

The Health Benefits of Soybeans and Bowman-Birk Inhibitor Concentrate

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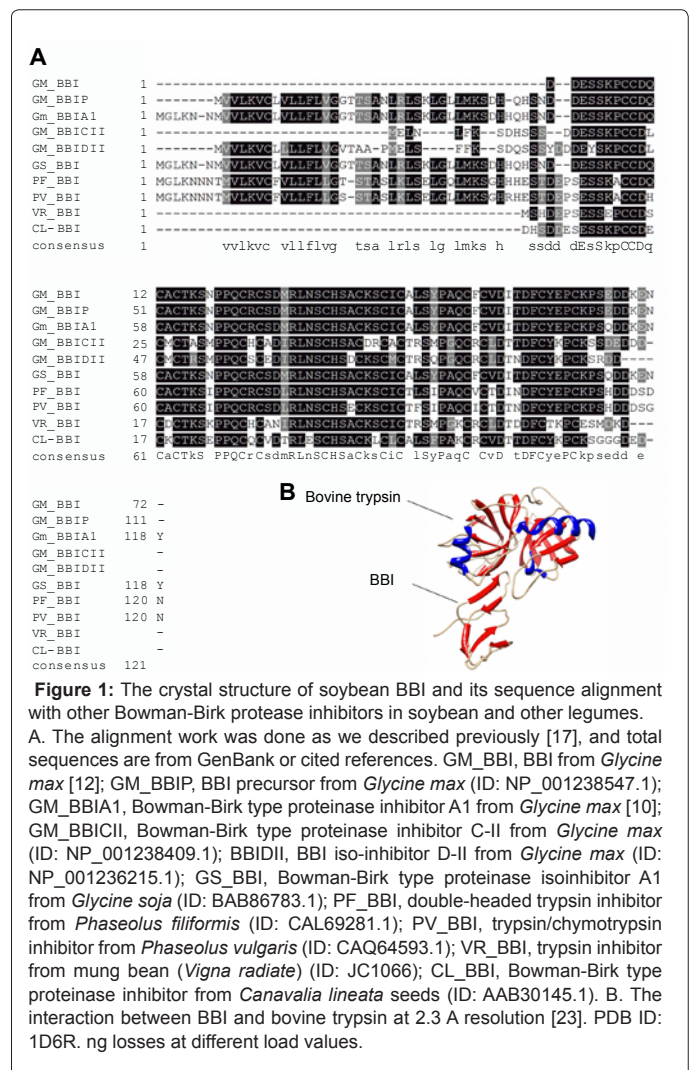
Introduction

Soybean (*Glycine max* L.) is a common and widely-distributed crop, its different dietary products are highly welcomed, especially to those living in the Orient [1,2]. Many medicinal components exist in soybean, such as lectins [3], Kunitz-type trypsin inhibitors (named under its pioneer investigator Moses Kunitz) [4-6], glyceollins [7], defensins [8], and Bowman-Birk inhibitor (BBI) and its isoforms [9,10], etc. Among them, BBI has attracted extensive investigations by an enlightening multi-group effort in the east and west. The first study traced back to 1946 when Dr. Donald Bowman isolated an inhibitor different from the soybean Kunitz trypsin inhibitors, and its further purification and characterization was accomplished by Dr. Yehudith Birk. In recognition of their pioneer work on this protein, Frattali in 1969 recommended to name it 'Bowman-Birk inhibitor' [11]. In 1972, the full sequence of BBI was revealed by Odani and Ikenaka [12]. Later, the numerous medicinal activities of BBI were evaluated in the laboratory of Ann Kennedy for over 3 decades [13].

There are many isoforms of BBI in soybean. It is estimated that protease inhibitors constitute 6% of all the soybean protein. BBI makes up about 4% while its isoforms and others account for 2%. In 1977, Odani and colleagues reported that there were five major BBIs, named A, B, C-II, D-II, and E-I [9,10]. Later, based on intensive work as well as sequence data, it was indicated that iso-inhibitor A is BBI [12], iso-inhibitor-B is similar to iso-inhibitor-A, and iso-inhibitor D-II is the precursor of iso-inhibitor E-I [10]. In view of this, Deshimaru and colleagues recommended that soybean BBI and its iso-inhibitors be classified into three major types: BBI (iso-inhibitor A which targets trypsin/chymotrypsin), iso-inhibitor C (which inhibits elastase/trypsin), and iso-inhibitor D with trypsin/trypsin inhibitory activity [10]. In addition to Bowman-Birk groups, the soybean Kunitz-type inhibitors are also in the limelight, details of their biochemical characteristics, sequences, structures, and medicinal activities are shown elsewhere [4,5,14,15].

Biochemical characteristics of BBI

Natural compounds are promising drug candidates [16-21]. BBI is a 8-kDa protein and consists of 71 amino acids linked by 7 disulfide bonds. It exhibits both trypsin- and chymotrypsin-inhibitory activities. Its chymotrypsin inhibitory activity is more pH-dependent: the activity is blocked at pH 5.3 but can be recovered at pH 7.0 [12]. BBI forms a 1:1 complex with either trypsin or chymotrypsin, in which Lys 16-Ser 17 bind to trypsin and Leu 43-ser 44 bind to chymotrypsin to exhibit its inhibitory activity [22]. In figure 1A, a sequence alignment among the soybean BBI, its iso-inhibitors, and Bowman-Birk type inhibitors of other origins, shows that there is high sequence homology among them which unveils the universal existence of this group of proteins. The crystal structure of BBI has been generated and its interaction with bovine trypsin is shown in figure 1B [23]. As shown in the fig, the triple-stranded β -hairpin and the surface loops surrounding the active site of the enzyme form a protein-protein interface [23].



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Health benefits of BBIC

The nutritional and health benefits of soybeans have been extensively investigated in the past decades. Results from laboratory, clinical, and epidemiological studies suggest that BBI is a potent chemopreventive natural compound for combating human cancer [24]. Twenty years ago, the purification of pure BBI (PBBI) was costly and by no means an easy task which in turn made clinical trials difficult to proceed. In 1993, Prof. Kennedy and coworkers reported a simple protocol to produce a BBI concentrate (BBIC) which contained predominantly BBI and manifested the same biochemical and antitumor activities as PBBI, and could serve as a substitute of BBI for later clinical trials [25]. A milestone for BBIC was set in 1992 when it achieved Investigational New Drug (IND) Status with the FDA.

The health benefits of BBI, especially prevention of carcinogenesis, have been extensively investigated. Studies on its antitumor potential have been conducted on different tumor cells, some tissues/organs, and three species, with satisfactory results [24]. Mechanisms of its action may include its protease inhibitory activity, and the inhibition of the initiating event in carcinogenesis, such as inhibition of *c-myc* and *c-fos* [22,24]. In addition to antitumor activity, BBI/BBIC also exhibits other health-promoting effects, such as suppression of experimental autoimmune encephalomyelitis up-regulation of IL-10, anti-inflammatory activity, prevention of hair and weight loss in animals with cancer, prevention of exencephaly caused by radiation, prevention of muscle atrophy in artificial microgravity and in animals with Duchenne muscular dystrophy, and most noteworthy of all, extension of life span [26-28].

The satisfactory bench top results on BBI/BBIC have risen to the human trial stage. Until now, 6 human trial works have been carried out by Dr. Kennedy's group and others for the following endpoints: cancer prevention on oral leukoplakia, treatment of benign prostatic hyperplasia, prostate cancer detection and treatment, treatment of ulcerative colitis, gingivitis, or esophagitis (and/or alleviation of adverse side effects of lung cancer treatment) [13, 27]. Patients were treated with doses of BBIC up to 1066 C.I. units per day for 6 months, and in Phase IIB trial of leukoplakia the duration was extended to 1.5 years, mostly with gratifying results though some side effects were noticed. Please see references [27,29-31] for details. Further work on these aspects will no doubt expand the applications of BBIC either as a drug/alternative medicine or special diet.

Closing remarks

Twenty years later, a new method to purify PBBI has been established. Very recently, Muzard and coworkers reported a simple and extremely fast method for purification of PBBI. By the application of a carboxyl-coated SMPs functionalized BBI antibody fragments, PBBI was acquired within 1 h as evidenced by two bands in SDS-PAGE (corresponding to monomer and dimer) and the same sequence using tandem mass spectrometry [32]. In addition, Dia and colleagues established a new process of preparing soy flour ingredients with lower levels of antinutritional components, and retaining biologically active components, including BBI, to increase the nutritional effects of soybean consumption. This is really good news which will facilitate future bench top and clinical investigations on BBI from soybean and other origins.

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This article was written in a personal capacity (E.F.F.) and does

not represent the opinions of the US Food and Drug Administration, the US Department of Health and Human Services, or the US Federal Government.

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