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Barley Lunasin Suppresses *ras*-Induced Colony Formation and Inhibits Core Histone Acetylation in Mammalian Cells

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Lunasin is a novel peptide originally identified in soybean that suppresses chemical carcinogen-induced transformation in mammalian cells and skin carcinogenesis in mice. Since the *lunasin* gene was cloned from soybean and the chemically synthesized form of the lunasin peptide has been used in experiments conducted so far, the isolation of lunasin from other natural sources and testing of its biological properties have not been carried out. We report here the isolation, purification, and biological assay of lunasin from barley, a newly found rich source of the peptide. The identity of lunasin was established by Western blot analysis and mass spectrometric peptide mapping of the in-gel tryptic digest of the putative protein band. Lunasin was partially purified with anion exchange and immunoaffinity chromatography. The crude and partially purified lunasin from barley suppressed colony formation in stably *ras*-transfected mouse fibroblast cells induced with IPTG. These fractions also inhibited histone acetylation in mouse fibroblast NIH 3T3 and human breast MCF-7 cells in the presence of the histone deacetylase inhibitor sodium butyrate.

KEYWORDS: Barley lunasin; chemopreventive peptide; isolation; purification and bioassay; mass spectrometric peptide mapping; suppression of colony formation; *ras* oncogene; inhibition of histone acetylation

INTRODUCTION

Epidemiological evidence suggests that dietary factors play an important role in the etiology of different kinds of cancer (*1*). For instance, diets rich in soybean products are associated with lower cancer mortality rates, particularly for cancers of the colon, breast, and prostate (*2*). Components of soybean believed to be capable of suppressing carcinogenesis include the Bowman-Birk protease inhibitor (BBI), inositol hexaphosphate, β-sitosterol, and isoflavones (*3*). BBI, now in clinical trials, has been shown to suppress carcinogenesis in laboratory animals and in in vitro transformation systems (*4*).

We isolated and cloned a cDNA encoding a post-translationally processed 2S albumin (Gm2S-1) from mid-maturation soybean seed in our search for methionine-rich proteins from soybean (*5*). Gm2S-1 codes for a signal peptide, a small subunit (we termed lunasin), a linker peptide, and a large subunit methionine-rich protein. Lunasin, is a unique 43-amino-acid peptide with (a) an acidic carboxyl end consisting of nine aspartic acids residues, (b) a cell adhesion motif −RGD− just before the poly-D, and (c) a predicted, conserved, helical region with structural homology to chromatin binding proteins. Transfection of the *lunasin* gene into mammalian cells leads to mitotic arrest and cell death characterized by cell lysis and chromosome fragmentation (*6*). Evidently, the large amount of lunasin peptide produced by the constitutive expression of the *lunasin* gene leads to binding to regions of hypoacetylated chromatin like those found in kinetochores in centromeres. As a result, the kinetochore complex does not form properly, and the microtubules fail to attach to the centromeres leading to mitotic arrest and eventually to cell death (*6*).

Using chemically synthesized lunasin, we have demonstrated the chemopreventive property of this unique peptide (*7*). Distinct from the antimitotic effect of the *lunasin* gene when tranfected into mammalian cells, exogenous addition of the lunasin peptide to mouse fibroblast cells C3H 10 T1/2 suppresses foci formation induced by the chemical carcinogens DMBA and MCA. Lunasin binds preferentially to deacetylated H4-histones in vitro and inhibits H3- and H4-histone acetylation in cells treated with a histone deacetylase inhibitor. The affinity of lunasin for deacetylated core histones suggests a role in chromatin modification, a process implicated in cell cycle control and in the role of tumor suppressors in carcinogenesis (*8*). Furthermore, in the first whole animal model, lunasin inhibits skin tumorigenesis in a mouse skin cancer model when applied topically (*7*).

The isolation of lunasin from soybean seeds 14 years ago led to a number of proposed biological functions, but none has

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been proven (*9*). An intriguing possible role of lunasin is in seed development. Early seed development in angiosperms is characterized by rapid cell division and differentiation. Cell division then ceases, and cell expansion begins when massive synthesis of storage proteins, carbohydrates, and lipids occurs in the endosperms of cereals and in the cotyledons of legumes (*10*). During the cell expansion phase, genomic DNA content increases as a result of DNA endoreduplication—a unique cell cycle of G1 and S phases without cell division that occurs only in terminally differentiated storage parenchyma cells (*11*). The molecular mechanisms underlying these early events are poorly understood. The ability of lunasin peptide to arrest cell division suggests a possible role in arresting cell division to initiate cell expansion in seed development.

The putative central role of lunasin peptide in seed development led us to screen for lunasin in other seeds. Using Western blot analysis, soybean and barley clearly showed the presence of the peptide, with barley showing a level higher than that of soybean on a protein basis. The serendipitous isolation of the *lunasin* gene as part of the Gm2S-1 cDNA from soybean resulted from the purification of methionine-rich proteins (*5*). Subsequently, chemically synthesized lunasin peptide based on the coded protein sequence was used to prove the chemopreventive property of the peptide. Thus, lunasin peptide has never been isolated directly from soybean or from any other natural source in our laboratory. Here, we report the isolation and partial purification of lunasin from barley and established its identity by Western blot and mass spectrometric peptide mass mapping. The crude and partially purified lunasin suppressed colony formation of stably *ras*-transfected NIH3T3 cells and inhibited histone acetylation in vivo in mouse fibroblast NIH3T3 and human cancer MCF-7 cells treated with the histone deacetylase inhibitor sodium butyrate.

MATERIALS AND METHODS

Seeds. Barley seeds (*Hordeum vulgare* L. cultivars Olbori, Daebackbori, Mirackbori, Ricebori, and Waxybori) were obtained from the Andong Seed Supply Institute, Andong, Kyungpook, Korea.

Isolation and Purification of Lunasin. *Isolation of Crude Protein.* Seeds were cracked individually, ground to a flour, and 30 g was extracted with 100 mL of phosphate-buffered saline (PBS, 0.1 M, pH 7.4) supplemented with fresh protease inhibitor cocktail at a concentration of 1% v/v (Sigma). The extraction mixture was shaken for 48 h, 4 °C, and the protein extract was dialyzed for 24 h at 4 °C against 2 L of distilled water using dialysis tubing with a MW cutoff of 1000 Da. The protein pellet obtained by centrifugation of the dialyzed protein extract at 12 000*g* for 30 min was re-extracted once with 10 mL of extraction buffer. The supernatants from the two extractions were combined and used for further purification.

Ion Exchange Column Chromatography. The protein extract from barley cultivar Olbori was further purified by ion exchange chromatography on Biogel resin AG 1-X4, mesh size 100−200 (Bio-Rad Laboratories). The column (5.0 × 50 cm, packed height of 40 cm) was equilibrated with PBS buffer (pH 7.0, 0.1 M), about 100 mg of concentrated protein in PBS buffer was applied, and the column was washed with 150 mL of equilibration buffer. Elution was carried out with straight 0.7 M NaCl solution at 4 °C at a flow rate of 30 mL/hr, and 24 mL fractions were collected. The column fractions were concentrated to a volume of 150 μL using YM3 Microcon centrifugal filter (Millipore Company). Each fraction was analyzed for protein concentration by Bradford assay (Bio-Rad Laboratories) and ran on SDS-PAGE and Western blot. Lunasin purified by the ion exchange column was analyzed by MALDI peptide mass mapping for confirmation of identity (see section below).

Immunoaffinity Column Chromatography. A rabbit polyclonal antibody against the carboxyl epitope (CEKHIMEKIQGRGDDDDD) of lunasin was custom produced (Zymed Inc., called Zymed R1) and provided by FilGen BioSciences, Inc. The coupling of the antibody to Affi-Gel Hz gel (Bio-Rad Laboratories) and subsequent sample application and elution were according to manufacturer's instructions. Basically, the coupling involved periodate oxidation of vicinal hydroxyls of the carbohydrate moiety in the Fc region of the antibody to form aldehyde groups for specific coupling to the agarose matrix in Affi Gel Hz gel. The immunoaffinity gel was contained in a 1.0 cm × 10 cm Econo-Pac chromatography column (Bio-Rad). After applying the concentrated sample from ion exchange chromatography, unbound proteins were removed by washing first with 2 bed volumes of 0.5 M NaCl and subsequently with 2 bed volumes of 0.1 M NaCl in the application buffer. Bound proteins were then eluted with 20 mL 0.2 M glycine−HCl (pH 2.5), and 1.0 mL fractions were collected for protein assays, SDS-PAGE, Western blot analysis, and bioassays.

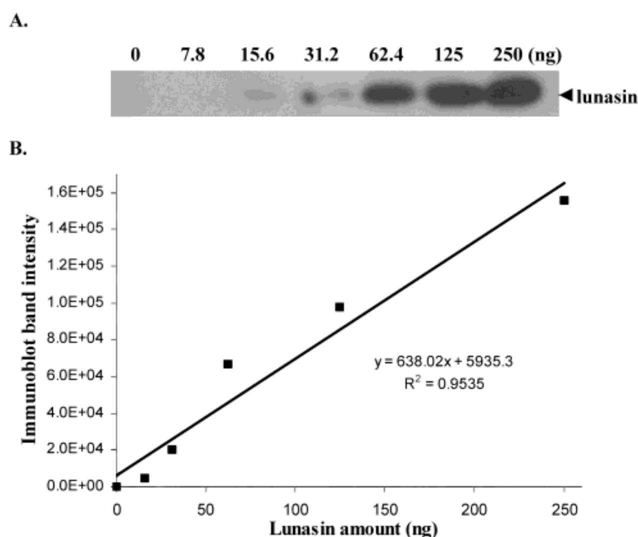


Figure 1. Typical Western blot (A) and standard curve (B) used for estimating amount of lunasin in protein extracts. As shown in panel A, the detection limit is approximately 15 ng synthetic lunasin with 1:4000 dilution of the primary Zymed R1 antibody and 1:3000 of the antirabbit secondary antibody. In other Western blots, a detection limit of 10 ng has been obtained.

Identification of Lunasin. *Gel Electrophoresis.* SDS-PAGE of seed extracts was performed using 15% Tris-HCl ready gel (Bio-Rad Laboratories) following manufacturer's instructions. Samples were diluted in Laemmli buffer (Bio-Rad Laboratories) and boiled for 5 min prior to loading. A goat antirabbit Western-compatible molecular weight standard (Santa Cruz Biotechnology) was used. Gels were stained with Coomassie brilliant blue and transblotted to PVDF membranes for Western blot analysis.

Western Blot. The PVDF membrane with the transferred protein was blocked for nonspecific binding for 1 h in Blotto A (5% nonfat 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 primary antibody Zymed R1 at 1:4000 dilution in Blotto B solution (3% nonfat milk and 1% Tween20 in TBS) for 1 h. After washing with 1% TBS-T, the membrane was incubated with an antirabbit secondary antibody at 1:3000 dilution in Blotto B solution for 1 h. The membrane was washed again with 1% TBS-T and prepared for detection using a chemiluminescence kit (Santa Cruz Biotechnology) following manufacturer's instructions. The intensities of the bands were quantified using the software ChemiImager 4,400 v. 5.5 (Alpha Innotech Corp.). Lunasin quantities in samples were determined by comparison to the standard curve established using known concentration of synthetic lunasin, as shown in **Figure 1**. The method has a detection limit of approximately 15 ng of lunasin, and the curve of signal intensity versus amount of lunasin has a high correlation coefficient of 0.95.

Identification of Lunasin by MALDI (Matrix-Assisted Laser Desorption Ionization) Peptide Mass Mapping. Lunasin purified by ion exchange column was analyzed by MALDI peptide mass mapping

for confirmation of identity. The band corresponding to the molecular weight of lunasin, based on comparison with chemically synthesized lunasin, was excised from SDS-PAGE gel and submitted for MALDI analysis (*12*) at the Protein Structure Laboratory at University of California, Davis. The identity of lunasin was established by comparison of the peptide mass map of the putative peptide obtained from in-gel tryptic digest with predicted masses of lunasin cleaved by trypsin (*13*).

Bioassay of Lunasin From Barley. Chemically synthesized lunasin has been shown in our lab to suppress anchorage-independent growth of mouse fibroblast cells in soft agar (colony assay) induced by *ras*-oncogene transfection and to inhibit histone acetylation in mammalian cells in the presence of sodium butyrate, a histone deacetylase inhibitor.

Colony Assay. Stably *ras*-transfected cells, (Dr. Peter Stambrook, Cincinnati Medical School) were used for the colony formation assay in 6-well plates. The cell line was established by introduction of a Ha-ras oncogene (valine mutation at codon 12) into NIH 3T3 cells (*13*). The Ha-*ras* oncogene, cloned from the human T24 bladder carcinoma cell line, is driven by an SV40 promoter with the *E. coli lac* repressor binding operator. Transfectants of one cell line designated as 2−12, after isopropyl β-D-thiogalactoside (IPTG, from Sigma) induction, show high expression level of Ha-*ras* oncogene and strong characteristics of transformation, including anchorage independence (*14*, *15*). The inducibility of 2−12 cells makes this cell line a simple yet convenient system to assay the effect of lunasin on anchorage independence of transformed cells. 2−12 cells were routinely grown and maintained in a 37 °C incubator at 5% CO_2 level. Normal growth medium was Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Calf Serum (CS). For each well, 1 mL of 0.6% base layer agar was prepared by diluting 120 μL of 5% agar in 880 μL of growth medium. The bottom agar layer was set at room temperature for 15 min. For each well, 1 mL of 0.33% upper agar layer was then prepared by combining 550 μL of 0.6% agar, 330 μL of growth medium, 20 μL of 1 M IPTG ,and 100 μL of 2−12 cells (40,000 cells/ml). When lunasin was used for treatment, the volume in the solution displaced the same volume of medium. Once the upper agar solution was transferred to the wells, cells were incubated for 14 days. Colonies were counted under a microscope at a magnification of 40×. The treatment concentrations varied from 1 nM to 10 μM for lunasin purified from ion-exchange and immunoaffinity column chromatography.

Histone Acetylation Assay. Mouse fibroblast cells NIH 3T3 (ATCC) were grown in DMEM + 10% FBS in a 37 °C incubator at 5% CO_2 level. Using standard protocols (Upstate Biotechnology), acid-extracted proteins enriched for histone proteins were isolated from NIH 3T3 cells, which were then treated with 10 μM of lunasin purified from immunoaffinity column chromatography in the presence and absence of 5 mM sodium butyrate (*16*). Approximately 0.8 mg of acid-extracted proteins were run on 16.5% Tris-Tricine gel (Bio-Rad Laboratories) and blotted onto Hybond-ECL membranes (Amersham). Immunoblot analysis was conducted using primary antibodies against tetraacetylated H4 (H4−Ac) and tetraacetylated H3 (H3−Ac) (Upstate Biotechnology), diluted 1:1000, and HRP-labeled ant-irabbit IgG secondary antibody, diluted 1:2000. Densitometer readings were taken on autoradiograph using the Biorad Molecular Imaging System GS525 and Molecular Analyst software to measure levels of acetylated H4 and H3.

RESULTS

Lunasin in Various Barley Varieties. Figure 2 shows the Coomassie blue staining (**Figure 2A**) and Western blot (**Figure 2B**) for five cultivars of barley, which all show a lunasin band on the Western blot. Three varieties of millet extracted and tested similarly showed no lunasin bands (data not shown). Coomassie blue staining shows that the majority of the proteins in barley range from 30 to 200 kDa in molecular weight with the rest in the 4−14 kDa range. The lunasin band from barley and that of synthetic lunasin are below the 5 kDa range as expected.

Lunasin Concentration in Barley Seeds. The concentration of lunasin in the crude extracts of five varieties of barley were calculated using the standard curve such as the one in **Figure 1** and expressed in ng lunasin/μg protein and μg lunasin/g seed as shown in **Table 1**. The values varied from 5.9 for Olbori cultivar to 8.7 ng lunasin/μg protein for Mirakbori. In comparison, our analysis of defatted soy flour shows an average of 5.5 ng lunasin/μg protein (unpublished results).

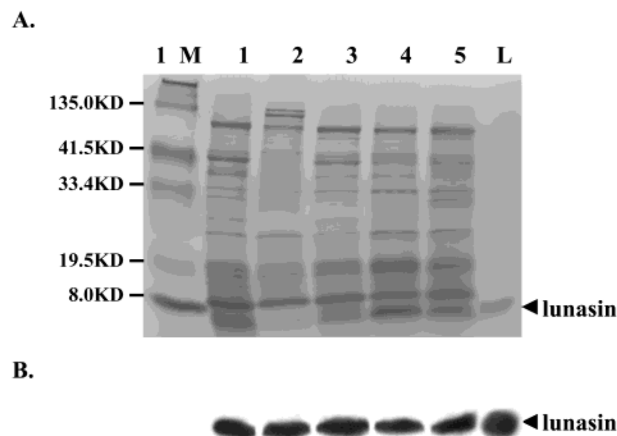


Figure 2. Coomassie blue staining (A) and Western blot (B) of protein extracts from different varieties of barley. Lanes are M (MW marker), 1 (cv. Olbori), 2 (cv. Daebackbori), 3 (cv. Mirackbori), 4 (cv. Ricebori), 5 (cv. Waxybori), and L (50 ng lunasin standard).

Table 1. Lunasin Contents of Extracts of Different Varieties of Barley[a]

varieties of barley	ng lunasin/μg protein	μg lunasin/g seed
Hordeum vulgare L.	5.93 ± 0.19	13.56 ± 0.43
cv. Olbori	7.51 ± 0.19	18.61 ± 0.48
cv. Daebackbori	8.71 ± 0.20	21.48 ± 0.49
cv. Mirakbori	5.99 ± 0.18	14.18 ± 0.42
cv. Ricebori	6.12 ± 0.17	17.14 ± 0.48
cv. Waxybori		

[a] Values represent averages ± standard deviations from triplicate determinations.

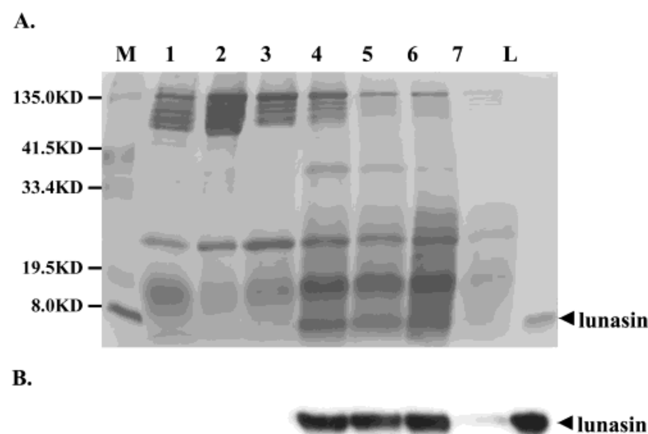
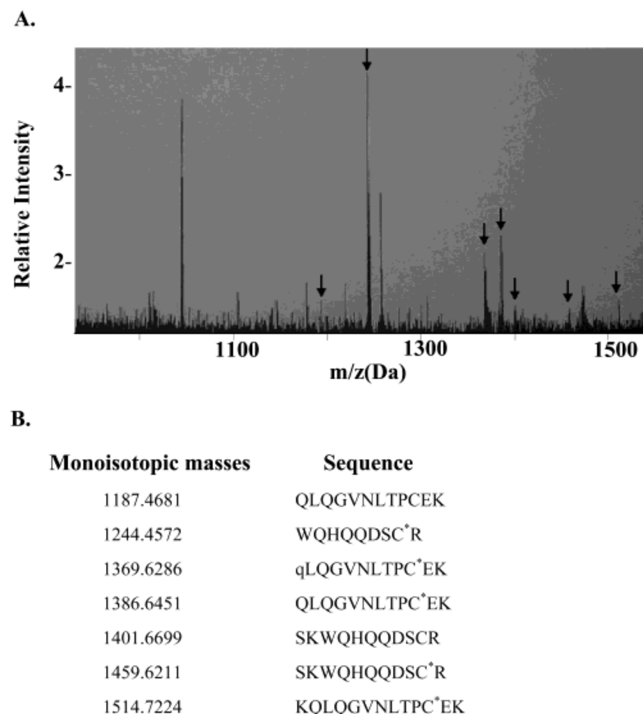


Figure 3. Coomassie blue staining (A) and Western blot (B) of protein extracts of barley (cv. Olbori) purified by ion-exchange column chromatography using various concentrations of NaCl as eluant. Lanes are M (MW markers); 1–7 are samples eluted at NaCl concentrations of 0, 0.1, 0.3, 0.5, 0.7, 0.8, and 0.9 M, respectively, and L (50 ng lunasin standard).

Purification of Lunasin from Barley by Ion Exchange Column Chromatography. Figure 3A shows that most of the low MW proteins were eluted at 0.5, 0.7, and 0.8 M NaCl in a batchwise elution mode. The presence of lunasin in these fractions was confirmed in the Western blot (**Figure 3B**), showing a single band for lunasin. For immunoaffinity purification, lunasin was eluted with 0.7 M NaCl from ion exchange column and fractions containing lunasin were pooled.



Monoisotopic masses	Sequence
1187.4681	QLQGVNLTPCEK
1244.4572	WQHQQDSC*R
1369.6286	qLQGVNLTPC*EK
1386.6451	QLQGVNLTPC*EK
1401.6699	SKWQHQQDSCR
1459.6211	SKWQHQQDSC*R
1514.7224	KQLQGVNLTPC*EK

Figure 4. (A) MALDI peptide mass map of putative lunasin band cut out from SDS-PAGE of ion exchange purified fraction of barley extract eluted with 0.7 M NaCl and digested with trypsin in-gel. The masses of seven peptide fragments marked with arrows match with those of predicted sequences deduced from tryptic cleavage within 30 ppm, unambiguously identifying lunasin as the major peptide in the band. (B) The masses of the seven fragments and their predicted sequences.Q refers to glutamine, and q is glutamine cyclized to pyroglutamic acid. C* refers to cys modified to carboxy amidomethyl-cysteine.

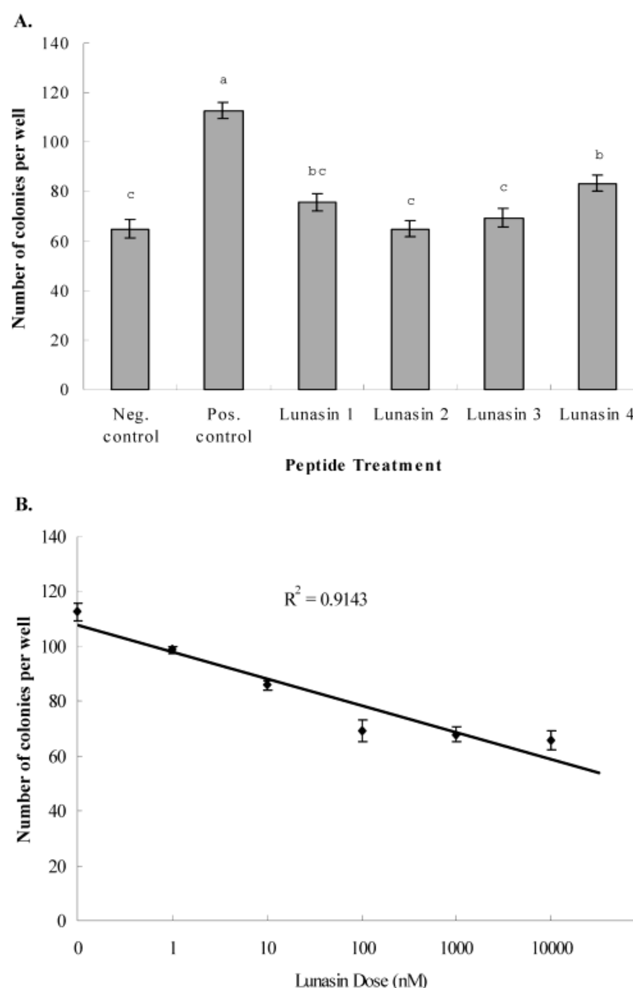


Figure 5. Purified lunasin inhibits colony formation in IPTG-induced *ras* stably transformed 2–12 cells. (A) At a concentration of 10 μM, lunasin purified using different methods was as effective in inhibiting colony formation as the synthetic lunasin. Negative control was not treated with IPTG, while the positive control was treated with IPTG without lunasin. Lunasin 1 is crude extract of barley, lunasin 2 is lunasin 1 purified by ion-exchange chromatography by elution at 0.7 M NaCl and not dialyzed before bioassay, lunasin 3 is lunasin 2 purified by immunoaffinity chromatography, and lunasin 4 is synthetic lunasin. Treatment means (± standard errors) with similar letters are not significantly different from each other as analyzed by One Way ANOVA followed by Duncan's Multiple Range Test. (B) Dose response of immuno-purified barley lunasin fraction in suppression of colony formation. Each lunasin dose represents the means (± standard error) of triplicate experiments.

MALDI Analysis of In-Gel Tryptic Digests of Lunasin Band. Figure 4A shows the Laser Desorption Ionizatin (LDI) mass spectrum for the in-gel tryptic digest of the putative lunasin band cut out from the SDS-PAGE of ion exchange column fraction eluted at 0.7 M NaCl. The arrows point to seven peptide masses that match the masses of predicted fragments based on tryptic cleavage of lunasin within 30 ppm (*12*), establishing unambiguously that lunasin was the major peptide in the band. The monoisotopic masses of the peptide fragments and the corresponding predicted sequences are shown in **Figure 4B**.

Purified Lunasin from Barley Suppresses Colony Formation of Stably *ras*-Transfected Cells. The colony assay provides a simple and rapid quantitative tool for analysis of the oncogene-induced anchorage independent growth of cells in culture. As shown in **Figure 5A**, lunasin barley fractions of different purification states inhibited colony formation in stably *ras*-transfected 2−12 cells just as effectively as synthetic lunasin at a concentration of 10 μM. Concentrations of lunasin in the barley fractions were determined by running Westerns and comparing lunasin band intensity with standard curve such as in **Figure 1B**. It is noteworthy that the crude extract was as effective as the other more purified fractions, including synthetic lunasin. In all the experiments, the negative control was a set of cells receiving no IPTG during the assay, while the positive control consisted of cells induced by IPTG but received no lunasin treatment. The concentrations of the barley lunasin fractions were determined by running Western blots and comparing the lunasin band intensities with a standard curve such as in **Figure 1**. As shown in **Figure 5B**, immunoaffinity-purified lunasin suppressed colony formation by 40% when present in the medium at a concentration as low as 100 nM. The dose-dependent colony suppressive effect of lunasin is a linear relationship with a R^2 value of 0.91 when plotted in a semilog scale. **Figure 5B** shows that the dose effect of lunasin is linear up to a 100 nM concentration; beyond this, the effect is nonlinear (i.e., logarithmic). This suggests that the internalization of lunasin at a concentration beyond 100 nM could be the limiting process in reduction of colony formation. Increasing the concentration of lunasin from 100 nM to 10 μM did not produce any further statistically significant reduction in colony formation. Whether the transport of lunasin through the cell membrane is through passive diffusion or active transport remains to be established. Overall, these results indicate that lunasin extracts from barley at different stages of purities retain their biological activities as measured by the colony assay. This is the first piece of evidence indicating that lunasin isolated from

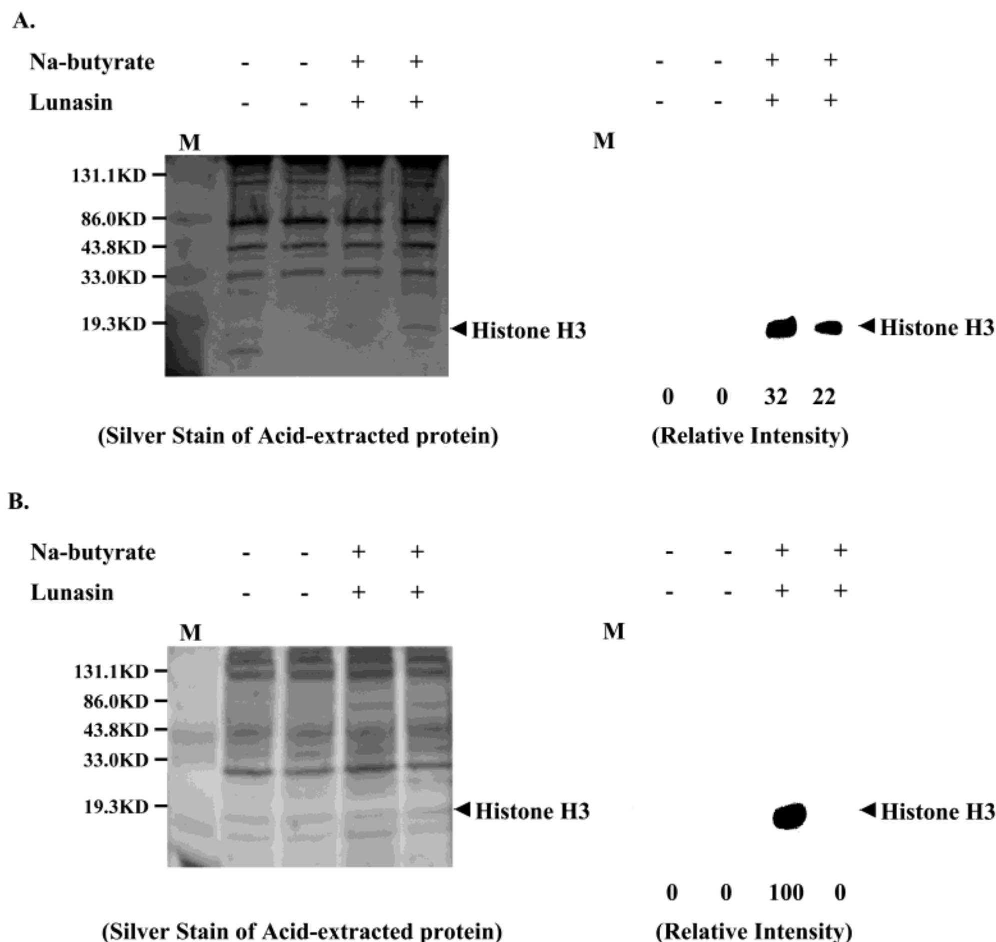


Figure 6. Lunasin inhibits in vivo acetylation of histone H3 in the presence of Na–butyrate in (A) NIH3T3 and (B) MCF-7 cells. Silver stain (left panels) and immunoblot (right panels) of acid-extracted proteins isolated from NIH3T3 cells treated with 100 μM of immunoaffinity-purified lunasin and 5 mM of Na–butyrate. Acid exracted proteins enriched for histone proteins from the different treatment combinations were probed with antiacetylated histone H3. Numbers underneath immunoblots correspond to densitometer readings normalized against that of the non-Na–butyrate treated controls.

a natural source (i.e., barley) can be an effective chemopreventive agent in cell culture systems. Studies on soybean extracts show similar results and will be reported elsewhere (in preparation).

Purified Lunasin Decreases in Vivo Histone Acetylation. Histone acetylation and deacetylation are involved in chromatin remodeling, which has been associated with eukaryotic transcriptional regulatory mechanisms (*17*, *18*). To demonstrate the effect of purified lunasin on histone acetylation in vivo, NIH3T3 cells were treated with a histone deacetylase inhibitor, Na−butyrate, in the presense and absence of immunoaffinity-purified lunasin. Na−butyrate is known to increase histone acetylation and the level of acetylated histones in the cell (*19*). **Figure 6A**,**B** shows Coomassie blue stains and Western blots of acid-extracted proteins in the absence and presence of lunasin. The immunoblot shows a significant reduction of tetraacetylated histone H3 (H3−Ac) in Na−butyrate-treated NIH3T3 cells when pretreated with 10 μM of immunoaffinity-purified lunasin peptide (**Figure 6A**). In the absence of lunasin, H3−Ac level increased about 40-fold with Na−butyrate treatment, whereas pretreatment with lunasin brought only an increase of 22-fold in NIH3T3 cells. In Na−butyrate-treated MCF-7 cells, the 100-fold increase in H3−Ac was completely inhibited by lunasin (**Figure 6B**). The extent of reduction in acetylation achieved here by immunoaffinity-purified lunasin is comparable to that achieved using synthetic lunasin (*7*). This confirms that naturally occurring barley lunasin purified by immnoaffinity chromatography is fully functional and retains its ability to inhibit histone acetylation in mammalian cells treated with a histone deacetylase inhibitor.

DISCUSSION

Lunasin, initially identified and isolated from soybean (*9*), has been proposed to have some important biological functions in seed development because of its unique sequence but none have been proven. Its ability to arrest cell division was demonstrated serendipitously in our laboratory first in bacteria and subsequently in mammalian cells, leading to cell death when the lunasin gene is transfected and constitutively expressed (*6*). More recently, it was shown that synthetic lunasin peptide suppresses chemical carcinogen-induced transformation of mammalian cells when added exogenously and inhibits chemical carcinogen-induced skin tumor formation in mice when applied topically (*7*). Evidence supports the proposed chemopreventive mechanism that lunasin selectively induces apoptosis in transformed cells by binding to deacetylated histones exposed by the transformation event and inhibiting acetylation (*7*). In all these experiments, chemically synthesized lunasin was used to demonstrate its chemopreventive properties. In search for natural sources of lunasin besides soybean, we report here the identification, isolation, and bioassay of lunasin from barley, a cereal grain whose lunasin content is higher on a protein basis.than that of soybean, a legume. This is the first report of lunasin in a cereal seed.

Our results clearly show that lunasin exists in significant amount in different varieties of barley. We established that ion-exchange and immunoaffinity column chromatography are suitable for purification of lunasin from barley. It is noteworthy that barley lunasin at different stages of purification, including the crude extracts, exhibit biological activities as measured by the two bioassays described. The extraction and purification methods provide us with the tools to isolate fully functional lunasin from natural sources that can be used for large scale animal studies and eventually human clinical trials to confirm its chemopreventive properties. In contrast to synthetic or recombinant lunasin, natural lunasin is expected to receive greater acceptance for chemoprevention from the general public.

The identification of lunasin in barley is highly significant, since it suggests that lunasin might be a universal chemopreventive agent in plant seeds. The intriguing proposed role of lunasin in arresting cell division to allow DNA endoreduplication during seed development (*10*, *11*) suggests the widespread presence of lunasin in angiosperm seeds, although our initial screening of a limited number of seeds suggests otherwise. A wider screening of plant seeds including those from traditional medicinal plants believed to fight cancer is underway.

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