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The cancer preventive peptide lunasin from wheat inhibits core histone acetylation

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Abstract

Lunasin is a unique 43-amino acid cancer preventive peptide initially reported in soybean and barley and has been shown to be chemopreventive in mammalian cells and in a skin cancer mouse model against oncogenes and chemical carcinogens. We report here the core histone H3- and H-acetylation inhibitory properties of lunasin from wheat, a new source of the peptide and from the livers of rats fed with lunasin-enriched wheat (LEW) to measure bioavailability. A non-radioactive histone acetyl transferase assay was used to measure inhibition of core histone acetylation. The presence of lunasin in wheat was established by Western blot and identified by liquid chromatography electrospray ionization mass spectrometry (LC–ESI-MS). Lunasin isolated from wheat seeds at different stages of development inhibited core histone H3 and H4 acetylation in a dose-dependent manner. Lunasin extracted from liver of rats fed with lunasin-enriched wheat (LEW) also inhibited histone acetylation confirming that the peptide is intact and bioactive. The amounts of lunasin in the developing seeds and in the rat liver correlated extremely well with the extent of inhibition of core histone acetylation.

Keywords: Lunasin; Core histones H3 and H4; Acetylation; Lunasin-enriched wheat (LEW)

1. Introduction

Advances in basic and clinical cancer research have alleviated the progression of a number of malignancies. Cancer however remains one of the leading causes of mortality in the Western world [1,2]. Studies indicate that adequate nutrition with certain types of food containing bioactive components might offer significant protection against carcinogenesis [3,4]. Wheat has been reported to contain supplementary substances possessing potential anticancer functions [5,6]. Soybean and barley were found to contain lunasin, a novel cancer preventive agent which is a 43-amino acid peptide with 9 Asp (D) residues at the carboxyl end, an Arg-Gly-

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Asp (RGD) cell adhesion motif and a predicted helix with structural homology to a conserved region of chromatin-binding proteins [7–11]. Lunasin suppresses carcinogenesis triggered by chemical carcinogens and oncogenes in vitro as well as in a mouse model for skin cancer [9,12].

Histone acetylation and deacetylation have been associated with eukaryotic transcriptional regulatory mechanisms [13]. The affinity of lunasin for hypoacetylated chromatin suggests its role in chromatin modification, a process implicated in cell cycle control and in the role of tumor suppressors in carcinogenesis [14]. Since lunasin inhibits core H3 and H4 histone acetylation in mammalian cells [9], we have postulated an epigenetic mechanism whereby lunasin selectively kills cells that are being transformed or newly transformed by disrupting the dynamics of histone acetylation–deacetylation triggered by the inactivation of tumor suppressors that operate through histone acetylation–deacetylation [12].

In the quest for other readily available natural sources of lunasin, we report here the identification and purification of lunasin from wheat and establishing its bioavailability by dietary administration of lunasin-enriched wheat (LEW) to rats. Consistent with our proposed epigenetic mechanism for lunasin, bioactivity of lunasin in wheat and in rat liver was measured by its ability to inhibit core H3 and H4 histone acetylation.

2. Material and methods

2.1. Isolation, purification and identification of lunasin from wheat

To determine levels of lunasin during seed development, wheat seeds (*Hordeum vulgare* cv. *Namhaemil*) obtained from the Rural Development Administration, Suwon, Gyunggido, Korea were grown in a greenhouse, and seeds were collected every week after flowering.

To isolate lunasin, seeds were ground to flour and 40 g was extracted with 80 ml of 0.1 M PBS, pH 7.4, supplemented with fresh protease inhibitor cocktail (Sigma, St. Louis, MO) at a concentration of 1% v/v (Sigma) by shaking for 48 h at 4 °C. The extract was centrifuged at 15,000 rpm for 30 min and the supernatants were collected. Protein contents were determined using Bradford assay [15]. Standard lunasin was synthesized by American Peptide Co., Sunnyvale, CA. Anti-lunasin polyclonal antibody was produced by Zymed Inc., South San Francisco, CA. The lunasin secondary antibody was purchased from Santa Cruz Biotechnology, Santa Cruz, CA. SDS–PAGE of extracts and column fractions were ran in 15% Tris–HCl ready gel (Bio-Rad, Hercules, CA) following the manufacturer's instructions. Gels were transblotted on to PVDF membranes for Western blot analysis, membranes were blocked for non-specific binding for 1 h in Blotto A (5% non-fat milk and 1% Tween 20 in Tris-buffered saline), washed with fresh changes of the 1% TBS-T solution (1% Tween 20 in Tris-buffered saline) and incubated with the lunasin primary antibody R1 at 1:4000 dilution in Blotto B solution (3% non-fat milk and 1% Tween 20 in TBS) for 1 h After washing, the membrane was incubated with an anti-rabbit secondary antibody at 1:3000 dilution in Blotto B solution for 1 h, washed again and treated with the detection agent (Amersham Biosciences) and immediately developed in Polaroid film.

To obtain LEW for the animal experiment and the purification of lunasin, 40 kg of wheat flour (*Hordeum vulgare* L. cv. *Namhaemil*) was dissolved in 250 L of distilled water by shaking for 48 h at 4 °C. The extracts were filtered with seven layers of cheese cloth and allowed to settle down for 48 h at 4 °C and the supernatant was freeze-dried. Approximately 3 kg of LEW protein was obtained.

LEW was extracted with water (1:10, g:mL) for 1 h, and centrifuged at 15,000 rpm for 1 h. The supernatants were dialyzed for 24 h at 4 °C against 2 L of distilled water using Spectra/Por 7 membrane (MWCO: 10,000) and the distilled water outside the bag was freeze-dried and redissolved in an appropriate volume of distilled water. To purify the extracts, 20 μl filtrate was injected into HPLC C18 column (DELTA PAK, 15 μm, 300A, 300 × 7.8 mm) equilibrated at ambient temperature and stabilized with the mobile phase (acetonitrile:water 4/6) at a flow rate of 2.5 ml/min for 15 min with the UV detector set at 295 nm. The wheat lunasin peak was identified by comparison with the lunasin standard peak that appeared at a retention time of 4 min. Lunasin content of the sample peak was quantified by Western blot using the software Un-SCAN-IT gel Version 5.1 (Silk Scientific, Inc.).

Lunasin was identified by liquid chromatography electrospray ionization mass spectrometry (LC–ESI-MS) (Korea Basic Science Institute). The putative lunasin peptide mass was compared with the predicted peptide mass map obtained from in-gel trypsin digestion using database search at National Center for Biotechnology Information (NCBI) with accession number AAP62458.

2.2. Isolation and purification of lunasin from rat liver

Four-week-old male Sprague–Dawley rats (Central Laboratories Animal Inc., Korea) were initially fed with AIN 76A diet for 48 h and then split into two groups with 12 rats each. The control group was fed with 280 g AIN76A and the treated group with 280 g AIN76A containing 50% (w/w) LEW for 4 weeks. The animals were sacrificed and the livers were immediately freeze-dried. One gram of the dried liver was extracted with 50 ml of 0.1 M phosphate buffer (pH 7.0) at 4 °C for 24 h, centri-