JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Inhibition of Core Histone Acetylation by the Cancer Preventive Peptide Lunasin

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Lunasin is a unique 43 amino acid soy peptide that has been shown to be chemopreventive in mammalian cells and in a skin cancer mouse model in this work against oncogenes and chemical carcinogens. The observation that lunasin inhibits core histone acetylation led to the proposal of an epigenetic mechanism by which lunasin selectively kills cells that are being transformed by disrupting the dynamics of cellular histone acetylation−deacetylation when the transformation event is triggered by the inactivation of tumor suppressors that function via histone deacetylation. Here is reported for the first time the core histone H3- and H4-acetylation inhibitory properties of lunasin from different Korean soybean varieties used for various food purposes and from tissues of rats fed with lunasin-enriched soy (LES) to measure bioavailability. Lunasin was analyzed by immunostaining and inhibition of core histone acetylation by a non-radioactive histone acetyl transferase assay. Various amounts of lunasin are found in the soybean varieties, which correlated with the extent of inhibition of core histone acetylation. Both soy lunasin and synthetic lunasin inhibit core histone acetylation in a dose-dependent manner. Lunasin in LES is protected from in vitro digestion by pepsin. Lunasin extracted from blood and liver of rats fed with LES is intact and inhibits core histone acetylation.

KEYWORDS: Lunasin; core histon acetylation; soy peptide; histone acetyl transferase

INTRODUCTION

Consumption of soybean products is associated with overall low mortality rates due to prostate, breast, and colon cancer (*1*, *2*). Two-thirds of the reported studies in the epidemiological literature associate soy intake with a reduction of cancer risk (*3*). More recent epidemiological studies, animal experiments, and in vitro studies show that soy products reduce cancer risks (reviewed in refs *4* and *5*). The most widely studied bioactive substances in soy are Bowman−Birk protease inhibitor (BBI) or its less pure form BBI concentrate (BBIC) and the isoflavones (*4*, *5*). BBIC has been shown to be cancer preventive in in vitro models of carcinogenesis as well as in a number of animal model systems (*6*). The biological effects of dietary isoflavones, which are phytoestrogens, have been attributed to their long-term estrogenic effects (*7*). However, the administration of soy isoflavones in a soy protein matrix has raised the possibility that other factors contribute to the observed preventive effects attributed to isoflavones mixtures (*8*).

A likely candidate is lunasin, a novel cancer preventive peptide, the efficacy of which has been demonstrated in our laboratory (*9*−*12*). Initially identified in soy, lunasin is also present in barley, a food crop known for its health effects (*11*). Lunasin is a unique 43 amino acid soybean peptide that contains at its carboxyl end 9 Asp (D) residues, an Arg-Gly-Asp (RGD) cell adhesion motif, and a predicted helix with structural homology to a conserved region of chromatin-binding proteins. It has been shown to suppress carcinogenesis caused by chemical carcinogens and oncogenes in in vitro models and 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 a role in chromatin modification, a process implicated in cell cycle control and in the role of tumor suppressors in carcinogenesis (*14*).

Transfection of the lunasin gene into mammalian cells leads to mitotic arrest and cell lysis, resulting in lunasin bound to the chromatin (*15*). Subsequently, we showed that exogenous addition of synthetic lunasin peptide to mammalian cells inhibits histone acetylation in the presence of sodium butyrate, a histone deacetylase inhibitor (*12*).

Because lunasin inhibits core H3 and H4 histone acetylation in mammalian cells (*12*), we propose an epigenetic mechanism whereby lunasin selectively kills cells that are being transformed or newly transformed cells by disrupting the dynamics of histone acetylation−deacetylation, which is triggered by the inactivation of tumor suppressors that operate through histone acetylation−deacetylation (*9*).

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10.1021/jf062405u CCC: $37.00
Published on Web 01/12/2007

In this paper, we used the inhibition of acetylation of core histones H3 and H4 by lunasin as a bioactivity assay for the first time. These were measured in lunasin extracted from a number of Korean soybean varieties used for different food purposes and from blood and liver of rats fed with lunasin-enriched soybean (LES) through oral administration to measure bioavailability.

MATERIALS AND METHODS

Materials. All electrophoresis chemicals were purchased from Bio-Rad (Hercules, CA). Synthetic lunasin (SynPep, Inc., Dublin, CA) was used as a standard, and lunasin polyclonal antibody was made against the carboxyl half of lunasin (Zymed, Inc., South San Francisco, CA). Monoclonal anti-BBI was generously provided to us initially by Dr. David Brandon of USDA-WRRL (Albany, CA) and later purchased from Agdia, Inc. (Elkhart, IN). Secondary antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). All HPLC solvents were purchased from J. T. Baker. The histone acetyl transferase (HAT) assay kit and PCAF for non-radioactive HAT assays were purchased from Upstate Biotechnology, Inc. (Chicago, IL). yGCN5 was prepared as described in a later section.

Protein Extracts from Various Soybean Seeds. Soybean (*Glycine max* cvs. Sowonkong, Seritae, Andongkumjungkong, Hwangkumkong, Pungsangnamulkong, Juinunikong, Milyang-139, and Chungjakong-1) were obtained from the Andong Seed Supply Institute and grown under usual greenhouse conditions at Andong National University, Andong, Kyungpook, Korea. Seeds were collected at 8 weeks after flowering, cracked, and ground to flour. Ten grams of the flour was extracted with 50 mL of phosphate-buffered saline (0.1 M PBS, pH 7.0) supplemented with fresh protease inhibitor cocktail (Sigma, St. Louis, MO). The mixture was centrifuged at 12000*g* for 30 min, and the supernatant protein extract was used for subsequent experiments. Protein content was determined using the Bradford assay (*16*).

Preparation of LES for Rat Feeding. Forty kilograms of the soybean flour (*G. max* cv. Hwangkumkong) was extracted for 48 h at 4 °C with 250 L of distilled water. The extract was filtered with seven layers of cheesecloth and allowed to settle for 48 h at 4 °C. The supernatant solution was freeze-dried. About 3 kg of LES protein were obtained from 40 kg of soybean flour.

Purification of Lunasin by HPLC. LES was extracted for 1 h with water (1:10, g/mL) and centrifuged at 15000 rpm for 1 h. The supernatant protein was dialyzed for 24 h at 4 °C against 2 L of distilled water using a Spectra/Por 7 membrane (MWCO = 50000) and freeze-dried. The protein extract was further purified by ion exchange chromatography on Biogel resin AG 1-X4, mesh size 100−200 (Bio-Rad Labs, Hercules, CA) as described by Jeong et al. (*17*). Lunasin was eluted at 0.7−0.9 M NaCl as shown in our previous work (*11*).

All of the fractions containing lunasin after ion exchange chromatography were pooled and further purified by HPLC. The 20 μL filtrate was injected into the HPLC system equipped with a C18 column (Delta Pak, 15 μm, 300A, 300 × 7.8 mm) after the system was equilibrated at ambient temperature and the UV detector (295 nM) stabilized with the mobile phase (acetonitrile/water = 4:6) at a flow rate of 2 mL/min for 15 min. Purified lunasin was obtained as compared with the standard lunasin peak at the same retention time of 4 min. The lunasin content of the samples was calculated by comparing the band intensities with those of known standards run under the same conditions using the software Un-SCAN-IT *gel* version 5.1 (Silk Scientific, Inc., Orem, UT). The purity of lunasin was about 98.8%.

Isolation and Purification of Lunasin from the Tissues of Rats. Rats (Sprague−Dawley, male, 4 weeks old) were purchased from Central Lab. Animal Inc. in Korea. All experiments on animals were done in accordance with humane guidelines of the Animal Center at Andong National University. The 12 rats were fed with 100% of AIN 76A for 48 h. Thereafter, the 12 rats were fed with AIN76A−LES-50%, and the rest remained in the 100% of AIN 76A for 4 weeks as the control group.

The rats were sacrificed, and the livers were freeze-dried immediately. Blood was centrifuged at 4500 rpm for 25 min, and the serum was freeze-dried. One gram of the dried tissues was extracted with 50 mL of 0.1 M phosphate buffer (pH 7.0) at 4 °C for 24 h. After the extract had been centrifuged at 15000 rpm for 1 h, the supernatant was dialyzed with a Spectra /Por 7 membrane (MWCO = 50000). Then, 0.1 g of the dried extract was dissolved with 3 mL of 0.1 M phosphate buffer (pH 7.0), and the lunasin peptide was isolated from this fraction by ion exchange column chromatography (resin AG1X4) as described previously (*17*) and purified by HPLC as described above. Collected fractions were freeze-dried.

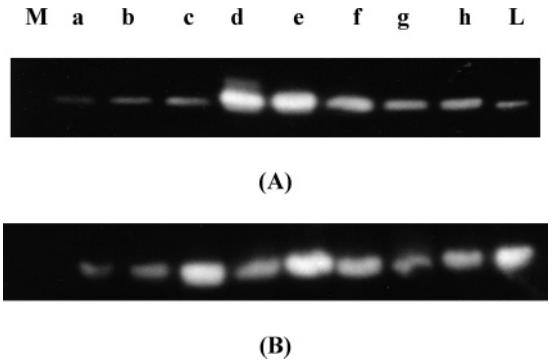

Figure 1. Western blot of lunasin (**A**) and BBI (**B**) in protein extracts from eight varieties of soybean. Lanes a–h contain protein extracts of eight Korean soybean cultivars in the following sequence: Sowonkong, Seritae, Andongkumjungkong, Hwangkumkong, Pungsangnamulkong, Juinunikong, Milyang-139, and Chungjakong-1; S, 200 ng of lunasin standard. Lane M is the MW marker, and lane L is 200 ng of synthetic lunasin and 3 μg of BBI. Each well contains 25 μg of proteins.

Determination of Lunasin and BBI. SDS-PAGE of protein extracts and Western blot for detection of lunasin peptide and BBI were performed on the various samples as described previously (*17*) except for the following modifications. The membrane for Western blot was washed with fresh changes of the TBS-1T at room temperature, incubated in either anti-lunasin or anti-BBI as the primary antibody with 3% nonfat milk in TBS-1T for 1 h, and then washed with fresh changes of the TBS-1T at room temperature. The membrane was then incubated using anti-rabbit (for lunasin) or anti-mouse (for BBI) streptavidin HRP as the secondary antibody with 3% nonfat milk in TBS-1T for 1 h and subsequently washed with fresh changes of the TBS-1T at room temperature.

Lunasin and BBI contents of the samples were calculated by comparing the band intensities with those of known standards run under the same conditions. The intensities of the bands were quantified using the software Un-SCAN-IT *gel* version 5.1 (Silk Scientific, Inc.).

Core Histone Acetylation Assay. The HATs yGCN5 and PCAF were used. yGCN5 uses acetyl-CoA to acetylate Lys-14 of H3 histone protein, whereas PCAF acetylates Lys-8 and Lys-16 of H4 histone to a lesser extent and generates acetylated histone and CoA (*18*). Non-radioactive HAT assays were done using a HAT assay kit (Upstate Biotechnology, Inc.) according to the manufacturer's protocol except for the following modifications. The catalytic domain of yGCN5 was recombinantly expressed as described by Tanner et al. (*19*) and purified from bacteria according to the method of Kim et al. (*18*) except for the following modifications. BL21 cell was used to express yGCN5. The expressed yGCN5 was extracted with distilled water and dialyzed with a Spectra/Por Membrane (MWCO = 50000)(Spectrum Laboratories Inc., Miami, FL) at 4 °C for 24 h. The dialyzed sample was then subjected to Ni-NTA Chelating Agarose CL-60 (Peptron, Inc., Daejeon, Korea) ion exchange chromatography and eluted with 200−400 mM imidazole gradient with yGCN5 eluting at 400 mM imidazole. Purified yGCN5 (assessed by SDS-PAGE) was pooled and concentrated and stored at −20 °C until use. Protein concentrations were determined according to the method of Bradford (*16*). PCAF for the non-radioactive HAT assay on histone H4 peptide was purchased from Upstate Biotechnology. Purified yGCN5 for H3 or PCAF for H4 was mixed with 100 μM acetyl-CoA and 1× HAT assay buffer and incubated on an enzyme-linked immunosorbent assay plate (the streptavidin-coated strip plate) precoated with histone H3 or H4 for 30 min. After several

Table 1. Lunasin and BBI Contents of Extracts of Different Varieties of Soybean[a]

	soybean cultivar	ng of lunasin/ μg of protein	mg of lunasin/ g of seed	ng of BBI/ μg of protein	mg of BBI/ g of seed	lunasin/BBI ratio based on μg of protein
A	Sowonkong	4.40 ± 0.32	0.50	30 ± 0.003	2.87	0.15
B	Seritae	7.02 ± 0.26	0.95	40 ± 0.005	5.84	0.18
C	Andongkumjungkong	11.56 ± 0.63	1.61	140 ± 0.011	19.78	0.08
D	cv. Hwangkumkong	70.49 ± 1.38	8.13	90 ± 0.006	9.99	0.78
E	Chungjakong-1	58.35 ± 0.95	5.65	170 ± 0.015	16.24	0.34
F	Pungsangnamulkong	36.57 ± 0.88	3.68	100 ± 0.008	10.54	0.37
G	Milyang-139	19.76 ± 0.72	2.26	60 ± 0.005	7.41	0.33
H	Juinunikong	19.52 ± 0.70	1.99	80 ± 0.006	8.38	0.24

[a] Values represent average ± standard deviation from triplicate determinations.

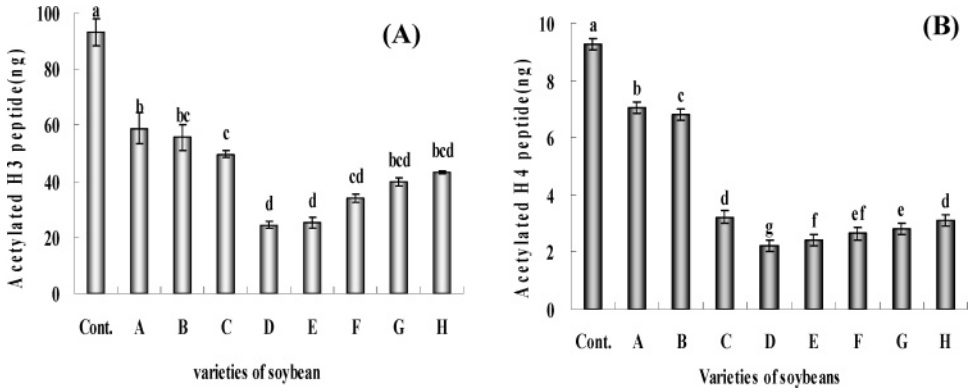

Figure 2. Amount of acetylated histone H3 (**A**) and H4 (**B**) measured after carrying out the HAT assay in the presence of crude protein extracts from various soybean varieties. Vertical lines on bars are ±SD, $n = 3$. yGCN5 and PCAF were used for the HAT assay for histone H3 and H4 peptide, respectively. Crude proteins were prepared as described under Materials and Methods, and 25 μg of crude protein/well for each cultivar waswere used in the HAT assay. Lanes: Cont., positive control for histones H3 and H4 without the addition of crude protein extract; A, cv. Sowonkong; B, cv. Seritae; C, cv. Andongkumjungkong; D, cv. Hwangkumkong; E, cv. Pungsangnamulkong; F, cv. Juinunikong; G, cv. Milyang-139; H, cv. Chungjakong-1. Vertical lines on bars are ±SD, $n = 3$. Bars with different letters are statistically significant from one another.

washes with TBS, the assay was initiated by adding 50 μL of HAT reaction cocktail (10 μL of 5× HAT assay buffer, 10 μL of 500 μM acetyl-CoA, and 1.5 μg of yGCN5 for H3 or 100 ng of PCAF for H4), and acetylated histones were detected using an anti-acetyl-lysine rabbit polyclonal antibody followed by the horseradish peroxidase (HRP) based colorimetric assay. The strip plate was read on a plate reader at a wavelength of 450 or 570 nm.

To verify that the coupled assays are accurate measures of yGCN5, reactions were performed as described under Materials and Methods. A linear relationship was obtained between quantities of acetylated H3 and H4 and reaction time and concentrations of yGCN5 with correlation coefficients R^2 of 0.99 and 0.97. The quantities of acetylated histone H3 or H4 peptide in the samples were determined by comparison with standard curves established using known concentrations of acetylated histone H3 and H4 peptide. The standard curves for histone H3 and H4 peptide showed linear relationships between quantities of acetylated H3 and H4 and optical density (450 nm) with correlation coefficients R^2 of 0.97 and 0.96, respectively.

In Vitro Digestion with Pepsin. The concentration of pepsin (Sigma) was 3.2 mg/mL in 0.03 M NaCl at pH 1.2. Two hundred microliters of pepsin was placed in 1.5 mL microcentrifuge tubes and incubated at 37 °C. Ten microliters of the test sample was added to pepsin-added tubes to start the digestion reaction. At intervals of 0, 2, 5, 15, 30, 60, and 120 min, 50 μL of 1 N NaOH was added to each tube to stop the reaction, and the sample was taken out and freeze-dried. Lunasin was analyzed using Western blot as described earlier.

RESULTS AND DISCUSSION

Lunasin and BBI in Different Korean Soybean Varieties. As in China and Japan, soybeans are widely used for different purposes in Korea, and certain varieties are chosen for specific applications. For instance, cv. Kongnamul is used for bean sprouts, cvs. Chungukjang and Deanjang are used for fermented soybeans, and cv. Babmitkong is used for beans cooked with rice in Korea. Among the eight varieties selected here, cvs. Hwangkumkong, Juinunikong, Milyang-139, and Chungjakong-1 are used for fermented soybeans, cvs. Sowonkong and Pungsangnamulkong are used for bean sprouts, and cvs. Seritae and Andongkumjungkong are chosen for cooking with rice.

Western blot patterns of lunasin and BBI are shown in **Figure 1**. The varying intensities of the bands representing 25 μg of protein for each sample show the varying amounts of lunasin and BBI in these samples. Calculated concentrations are presented in **Table 1**. Values for lunasin vary from a high of 70.49 ng/μg of protein (or 8.13 mg/g of seed) for cv. Hwangkumkong to a low of 4.40 (or 0.50) for cv. Sowonkong. The BBI concentrations varied from a high of 170 ng/μg of protein (or 16.24 mg/g of seed) for cv. Chungjakong-1 to 30 ng/μg of protein (or 2.87) for cv. Sowonkong. In comparison, our analysis of defatted soy flour shows an average of 5.5 ng of lunasin/μg of protein, and the values we obtained from the analyses of 144 varieties from the U.S. Germplasm Collection at the University of Illinois show a range of 5.9−7.2 ng of lunasin/μg of protein (*20*, *21*).

The ratios of lunasin to BBI based on micrograms of protein are shown in the last column of **Table 1**. The highest, 12.11, is found in cv. Andongkumjungkong, and the lowest is 1.28 in cv. Hwangkumkong. This ratio is relevant considering that lunasin is evidently protected from digestion by naturally occurring soy protease inhibitors such as BBI and Kunitz trypsin inhibitor (KTI). Although the ratios vary significantly among different cultivars, our tests based on in vitro digestibility studies

show that lunasin is protected from digestion in all of the soybean cultivars that we have tested so far.

Inhibition of Core Histone Acetylation Crude Protein Extracts of Soybean Varieties. Figure 2 shows the content of acetylated histones H3 (**Figure 2A**) and H4 (**Figure 2B**) after the HAT assay had been carried out with 25 μg of protein from each of the varieties. The inhibition patterns correlate exactly with the lunasin contents of the different varieties. Cv. Hwang-kumkong, which has the highest concentration of lunasin, inhibits acetylation of H3 and H4 by 74.0 and 76.1%, respectively, whereas cv. Sowonkong, with the lowest lunasin content, inhibits the acetylation by 36.8 and 23.7%, respectively. Interestingly, the highest inhibition of H3 histone acetylation is found among the varieties used for fermentation, but no definitive conclusion can be made because of the small number of samples analyzed.

This is the first report of the histone acetylation inhibitory properties of different soybean varieties. As expected, the degree of inhibition correlates with the lunasin concentration. This property is relevant to the proposed epigenetic mechanism for the cancer preventive property of lunasin and for the postulated role of lunasin in seed development (*9*, *12*, *17*, *22*, *23*). As stated earlier, the mechanism we proposed stipulates that lunasin selectively kills cells that are being transformed by disrupting the dynamics of histone acetylation−deacetylation when a transforming agent such as E1A inactivates the tumor suppressor Rb, which functions by keeping the core histones in the Sp1 promoter region deacetylated (*9*, *14*, *23*). The inactivation of Rb decouples the Rb-HDAC (histone deacetylase), exposing the deacetylated histone for acetylation by HATs and binding by lunasin. The tight binding of lunasin to deacetylated histones disrupts the dynamics of histone acetylation−deacetylation, which is perceived as abnormal by the cell and leads to cell death (*23*). This happens only to cells being transformed, leaving normal cells unaffected. In addition to Rb, p53 and pp32, nuclear proteins found in prostate cells, partly operate via histone acetylation−deacetylation mechanisms (*24*, *25*).

The mechanism by which lunasin inhibits histone acetylation is not definitively known. It is likely that lunasin binds to deacetylated histone by ionic interaction with its negatively charged poly-D end as shown by our earlier work (*20*). The N terminus of lunasin that includes a helical region may play a role in targeting lunasin to deacetylated histones (*20*). Inhibition experiments using various concentrations of lunasin and deacetylated histones are being planned in our laboratory to elucidate the inhibitory mechanism.

We also propose that lunasin plays a role in seed development by binding to deacetylated histones and arresting cell division to allow DNA endoreduplication during seed development (*22*, *26*). This suggests the widespread presence of lunasin in angiosperm seeds, although our initial screening of a limited number of seeds suggests otherwise (*17*).

Inhibition of Acetylation of H3 and H4 Core Histones by Purified Soybean Lunasin. The lunasin purified by HPLC from soybean (cv. Chungjakong-1) was used in the HAT assay to determine the effect of dose in comparison with synthetic lunasin and BBI (**Figure 3**). **Figure 3A** shows the Western blot of HPLC-purified lunasin with different loadings per well. The inhibitory effects of purified soy lunasin, synthetic lunasin, and BBI at different doses are shown in **Figure 3B** for core histone H3 and in **Figure 3C** for core histone H4.

The dosage effect of purified soy lunasin on histone acetylation inhibition is indistinguishable from that of synthetic lunasin. Both are effective at 10 nM, bringing about a 20%

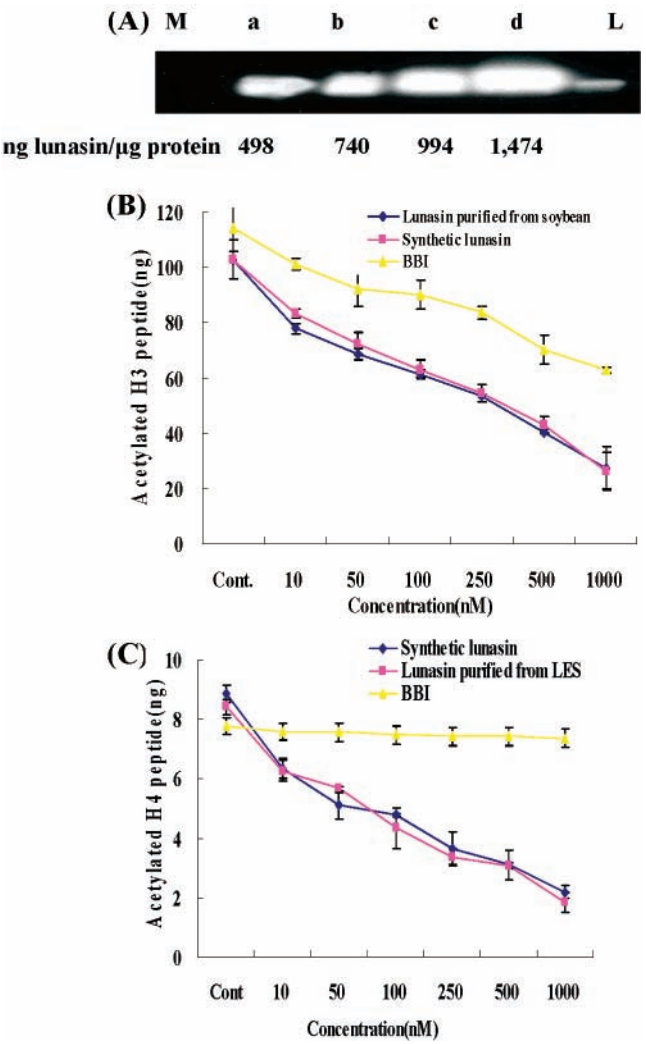

Figure 3. (**A**) Western blot of lunasin purified from lunasin-enriched soybean by HPLC. Lanes: M, MW markers; a–d are loaded with volumes of 0.5, 0.75, 1.0, and 1.5 μg of protein per well, as lunasin peptide purified by anion exchange column chromatography–HPLC and L (200 ng of lunasin standard). (**B**) Inhibition of core H3-histone acetylation by synthetic lunasin, lunasin purified from soybean, and synthetic BBI. (**C**) Inhibition of core H4-histone acetylation by synthetic lunasin, lunasin purified from soybean, and synthetic BBI. Vertical lines on bars are ±SD, $n = 3$. The concentration on the *X*-axis refers to either lunasin purified from soy, synthetic lunasin, or BBI.

reduction in acetylated H3 histone relative to the control. At 1000 nM, both bring about a reduction of 80%. For core histone H4, at 10 nM, both purified soy lunasin and synthetic lunasin bring about a reduction of 25%, whereas at 1000 nM, a reduction of 75% is seen. When plotted on a semilog scale, the concentration is linear with respect to the amount of acetylated histone with a negative correlation coefficient, $R^2 = -0.96$. This is the first study showing that the bioactivities of both natural lunasin and synthetic lunasin are similar with respect to inhibition of core histone acetylation. BBI shows a slight inhibition of core histone H3 acetylation and none on H4 acetylation, suggesting that BBI might inhibit a lysine acetylation site in H3 different from that of H4.

In Vitro Digestion of Lunasin and in Vivo Bioavailability in Rats. Oral administration is an important feature of an ideal cancer preventive agent. Because lunasin is a peptide, it is crucial to establish whether it survives digestion, gets absorbed into

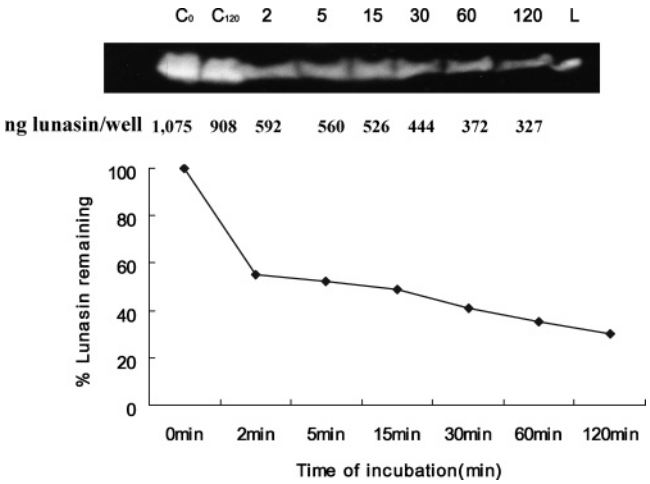

Figure 4. Western blot analysis (top) of samples taken at various times (minutes) after incubation with pepsin. The numbers above the blots indicate the minutes of incubation, and the numbers below represent the amount remaining (nanograms of lunasin) for each incubation period. C_0, control (no pepsin) at zero time of incubation; C_{120}, control at 120 min of incubation; L, synthetic lunasin, 200 ng.

the tissues, and remains bioactive if it is to be developed into a widely used orally administered cancer preventive agent.

First, we determined whether lunasin in LES is protected from pepsin digestion in an in vitro digestion experiment. **Figure 4** shows the Western blot of samples taken at various times (minutes) after incubation with pepsin (top) and the plot of the amount of lunasin remaining versus time in minutes (bottom). The bottom figure shows that lunasin is afforded some protection against pepsin. This suggests that BBI and other naturally present protease inhibitors protect lunasin from digestion by pepsin. In another set of experiments our laboratory has shown that lunasin in LES is bioavailable, being absorbed and ending up in various tissues, and is protected from in vitro pancreatin digestion by BBI and KTI (unpublished results, to be submitted).

Bioactivity of Lunasin Extracted from Tissues of Rats Fed LES. In the next set of experiments, rats were fed LES for 4 weeks to determine whether orally ingested lunasin survives digestion, ends up in the tissues, and remains intact and bioactive as measured by the HAT assay.

Lunasin is present in the liver and blood of LES-fed rats but not in the rats not fed LES -unfed rats (not shown) and is detectable by Western blot only after ion exchange HPLC purification. The bands have the same MW as the lunasin standard and show a proportional increase in signal with increasing loading (**Figure 5A**,**B**).

We then ask whether lunasin extracted and purified from the tissues is bioactive using the HAT bioassay. **Figure 5C** shows that the purified lunasin from the liver and blood inhibits H3-core histone acetylation, reducing the level of acetylated H3 histone by 68% at 1 μM lunasin concentration. **Figure 5D** shows the same pattern of inhibition of H4-core histone acetylation by lunasin extracted and purified from the blood and liver of LES-fed rats. The lunasin extracted from the liver and blood of rats not fed LES did not inhibit histone acetylation. All of these findings clearly demonstrate that the lunasin extracted and purified from the blood and liver of LES-fed rats is intact and bioactive.

In summary, the data presented here document for the first time the core histone acetylation inhibitory properties of lunasin from different varieties of soybean and from the liver and blood of rats fed LES. These observations are relevant to our hypothesis that lunasin as a cancer preventive agent functions via an epigenetic mechanism to modify chromatin.

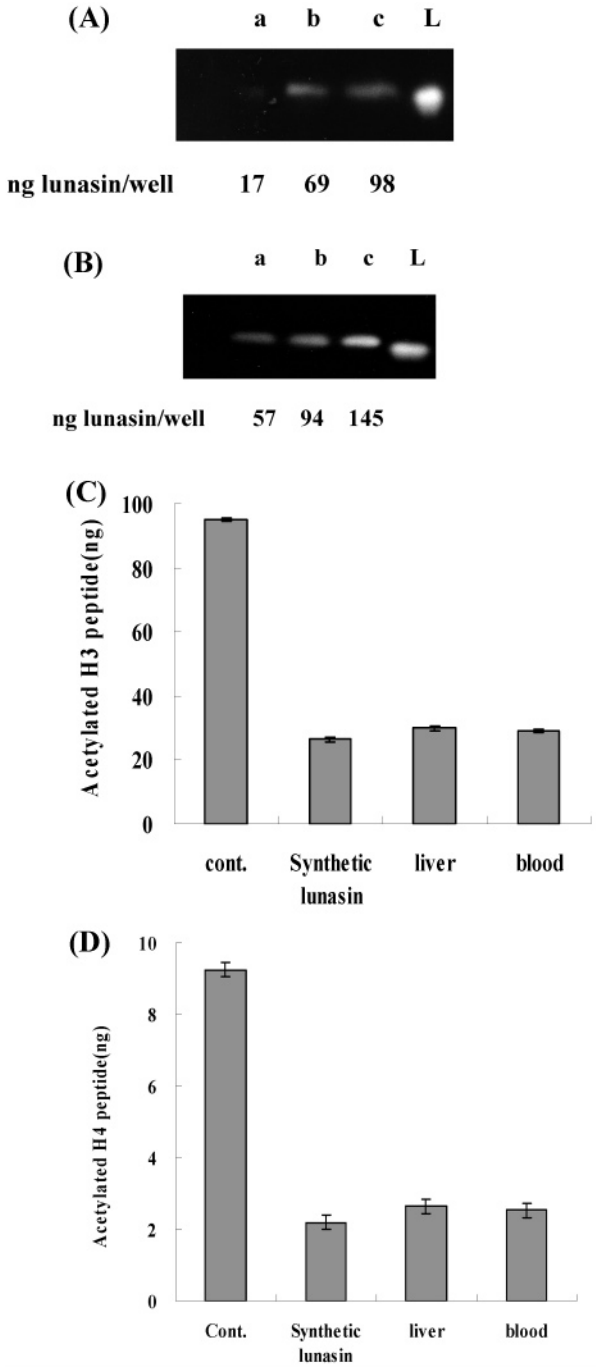

Figure 5. Western blot of lunasin extracted and purified from liver (**A**) and blood (**B**) of rats fed LES and purified by sequential anion exchange HPLC. The lanes show different protein loadings per well: a, 0.06 μg of protein; b, 0.12 μg of protein; c, 0.24 μg of protein; L, synthetic lunasin, 200 ng. The purified lunasin was used in the HAT assay, and the results are shown in the lower figures for H3-core histone (**C**) and H4-core histone (**D**), using 1 μM lunasin.

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Received for review August 21, 2006. Revised manuscript received November 21, 2006. Accepted December 5, 2006. We acknowledge the support provided by the U.S. Department of the Army Congressionally Directed Medical Research Program (W81XWH-04-1-0128), University of California Discovery Grants, University of California−Berkeley Committee on Research, and the second stage of BK21 grants from the Ministry of Education and Human Resources Development, Korea.

JF062405U