (Andrews et al., 2009; Cao et al., 2013; Nanga et al., 2008). Work by Eisenberg and coworkers and Ramamoorthy and coworkers suggested that the most toxic form of IAPP may be a cylindrin structure (Laganowsky et al., 2012; Liu et al., 2012; Patel et al., 2014). Regardless of the exact structure, IAPP aggregates play a role in the loss of pancreatic β-cells and the progression of type 2 diabetes (Ritzel et al., 2007).

The extracts of 27 vegetables, spices and herbs were screened for their ability to inhibit IAPP aggregation (Table 1). These plant products were chosen because of their relative abundance within the United States and Europe and because of their distribution across different families of plant products (Table 1). The extraction

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Table 1
Plant extracts screened for their ability to inhibit IAPP aggregation.

Spices/herbs	Genus	Species	Family
Red pepper	*Capsicum*	*annum*	Solanaceae
Mint	*Mentha*	*sachalinensis*	Lamiaceae
Cumin	*Cuminum*	*cyminum*	Apiaceae
Turmeric	*Curcuma*	*longa*	Zingiberaceae
Cilantro	*Coriandrum*	*sativum*	Apiaceae
Parsley	*Petroselenium*	*crispum*	Apiaceae
Sage	*Salvia*	*officinalis*	Lamiaceae
Thyme	*Thymus*	*vulgaris*	Lamiaceae
Dill	*Anethum*	*graveolens*	Apiaceae
Rosemary	*Rosmarinus*	*officinialis*	Lamiaceae
Garlic	*Allium*	*sativum*	Amaryllidaceae
Huacatay	*Tagetes*	*minuta*	Asteracease
Peppermint	*Mentha*	*piperita*	Lamiaceae
Vegetables			
Eggplant	*Solanum*	*melongena*	Solanaceae
Potato	*Solanum*	*tuberosum*	Solanaceae
Onion	*Allium*	*cepa*	Amaryllidaceae
Kale	*Brassica*	*oleracea*	Brassicaceae
Red bell pepper	*Capsicum*	*annuum*	Solanaceae
Brussel sprouts	*Brassica*	*oleracea*	Brassicaceae
Green bean	*Phaseolus*	*vulgaris*	Fabaceae
Arugula	*Eruca*	*sativa*	Brassicaceae
Carrot	*Daucus*	*carota*	Apiaceae
Bok choy	*Brassica*	*rapa*	Brassicaceae
Yellow bell pepper	*Capsicum*	*annuum*	Solanaceae
Jalapeno	*Capsicum*	*annuum*	Solanaceae
Broccoli	*Brassica*	*oleracea*	Brassicaceae
Cauliflower	*Brassica*	*oleracea*	Brassicaceae

method, described below, produced active extracts while removing over 99% of the carbohydrates. As people afflicted with type 2 diabetes must limit their intake of carbohydrates, this extraction method would be suitable for yielding bioactive products with very low carbohydrate content.

2. Materials and methods

2.1. Preparation of the extracts

2.1.1. The vegetable extracts

100 g of each individual fresh vegetable was ground using a mortar and pestle mixed with 100 mL of ethyl acetate. The resulting slurry was filtered and the ethyl acetate layer separated. The ethyl acetate fraction for each vegetable was separated into 15 equal aliquots and speed vacuumed to dryness. These dehydrated extracts were stored at −20 °C. Extracts were rehydrated by re-suspending each aliquot in 150 μL of 20 mM tris buffer (pH 7.4).

2.1.2. The herb extracts

Samples were prepared as described above for vegetable extracts, except that 25 g of each individual herb/spice was ground using a mortar and pestle mixed with 100 mL of ethyl acetate. 2-mL aliquots were speed vacuumed to dryness and stored at −20 °C. These samples were rehydrated by re-suspension in 150 mL of 20 mM tris buffer (pH 7.4).

2.2. Preparation of IAPP stock solutions

IAPP stock solutions were prepared by dissolving 1 mg of synthetic amylin (Anaspec Corp. Fremont, CA, USA) in 8 mL of hexafluorisopropanol (HFIP, Sigma–Aldrich, St. Louis, MO, USA). This IAPP solution was fully disaggregated by sonicating in a 25 °C water bath for 5 min. The resulting IAPP stock solution was stored at −80 °C.

2.3. Thioflavin T binding assays

Aliquots of the IAPP stock solution were placed in glass tubes and placed under speed vacuum to remove the HFIP solvent. The resulting dry IAPP samples were re-suspended in 20 mM tris buffer

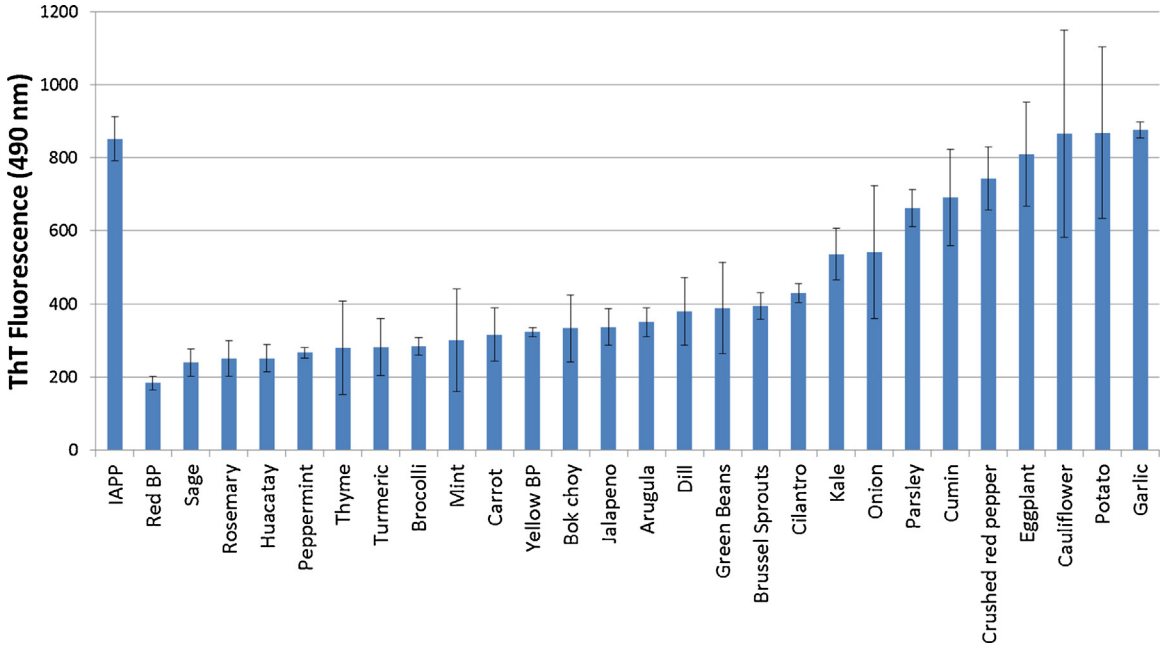

Fig. 1. Thioflavin T fluorescence of IAPP alone and IAPP in the presence of each food extract. Fluorescence was measured after 25 min incubation under conditions known to promote IAPP aggregation. IAPP concentration was 106 μM for each sample. Data is the average of at least 3 data sets. Error bars show the standard deviation from the average of three or more data sets.