Ascorbigen: chemistry, occurrence, and biologic properties
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Abstract Ascorbigen (ABG) belongs to the glucosinolate family and occurs mainly in Brassica vegetables. It is formed by its precursor glucobrassicin. Glucobrassicin is enzymatically hydrolyzed to indole-3-carbinol, which in turn reacts with L-ascorbic acid to ABG. The degradation of glucobrassicin is induced by plant tissue disruption. The ABG formation depends on pH and temperature. The degradation of ABG in acidic medium causes a release of L-ascorbic acid and a formation of methylideneindolenine; in more alkaline medium, the degradation of ABG causes the formation of 1-deoxy-1-(3-indolyl)-α-L-sorbopyranose and 1-deoxy-1-(3-indolyl)-α-L-tagatopyranose. ABG may partly mediate the known anticarcinogenic effect of diets rich in Brassicaceae. Furthermore, ABG is able to induce phase I and II enzymes that are centrally involved in the detoxification of xenobiotics. Cosmeceuticals containing ABG as an active principle are becoming increasingly popular, although the underlying cellular and molecular mechanisms regarding its potential antiaging and ultraviolet-protective properties have not been fully established.
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Introduction

Epidemiologic studies revealed an inverse relationship between Brassica vegetable intake and the development of cancer.1-4 All kinds of cabbages, brussels sprout, broccoli, cauliflower, kohlrabi, turnip, and suede belong to the family of Cruciferae and are botanically known as Brassicaceae. Their anticarcinogenic effect is probably due to their high amounts of glucosinolates.5 One of the most commonly studied glucosinolate is glucobrassicin (GB).6 This compound is a precursor of indole-3-carbinol (I3C) and ascorbigen (ABG), which are both considered to be potent anticarcinogens of the glucosinolate family. The present review summarizes the current literature regarding the chemistry, occurrence, and biologic activity of ABG.

Chemistry

Structure

Glucosinolates consist of a β-thioglucopyranoside group and a side chain that is attached to the carbon atom number 0 in the (Z)-N-hydroximine-O-sulfonate group.7 In all identified glucosinolates, this structure has been found and validated by chemical, x-ray, crystallographic, and nuclear magnetic resonance measurements.7 Structural variations are caused by differences in the R group and by acylsubstituents on the thioglucose group.7 Shortly after the discovery of the chemical structure of vitamin C by Svirbely and Szent-Györgyi8 in 1932, different research groups found a
ABG is not present in intact plant tissues. Its formation takes two consecutive steps. The first reaction is the enzymatic hydrolysis of GB, and the second step is a spontaneous reaction of the emerging intermediate (I3C) with AA. The breakdown of GB is induced by plant tissue disruption (e.g., culinary procedures) and catalyzed by the enzyme myrosinase. This enzymatic degradation of GB is a fast process depending on the myrosinase activity (Figure 2). The product formation is regulated by the hydrolysis conditions, including pH and the presence of metal ions. I3C is the major product at neutral pH, whereas in acidic conditions 3-indolyl-acetonitrile is the main compound formed. In the presence of AA, 3-indolyl-acetonitrile and I3C form ABG.

Biosynthesis

ABG and I3C are unstable compounds that can be transformed into different products. There are numerous studies focusing on the transformation of I3C in an acidic environment and the identification of new breakdown compounds. The major identified compounds are 3,3'-diindolylmethane, 2-(3-indolylmethyl)-3,3'-diindolylmethane, and 5,11-dihydroindol[3,2-b]arbazole (ICZ).

Hrncirik et al investigated the formation of ABG at 25°C in buffers of different pH values containing I3C and AA in a molar ratio of 1:1. The pH values of Brassica vegetables ranged between 5.0 and 6.5; lower pH conditions were tested because of ABG formation during culinary and industrial processing of vegetables. Obviously, ABG formation was strongly dependent on the pH in the medium: In a medium with a pH of 3, 54% of the theoretic amount of ABG forms.
was reached in 5 minutes. At pH 4, 82% was achieved after 15 minutes, and pH 5 resulted in 89% of the theoretic amount of ABG. The corresponding reaction scheme is presented in Figure 2. In solutions with a pH of 3, the main products found were ABG, ABG-dimer (2′-skatylascorbigen), and ABG-trimer ((2′-2′′-(skatyl-3′′′)skatyl)ascorbigen), reaching 13% and 11%, respectively. Similar results were obtained at conditions of pH 4, whereas at a pH of 5 only the ABG dimer was detected. No ABG polymers were found in solutions of pH 6 and 7. In contrast with the results found by Kiss and Neukom,14 who detected the highest amounts of ABG at pH 4.0, Hrncirik and coworkers found the maximum levels between pH 4.5 and 5.0 after 60 minutes.

Degradation

During the degradation of ABG in an acidic medium, AA is released and methylideneindolenine is formed. The 3-indolylmethyl moiety binds to another molecule of ABG to form ABG-dimer, ABG-trimer,28 and 5,11-dihydroindolo[3,2-b]carbazole.29 Preobrazhenskaya et al30 demonstrated a transformation of ABG in mild alkaline media into indole-derived carbohydrates 1-deoxy-1-(3-indolyl)-α-L-sorbopyranose and 1-deoxy-1-(3-indolyl)-α-L-tagatopyranose resulting from the opening of the lactone ring and decarboxylation. Recent investigations by Reznikova et al31 in bovine blood serum and mouse liver homogenates revealed that these 1-deoxy-1-(indol-3-yl)-ketoses are the main products of ABG transformation in vivo.

The stability of ABG significantly depends on the pH value and temperature. Thermal treatment for vegetable processing increases the degradation of ABG. During the first 2 hours in solutions of pH 3 to 6 at 25°C, ABG is relatively stable with a loss of only 3% to 5%. A pH value of 7 caused a decomposition of ABG of approximately 25%. The storage at 25°C for 10 hours resulted in a 12% to 20% degradation of ABG at pH 3 to 6 and in a degradation of 75% at a pH of 7. In solutions of pH 4 the highest stability of ABG was observed, whereas at more alkaline conditions ABG was dramatically decreased. Heating at 80°C for 20 minutes resulted in a loss of 54%, 39%, 56%, 95%, and 100% of ABG at pH values of pH 3, 4, 5, 6, and 7, respectively.

Content of ascorbigen in different Brassica vegetables

Hrncirik and coworkers32 established a high-performance liquid chromatography method to measure the ABG concentrations of different members of the Brassica genus. White Cabbage, Chinese cabbage, broccoli, and cauliflower were first analyzed regarding their content of AA and GB. The highest amounts of AA (840 mg/kg) were found in broccoli, whereas the lowest amounts of AA (110 mg/kg) were found in Chinese cabbage. The AA amounts in white cabbage and cauliflower ranged in between. The GB amounts ranged between 25 mg/kg for Chinese cabbage and 142 mg/kg for cauliflower. In the next step they analyzed the concentration of ABG in aqueous vegetable homogenates in 2-hour intervals. The concentrations of ABG in homogenized vegetables increased during the incubation at room temperature (RT), reached the maximum after 4 to 6 hours of incubation, and finally decreased slowly (Figure 3). Highest amounts were obtained in homogenized white cabbage with 16 mg/kg after 2 hours of incubation at RT. The lowest amounts of ABG were found in Chinese cabbage (5.3 mg/kg; 2 hours at RT), whereas the amounts in broccoli (6.8 mg/kg; 2 hours at RT) and cauliflower (13 mg/kg; 2 hours at RT) were in between. The absolute value of white cabbage in the study of Hrncirik and coworkers32 was lower than the levels found by Aleksandrova et al,28 which ranged from 31 to 55 mg/kg in fresh chopped cabbage. The different levels of ABG found by both studies could be caused by different initial levels of GB in the cabbage used. As already pointed out, the pH value of the solution is a critical point for the formation and stability of ABG.17 For ABG formation, the initial GB levels in vegetables are an important factor32 in why the amount of ABG can significantly vary between different species. The yield of ABG depends on small differences in natural pH values of the vegetable homogenates. Chinese cabbage homogenates exhibited the lowest pH value, thus transforming approximately 50% of GB into ABG, whereas only 20% of GB in

![Image](Link to image)
Cauliflower is converted. Ciska and Pathak investigated fermented cabbage on its concentration of glucosinolates. With concentrations of 43 mg/kg, ABG represented the most prominent compound of glucosinolates in fermented cabbage. During the storage of up to 17 weeks, the amount of ABG remained stable. Therefore, fermented cabbage seems to be a good source of ABG, especially during the winter period when the availability of fresh vegetables is lower. Another benefit of fermented cabbage is the stability of ABG for a long period of time.

Cellular effects of ascorbigen

Anticarcinogenic effects of ascorbigen

There are a few studies focusing on the anticarcinogenic effect of ABG (Table 1). Studies by Sepkovic et al. on catechol estrogen production in rat microsomes revealed an anticarcinogenic effect of ABG, I3C, and the cytochrome P450 1A1 inducer β-naphthaflavone. 16α-hydroxyestrone (16α-OHE1) is a genotoxic compound that can be formed from estrogen. The protective effect of I3C, ABG, and β-naphthaflavone is probably due to a decrease of the estrogen pool, thereby reducing the possibility to generate 16α-OHE1 (Figure 4). The study by Sepkovic and coworkers showed an increase of 2-hydroxylation of estradiol in the microsomes of Sprague-Dawley rats after dietary I3C intake. A pretreatment of microsomal incubates from rats with ABG and β-naphthoflavone demonstrated an increase of estradiol 2-hydroxylation. The ability of ABG and I3C to induce the C-2 hydroxylation of estradiol and therefore to decrease the circulating levels of 16α-OHE1 may be the potential mechanism by which compounds reduce the risk of developing mammary tumors.

These results indicate that I3C itself is not able to induce phase 1 enzymes that are responsible for the increasing estradiol 2-hydroxylation. Bradfield and Bjeldanes observed that I3C must be first activated by a low pH environment, as in the stomach, to form 3,3′-diindolylmethane, ICZ, and various other compounds that are able to activate the cytochrome P450 monoxygenase, mediating the activation of phase 1 enzymes.

Stephenson et al. investigated the mechanism of the in vitro modulation of CYP1A1 activity by ABG. CYP1A1 is an enzyme involved in xenobiotic metabolism. In a mouse hepatoma cell line, Hepa 1c1c7, the authors observed an induction of CYP1A1 activity by ABG in a concentration-dependent manner. In addition, the authors found an increased activity of 7-ethoxyresorufin O-deethylase that is caused by an ABG-arranged induction of CYP1A1 protein. By conducting chloramphenicol acetyl transferase (CAT)-reporter gene experiments, it became apparent that ABG induced aryl hydrocarbon (Ah) receptor-driven CAT activity. The activation of the Ah receptor is a well-known mechanism for the induction of CYP1A1 protein and enzyme activity. Although Gillner et al. detected a decreased binding affinity of ABG to the Ah receptor, Stephenson and coworkers noticed that ABG is transformed into a more potent ligand and CYP1A1 inducer, such as ICZ.

Another study, conducted by Kravchenko et al., focused on the effects of indoles on the activity of xenobiotic metabolizing enzymes. The authors fed two groups of rats, one with the basal control diet and one with an indole-enriched diet, containing I3C, ABG, and sulforaphane (SUL) from broccoli. Eight days before the study was terminated, half of the animals of each group received 0.8 mg/kg T-2

<table>
<thead>
<tr>
<th>Organism</th>
<th>Ascorbigen (concentration)</th>
<th>Finding</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Rat microsomes</td>
<td>7 mmol/L</td>
<td>Increases C2-hydroxylation of estradiol to 2-hydroxyestrone, thereby decreasing the circulating levels of 16α-hydroxyestrone</td>
<td>34</td>
</tr>
<tr>
<td>Hepa1c1c7 (mouse hepatoma cell line)</td>
<td>1–1000 μmol/L</td>
<td>CYP1A1 activity increased dose-dependently; EROD activity is inhibited</td>
<td>48</td>
</tr>
<tr>
<td>LS-174 cells</td>
<td>700 μmol/L</td>
<td>The detoxification enzymes CYP1A1, AKR1C1, and NQO1 were induced</td>
<td>5</td>
</tr>
<tr>
<td>Caco-2 cells</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Human colon epithelial cells</td>
<td></td>
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<tr>
<td>SV40-transformed Indian muntjac cells</td>
<td>688 μmol/L</td>
<td>ABG did not cause any chromosome aberrations or sister chromatid exchanges Increase of xenobiotic enzymes (cytochrome P450, benz(o)arene hydroxylase, epoxide hydrolase, carboxylesterase, pNP-UDP-glucuronosyl transferase, HBP-UDP-glucuronosyl transferase, and glutathione-S-transferase)</td>
<td>38</td>
</tr>
<tr>
<td>Male Wistar rats</td>
<td>Indole-enriched diet containing indole-3-carbinol, ABG, and SUL (final concentration 0.1% indoles)</td>
<td></td>
<td>37</td>
</tr>
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ABG, ascorbigen; AKR1C1, aldo-keto-reductase 1C1; EROD, 7-ethoxyresorufin-O-deethylase; HBP-UDP, 4-hydroxy-biphenyl-uridindiphosphate; pNP-UDP, p-nitrophenyl-uridindiphosphate; SUL, sulforaphane.
toxin, a trichothecene mycotoxin, whereas the control animals received an equal volume of solvent (0.1% aqueous solution of ethanol). The 3-week indole-enriched diet application did not have a significant influence on animal growth, relative weight of internal organs, enzyme activity in liver lysosomes, and morphology of internal organs and tissues. The activity of the xenobiotic metabolizing enzymes increased during the consumption of an indole-enriched diet. T-2 treatment of rats fed the basal diet without ABG or SUL caused signs of toxicity: The body weight decreased, the liver weight increased significantly, and the xenobiotic metabolizing enzymes showed a decreased activity. Morphologic changes were also apparent in response to the T-2 treatment. However, the symptoms of T-2 toxicity decreased when the animals were treated with T-2 and fed an indole-enriched diet. No changes in body weight of the animals were then detectable. Only 11% of the rats exhibited an increased liver weight, and macroscopic changes were obvious in only 50% of the animals. Compared with T-2-treated rats receiving the basal diet, the xenobiotic metabolizing enzyme activities were 1.5 to 2.0-fold higher in ABG-treated animals.

Recent studies by Bonnesen et al. focus on the mechanism by which glucosinolate hydrolysis products prevent colon cancer. The authors investigated different adenocarcinoma cell lines, including LS-174, Caco-2, and the human colon epithelial cell on the toxicity of indoles and isothiocyanates. There was no hint of toxicity of ABG and I3C on LS-174, Caco-2, and human colon epithelial cell lines; the IC_{50} values were more than 500 μmol/L. 3,3’-diindolylmethane and indole [3,2-b] carbazole (ICZ) were more toxic in human colon cells compared with ABG or I3C. All three cell lines tolerated the aliphatic isothiocyanate SUL better than the aromatic isothiocyanates benzyl isothiocyanate and phenethyl isothiocyanate. All phytochemicals examined induced apoptosis in transformed LS-174 and Caco-2 cell lines, but none of the test components stimulated apoptosis in untransformed human colon epithelial cells. Furthermore, Bonnesen and coworkers demonstrated that the detoxication enzyme activity of the analyzed cell lines could be enhanced by 3,3’-diindolylmethane, ABG, I3C, ICZ, SUL, benzyl isothiocyanate, and phenethyl isothiocyanate. In another experiment, the authors studied the drug-metabolizing enzyme CYP1A1 in colon cells; indoles and isothiocyanates induced this enzyme. In CAT-reporter gene assays, it was shown that the induction of gene expression by dietary indoles and isothiocyanates is mediated through different molecular mechanisms. Indoles induce the xenobiotic response element-driven CAT activity, whereas the isothiocyanates induce the antioxidant response element-driven CAT activity. Bonnesen et al. investigated the protection of DNA damage by the application of phytochemicals (1 μmol/L ICZ or 5 μmol/L SUL): LS-174 cells were treated with benz(a)pyrene or H₂O₂ for the induction of DNA damage. The amount of DNA damage was measured by the Comet assay. After pretreatment with ICZ and SUL, the colon cells were significantly protected from DNA single-strand breaks compared with the non-supplemented cells.
Clastogenic and mutagenic properties of ascorbigen

In SV40-transformed Indian muntjac cells, ABG and Me-ABG were tested for potential clastogenic activities. The mutagenic activity was determined by the Ames test with Salmonella typhimurium strains TA-98 and TA-100. For ABG there was no induction of either chromosome aberrations or sister chromatid exchanges at any concentrations tested, or an induction of mutations in the above-mentioned Salmonella strains. Furthermore, no effect on the clonal survival of SV40-transformed Indian muntjac cells at concentrations less than 6.88 μmol/L was detected. However, Me-ABG was more cytotoxic and induced sister chromatid exchanges and mutations in the Ames test, but no chromosome aberrations were observed. Studies conducted by Musk and coworkers investigated the breakdown products of ABG on their genotoxic properties. ABG-dimer and Me-ABG-dimer, the products formed in acidic solutions, did not show any genotoxicity, whereas ISPP and Me-ISP that are formed in alkaline media tested positive at levels greater than 0.125 μg/mL. These findings should be taken into consideration when using ABG as a dietary compound. Figure 5 summarizes the different properties of ABG.

Skin protective properties of ascorbigen

Cosmeceuticals containing ABG as an active principle are becoming increasingly popular, although the mode of action regarding its potential ultraviolet (UV)-protective and antiaging properties has not been established.

It is possible that ABG mediates some of its biologic activity in the skin as the result of AA, its precursor GB, or I3C. Furthermore, ABG may affect signal transduction pathways similar to SUL. Similar to AA, ABG may promote collagen synthesis, photoprotection from UVA and UVB light, and an improvement of a variety of inflammatory dermatoses. UV radiation is the main factor responsible for most nonmelanoma skin cancers. Dinkova-Kostova et al supplemented HaCaT human or PE murine keratinocytes with SUL and observed a dose-dependent induction of intracellular nicotinamide adenine dinucleotide phosphateQO1 and glutathione levels. The topical application of SUL in the form of broccoli sprouts extracts elevated NQO1 activity in mouse skin. In another experiment, Dinkova-Kostova and coworkers exposed SKH-1 hairless mice to relatively low doses of UVB radiation (30 mJ/cm²) twice per week for 20 weeks; this resulted in “high-risk mice” that subsequently developed skin cancer in the absence of further UV treatment. This animal model is able to represent humans who have been heavily exposed to the sun in their childhood but have limited their exposure as adults. The treatment was terminated after 11 weeks; 100% of the animals in the control group developed tumors, whereas 48% of the animals obtaining 1 μmol SUL in the form of sprout extract did not generate cancer. Similarly, the tumor multiplicity was reduced by 58% in the group treated with 1 μmol SUL.

It is known that UV light induces activator protein (AP)-1 involved in skin carcinogenesis. Zhu et al treated HCL14 cells (human keratinocytes) that were stably transfected with an AP-1-luciferase reporter gene with SUL or tert-butylhydroquinone. The authors found an increase of quinone reductase, a marker of global cellular
phase II enzymes, and cellular glutathione levels. In electrophoretic mobility shift assays, a direct application of SUL inhibited the AP-1 DNA binding, whereas tert-butylhydroquinone was ineffective. The elevated phase II enzymes and glutathione levels in human keratinocytes are not able to inhibit the activation of AP-1 through UVB. One mechanism of how SUL exhibits its inhibitory effect on UVB-induced AP-1 activation is the direct inhibition of AP-1-DNA-binding.

The nuclear factor E2-related factor 2 (Nrf2) is a transcription factor involved in the regulation of many detoxifying antioxidant enzymes in response to oxidative or electrophilic stress. Xu and coworkers' study on Nrf2(+/+) and Nrf2(-/-)-mice showed that the Nrf(-/-) mice were more vulnerable to skin tumorigenesis. The treatment of Nrf(+/+)- and Nrf (-/-) mice with SUL caused a significant inhibition of skin tumorigenesis in Nrf(+/+) mice but not in Nrf(-/-) mice. In addition, when 100 nmol SUL was topically applied to the Nrf2(+/+) mice once per day for 14 days, a robust increase of Nrf2 protein levels was detected. These results indicate that the chemopreventive effect of SUL is at least in part mediated by Nrf2. ABG may have effects similar to those of SUL on skin. This should be investigated in future studies.

A study conducted by Srivastava and Shukla' showed that topical I3C treatment, the precursor of ABG, inhibited the tetradecanoylphorbol-13-acetate promotion of 7,12-dimethylbenz[a]anthracene–initiated mouse skin tumors. Cope and coworkers' irradiated Crl:SKH1:hr-BR hairless mice and fed them a control diet, a chlorophyllin (a cancer chemopreventative), or a 13C diet. Although the chlorophyllin group did not show a significant effect, the I3C group showed a significantly reduced overall tumor multiplicity. In addition, the different diets did not show any effect on the UV-induced dorsal skin swelling response at doses of 0.75 and 1 kJ UVB. Doses of 1.5 kJ UVB in the chlorophyllin diet significantly decreased the dorsal skin swelling responses of the mice, whereas the I3C and control diets had no effect. Cope and coworkers' observed that dietary supplementation with I3C exerted significant protection from photocarcinogenesis related to overall tumor multiplicity but not from squamous cell carcinoma.

Conclusions

ABG is the major product of the GB degradation pathway. It is formed in two consecutive steps: 1) GB is hydrolyzed by the enzyme myrosinase, and 2) a spontaneous reaction of the emerging intermediate I3C with AA forms ABG. ABG induces phase I enzymes via the Ah receptor and caused apoptosis in transformed colon cancer cell lines. Another study revealed an increase of C-2-hydroxylation of estradiol, thereby causing a decrease of the circulating levels of 16α-OHE1; this could be one reason for the observed anticarcinogenic effects of ABG. In rats fed an indole-enriched diet (containing I3C, ABG, and SUL), an induction of xenobiotic metabolizing enzymes, including cytochrome P450, glutathione-S-transferase, and uridindiphosphate-glucuronosyl transferase, was found. Systematic investigations regarding the potential photoprotective and antiaging properties of ABG in cultured cells and in vivo are warranted. Furthermore, human studies are necessary to obtain robust data regarding the bioavailability and metabolism of ABG.

References

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