Confidential information

Technical Information on APM

INCI Name: Magnesium Ascorbyl Phosphate

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Functional Chemicals Division

Ver1703NA

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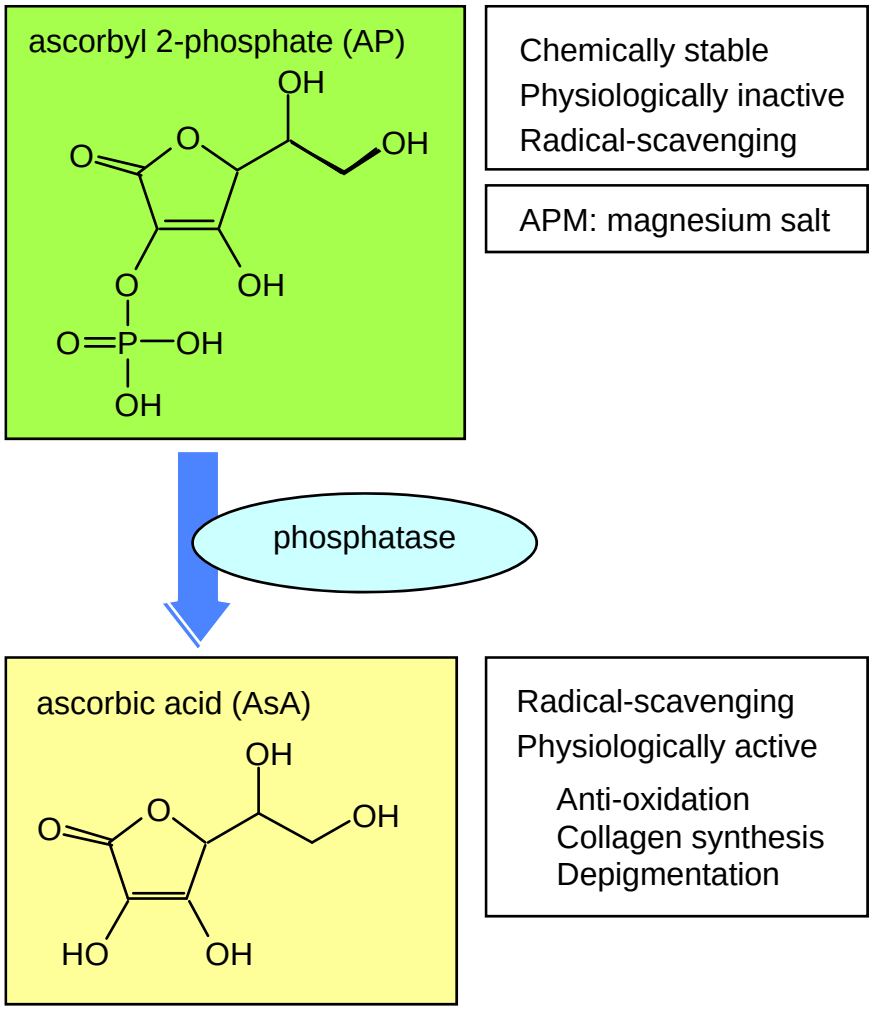
Introduction ;
Ascorbyl 2-phosphate (AP)

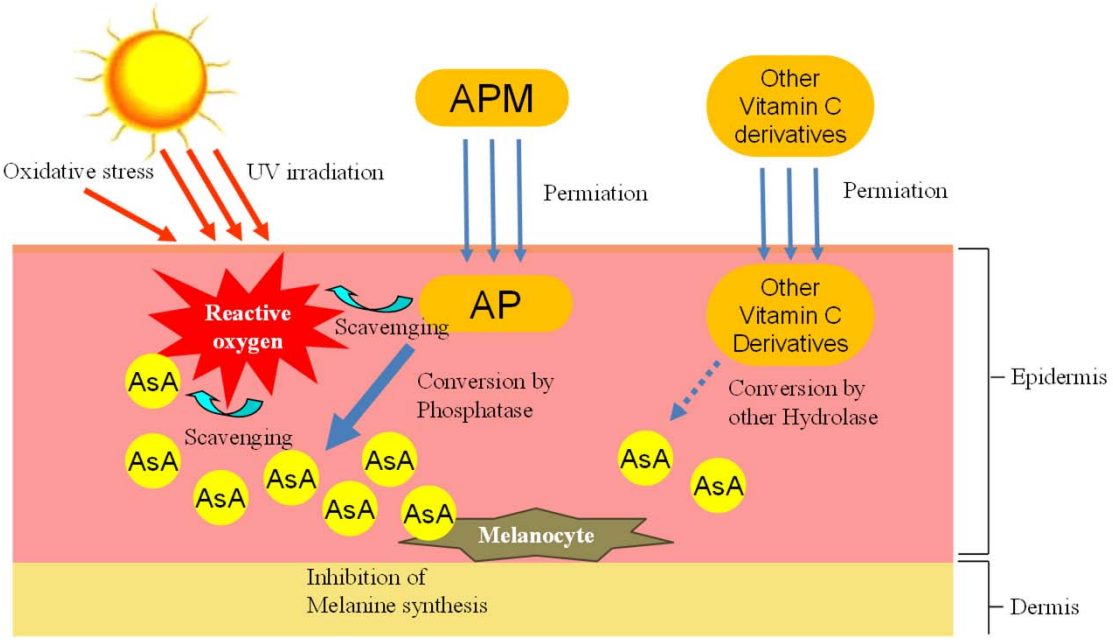


A provitamin C: Magnesium Ascorbyl Phosphate (APM).

Ascorbyl 2-phosphate (AP) is a stable, aqua-soluble provitamin C. APM is a magnesium salt of AP. With ascorbate's sensitive hydroxyl group modified by phosphoric ester, AP is resistant to atmospheric oxygen. AP, being a modified form, still has anti-oxidant activities such as radical-scavenging and dechlorination. However, it does not have any physiological effects. They appear after AP is converted to ascorbate via hydrolysis catalyzed by phosphatase. Those effects include enhanced collagen synthesis and inhibition of skin pigmentation. With the chemical stability, AP enables not only an easy formulation of vitamin C in cosmetics, but also its effective delivery into the skin.

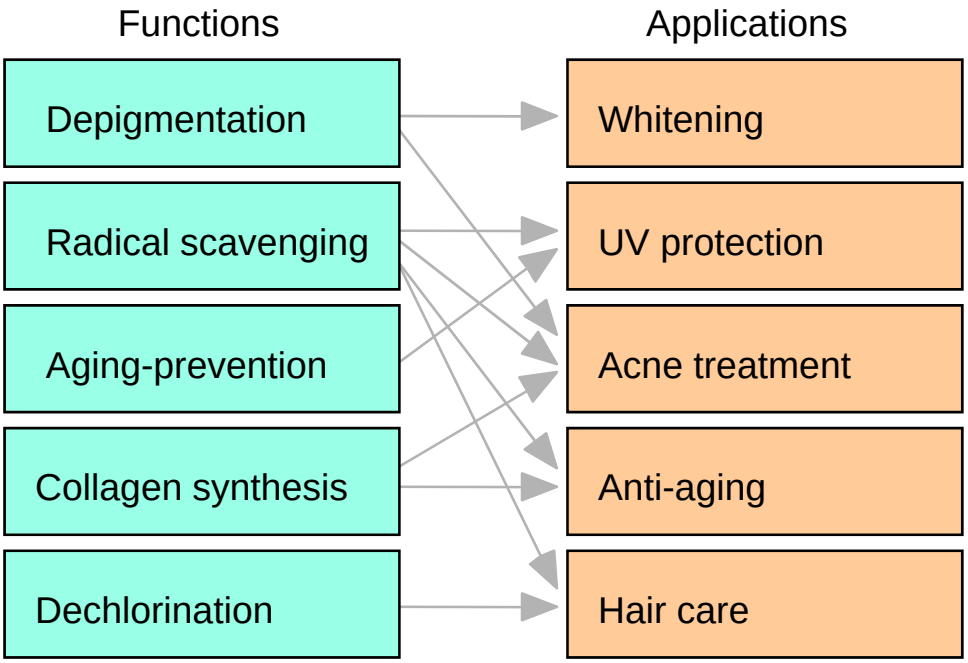
A001(00rev2)ti

Introduction ;
Mechanism of actions



APM enriches ascorbic acid content in the skin.

An ascorbic acid derivative is converted to its bioactive form, AsA, by enzyme in the skin.
There are some types of AsA derivatives. APM is derivatized with phosphate group and is hydrolyzed by phosphatase. The others are modified glucoside group, acyl group and sulfate group etc.. They are converted by glucosidase or esterase etc.. Since phosphatase acts more effectively than these other enzymes, APM can supply more AsA and shows high and various effects in the skin.



Proposed applications of APM, based on the fundamental studies.

Although APM was mainly utilized as an active ingredient for whitening cosmetics, recent investigations suggest various possibilities to utilize the multi-functioning, stable pro-vitamin C, AP. Intradermal and extradermal protective effects against UV-generated radicals suggest that the use for UV-care products. Acne, being re-identified as a radical disease, is another candidate that AP should be applied for. Clinical studies strongly support this idea. Enhancement of collagen synthesis and prolongation of life span of cells support AP's anti-aging efficacies. Furthermore, as AP protects hair cuticles against chlorine-damege, it also should be applied for hair care products.
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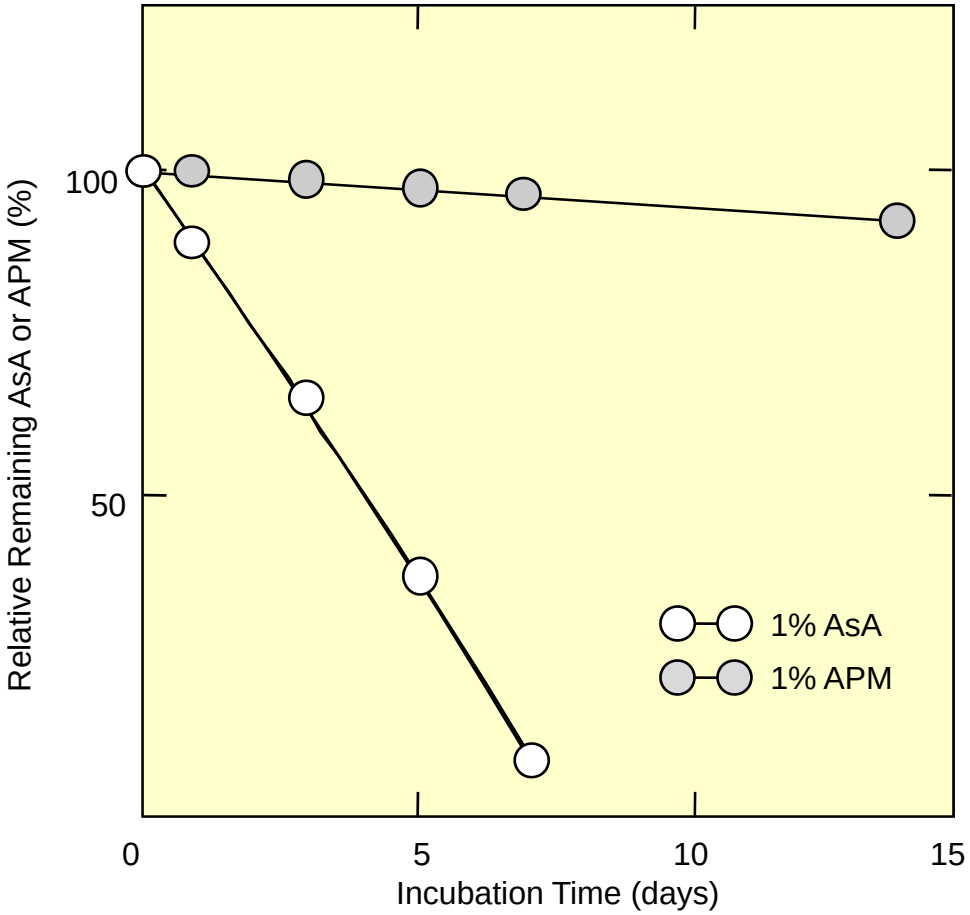
Physical properties of APM ;
Chemical Stability

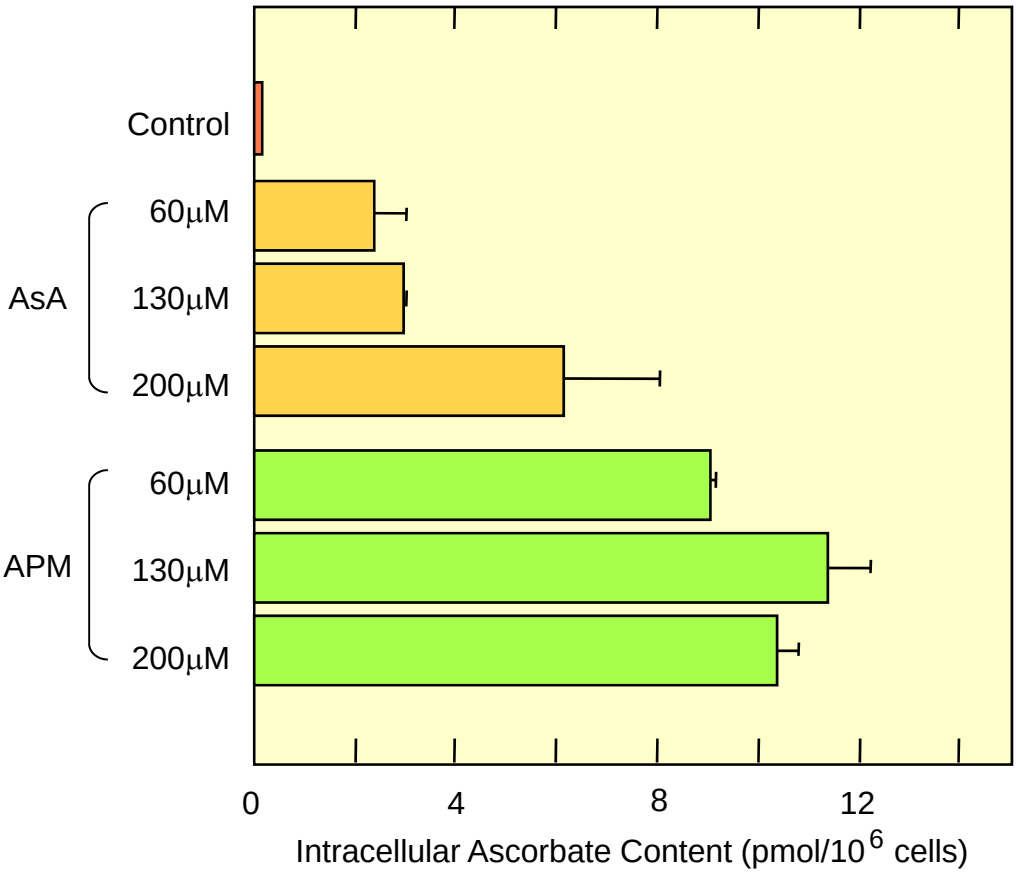


APM is highly stable in aqueous solutions.

The chemical stabilities of APM was compared with that of ascorbate in aqueous solutions. Each substance was dissolved in distilled water at a concentration of 10 mg/ml (1%) and incubated at 60˚C in a test tube with a screw cap tightly fitted. Ascorbate (AsA) was very unstable; it almost disappeared within a week. On the contrary, more than ninety percent of APM remained unoxidized even after the two-week incubation.

A002(00a)ti

Physical properties of APM ;
Intracellular Enrichment of AsA

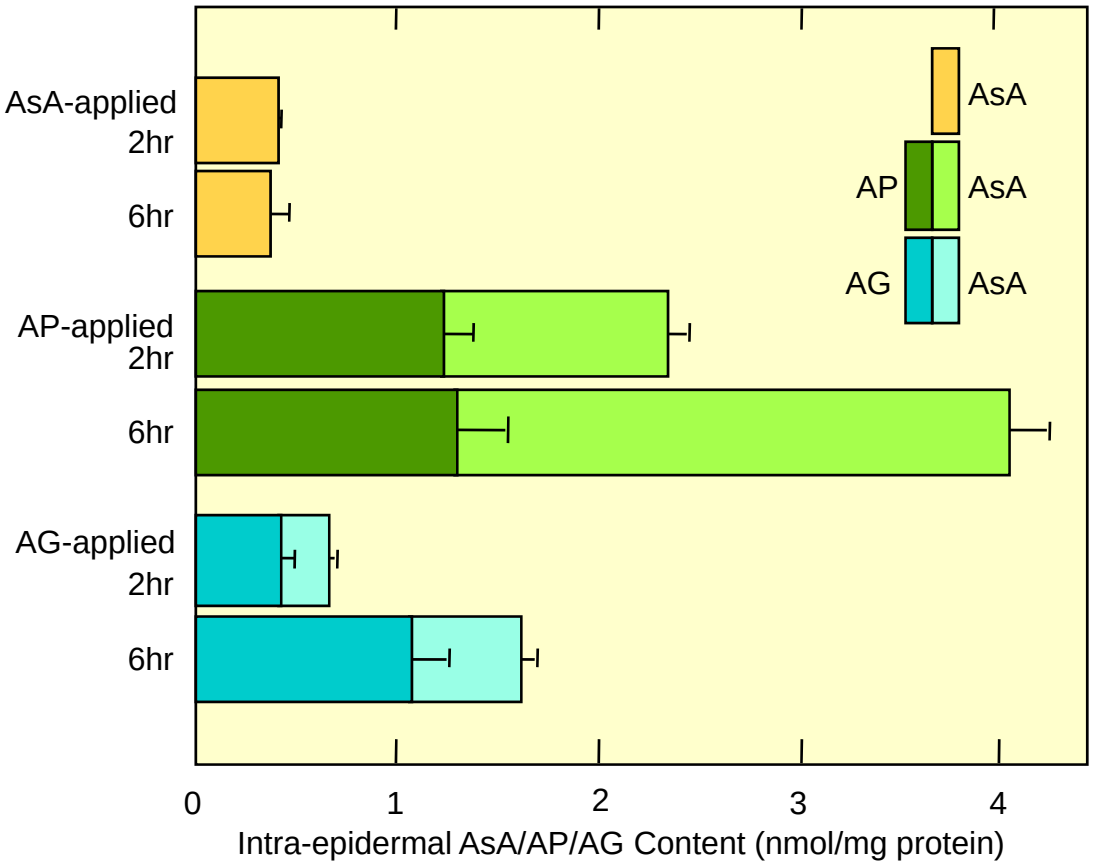


APM enriches intracellular ascorbate content.

Normal human umbilical vein endothelial cells (HUVEC) were kept subconfluent by transferring cells into fresh medium (2500 cells/ml) every 48-72 hours. At each transfer, 60-200 μM ascorbic acid (AsA) or APM were added to the fresh medium. Intracellular concentrations of ascorbate were determined by HPLC: Constant amount of the cells were harvested, homogenized and centrifuged under ice-cold condition.
It was shown that ascorbate was enriched in the cells significantly by addition of APM. Fed at the optimal concentration of 130 μM, intracellular content was 3.9-fold more than that of the cells fed by ascorbate.

A003(03a)ti

Physical properties of APM ;
AsA Enrichment in Human Epidermis



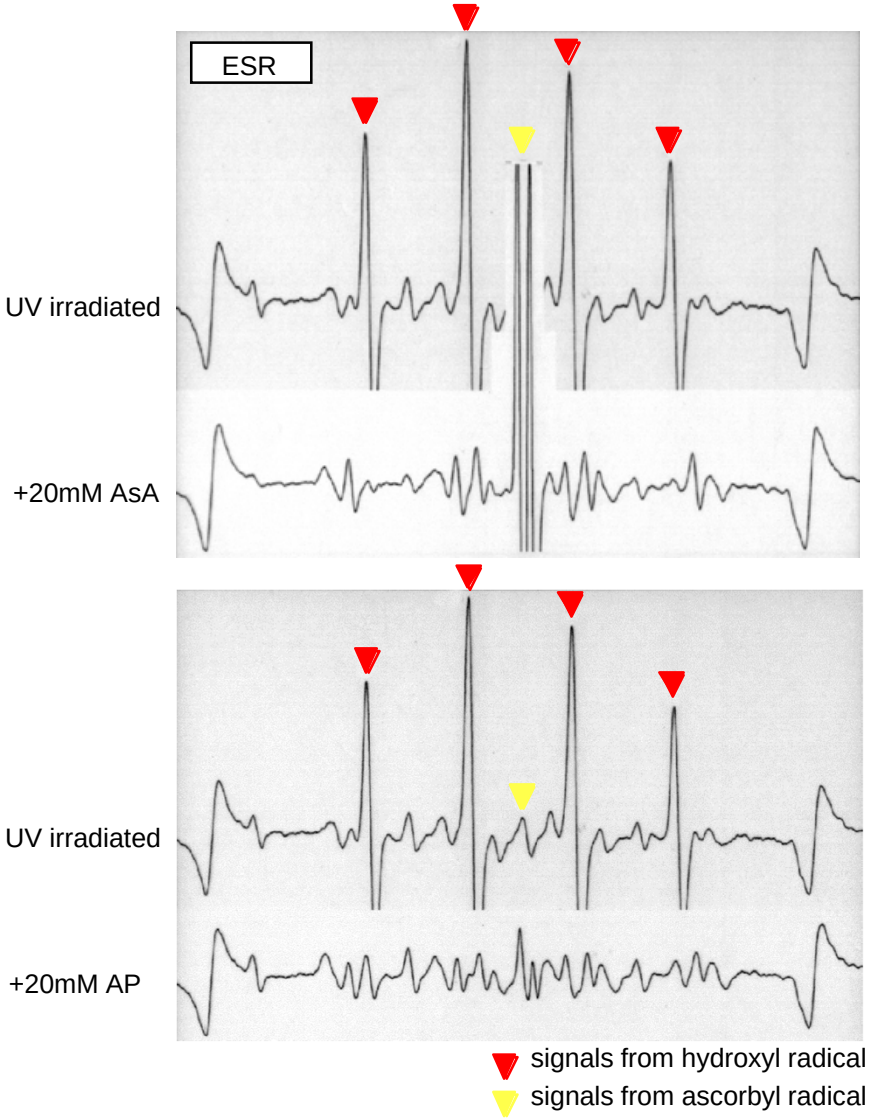
AG: Ascorbyl 2-glucoside

Ascorbate enrichment by APM is significant in human skin.

Tiny skin biopsy samples were prepared from volunteers. The samples were divided into three equal parts and used for the comparison of the permeation and conversion of ascorbate, AP and ascorbyl glucoside (AG). Hydrophilic ointment containing 20 mg/g of each substance was placed onto epidermis side of skin sample. After the incubation for 2 to 6 hours, the skin was homogenized under a strictly anaerobic condition. The content of ascorbate and AP/AG was determined by HPLC.
The ascorbate enrichment by AP was outstanding: At 6hr the 'free' ascorbate content in AP-applied skin was eight times higher than that of ascorbate-applied and five times higher than that of AG-applied.

A009(03c)NAti

UV-protection properties of APM ;
Radical Scavenging Activity (1)

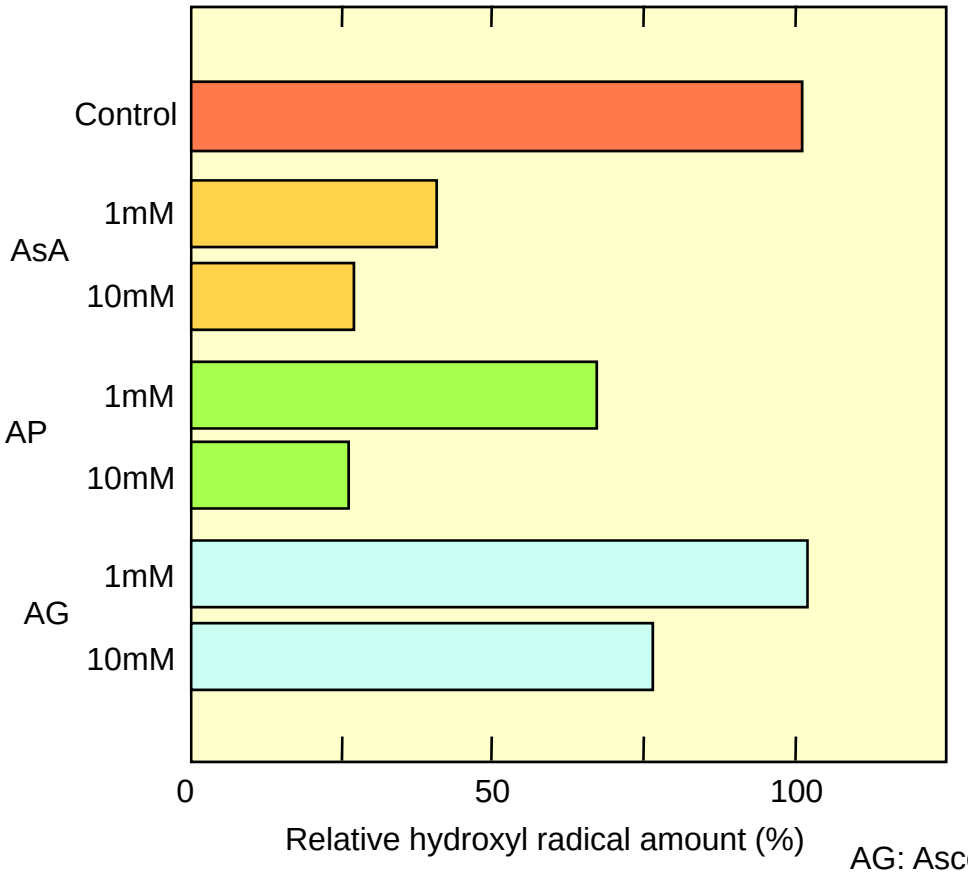


APM scavenges hydroxyl radicals.

When unpurified water is irradiated with ultraviolet light, there occur hydroxyl radicals in it. In this experiment HEPES buffere (pH 7.2) prepared with tap water was irradiated with UVB and generated radical species were detected by ESR spin-trap method.
The spectrum of irradiated sample showed strong quadruplet signals (pointed with red triangles) of hydroxyl radicals. The peaks disappeared upon the addition of ascorbate (AsA). However, unique doublet signals showed up instead, which were identified as those of ascorbyl radicals. They might cause another physiological harm on the skin.
On the contrary, AP reduced hydroxyl radicals' signals almost completely with no significant radicals newly generated.

A31(4a2)ti

UV-protection properties of APM ;
Radical Scavenging Activity (2)



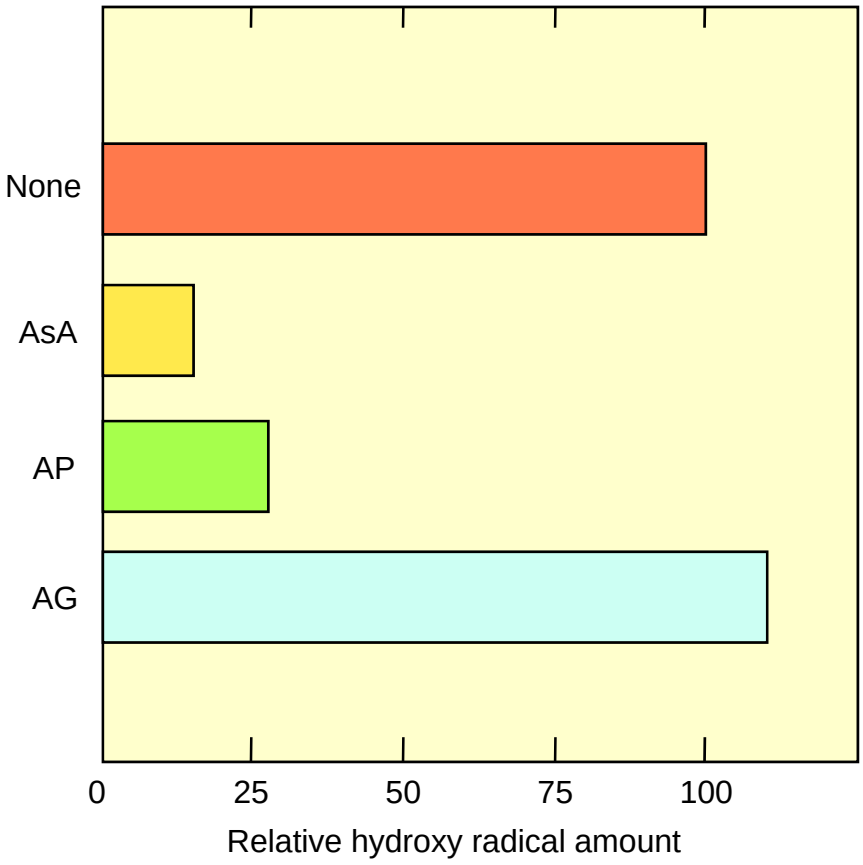
AG: Ascorbyl 2-glucoside

APM effectively scavenges hydroxyl radicals generated by UVB irradiation.

Radical scavenging activities of AP, AsA and AG were compared by the method described in the previous figure. At 10 mM, AP showed a comparable scavenging activity to AsA. The detected amount of hydroxyl radicals were reduced by 75% of non-additive control. AG showed less efficiency; it reduced only by 25% at the same concentration.

A32(04b2)ti

UV-protection properties of APM ;
Radical Scavenging in Human Skin Model

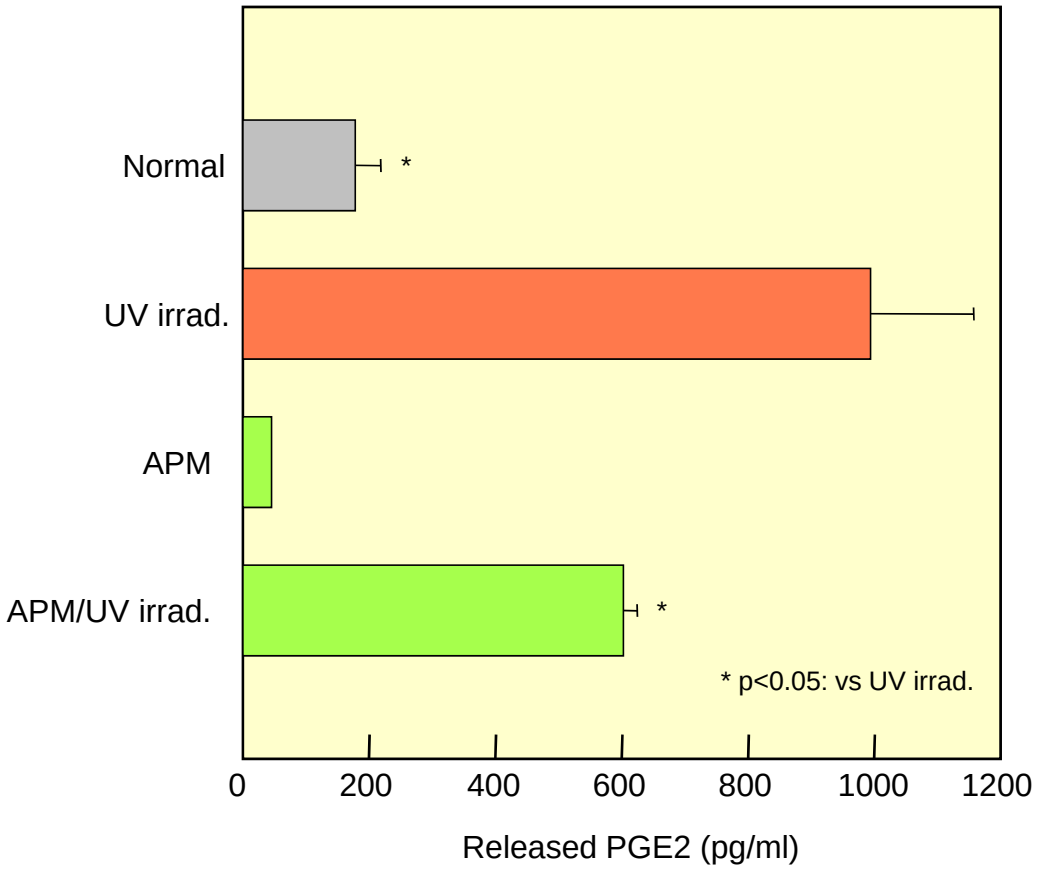


AG: Ascorbyl 2-glucoside

APM effectively scavenges hydroxyl radicals in human skin.

The radical-scavenging effect of AP was confirmed by ESR in human skin models. All the measurement conditions were the same as the experiment shown in the previous figure except that a three dimensional human skin cell culture (TESTSKIN) was used instead of hairless mouse skin sample. Each sample solution (200 mM) was placed onto the epidermis of the skin model and incubated for two hours at 37˚C under 5% carbon dioxide atmosphere. The hydroxyl radicals generated by UV irradiation were effectively scavenged by the pretreatment with ascorbic acid (AsA) or AP. As expected from the fact that AP enriched the intradermal ascorbate more effectively and AP molecule scavenged intradermal radicals more quickly than AG did, there was a significant difference observed in skin-protecting capability between them.

A38(05)ti

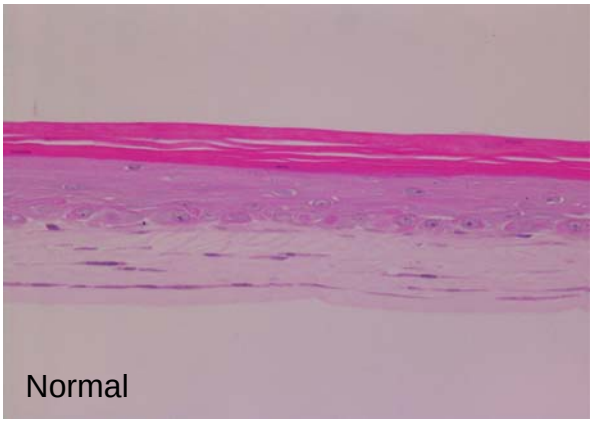


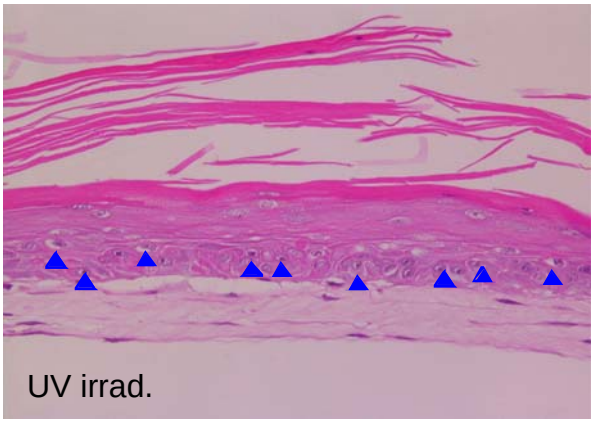
Suppression of inflammation caused by UV irradiation in skin model.

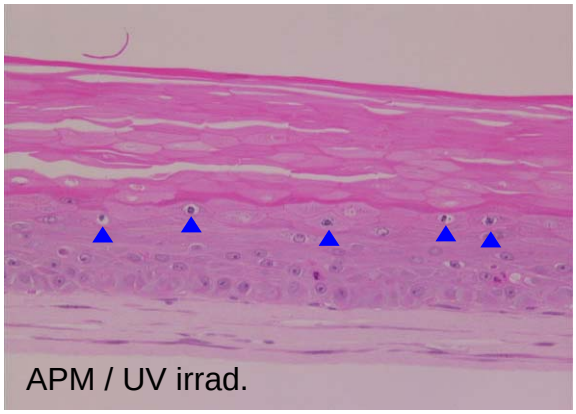
The suppresive effect of APM on inflammation in the skin was examined using a three dimensional restructured human skin model (TESTSKIN^{TM} LSE-high, TOYOBO, Japan).
After irradiation by UVB at an energy of 80 mJ/m^2, the skin models were cultivated with 3% solution of APM on its surface. After 2 hours, APM solution was removed and the skin models were cultivated for another 22 hours.
After 24 hours cultivation, the amount of secreted prostaglandin E2 (PGE2) in the medium was determined by ELISA method.
The PGE2 generation was significantly enhanced by UVB irradiation, while it was suppressed by the post-treatment with APM.

A40/08NA/en

UV-protection properties of APM ;
Skin Protection against UV



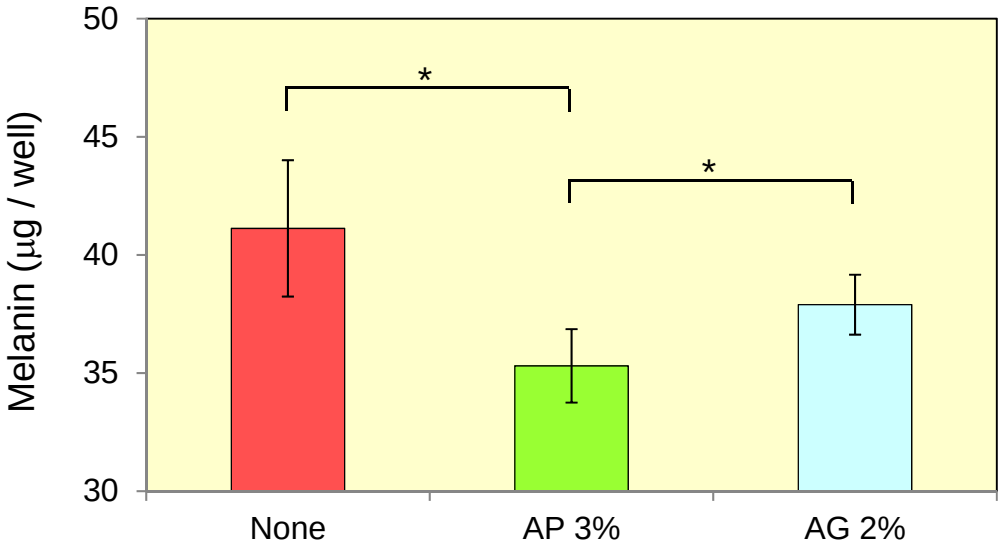




APM protects the skin from UV irradiation.

The protective effect of APM against UV irradiation was examined using a three dimensional restructured human skin model (TESTSKIN™ LSE-high, TOYOBO, Japan).
After irradiation by UVB at an energy of 80 mJ/m^2, the skin models were cultivated with 3% solution of APM on its surface. After 2 hours, APM solution was removed and the skin models were cultivated for another 22 hours.
After 24 hours cultivation, the skin models were fixed and stained with 1% hematoxylin and eosin by the ordinary method.
The UV-damaged cells were stained darker (sunburn cells, typically indicated by blue arrows).
The observations suggested that the damage of corneous layer and the sunburn cells formation was effectively suppressed by the treatment with APM.

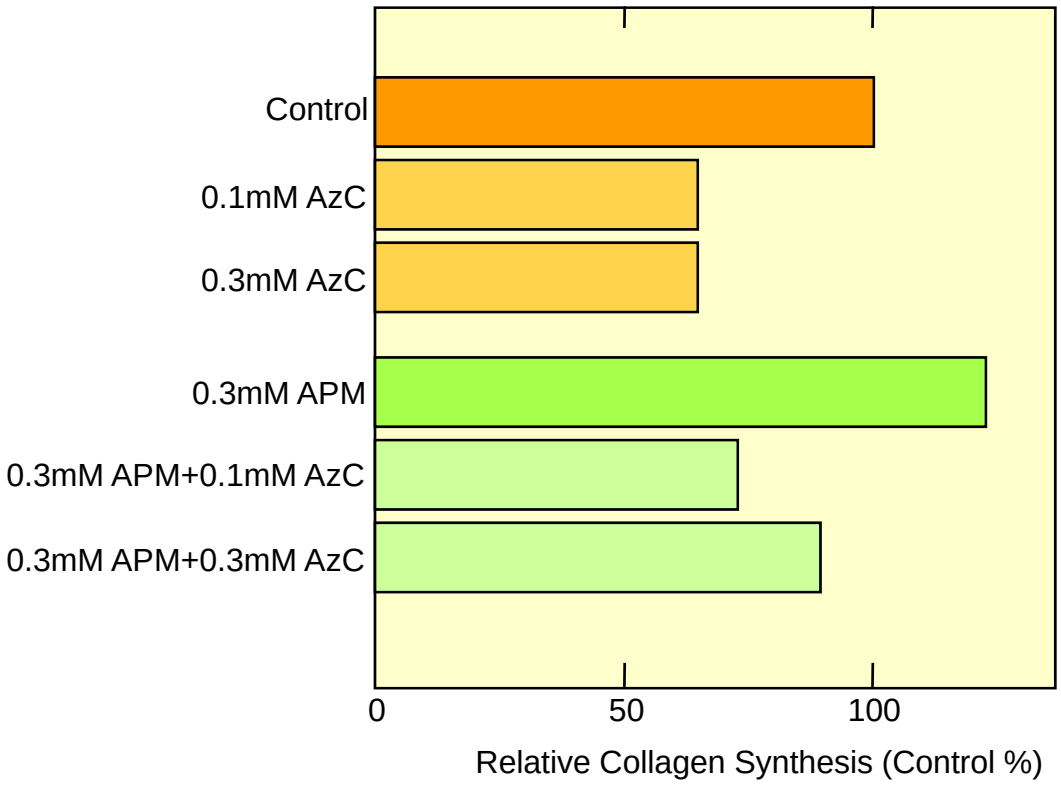
Whitening properties of APM ;
Inhibition of melanin synthesis



Inhibitory effect of APM on melanin production in cultivated skin model.

Ascorbic acid is a known tyrosinase inhibitor. It blocks tyrosinase from working to produce melanin in melanoncyte and shows whitening effect.
Here, inhibitory effect of APM on melanin formation in the skin was verified using three dimensional human keratinocyte/melanocyte co-cultivation system (MEL-300/Asian). The skin models were cultivated with or without test substances for 15 days and then the melanin contents were measured. APM application reduced melanin content in the skin model significantly relative to control. Moreover, the whitening effect of 3% APM, which is the approved lowest concentration as an active for skin whitening Japanese QDs, was higher than that of 2% AG, which is also the approved concentration as an active ingredient for skin whitening Japanese QDs.

Anti-aging properties of APM ;
Collagen Synthesis in Cells



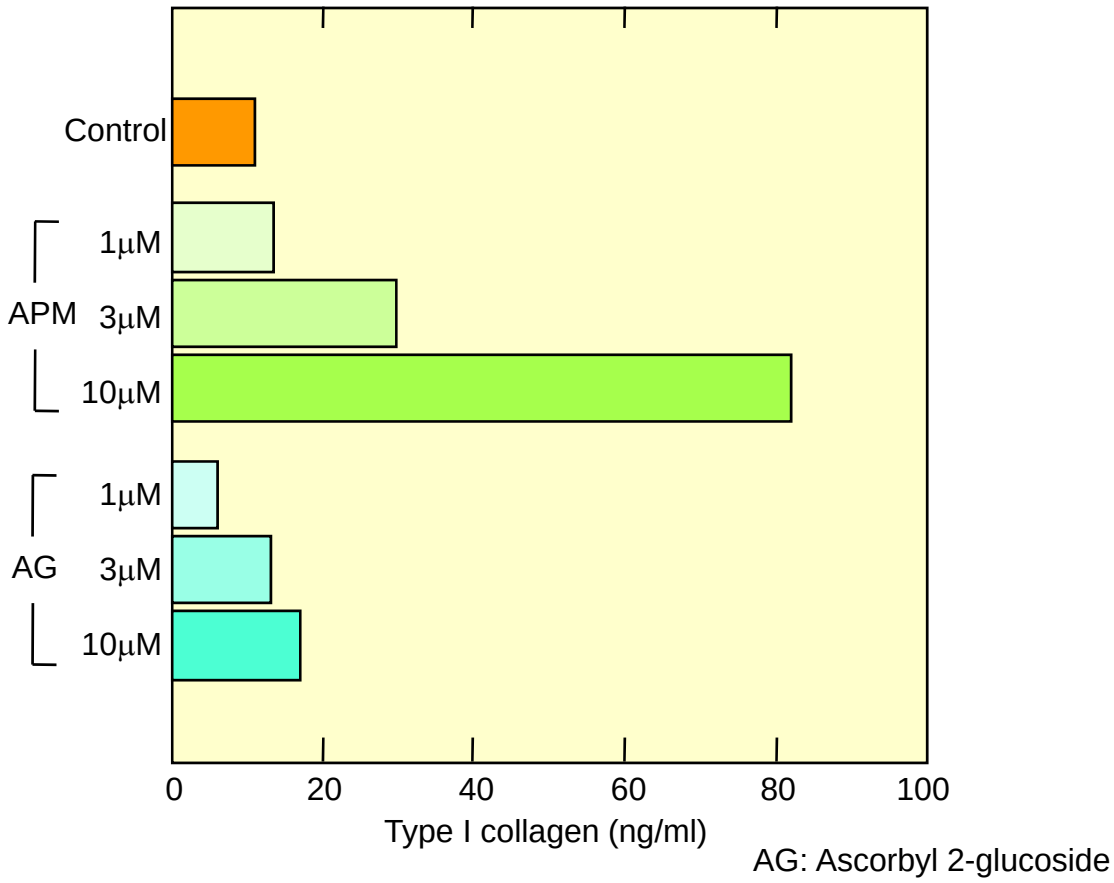
APM enhances the collagen synthesis in cultured cells.

Human fibrosarcoma HT1080 cells were cultivated with tritium-labeled proline fed in the medium, in the absence or in the presence of APM and/or azetidinecarboxylic acid (AzC), which is a proline-analogue, therefore, is a strong inhibitor of collagen synthesis. After the cultivation for one week the synthesized collagen amount was estimated by measuring the radioactivity of the cell components. 0.3 mM APM enhanced the uptake of proline by 20%. The inhibition by AzC confirmed that the observed uptake of radioactive proline was utilized for collagen synthesis.

Data obtained by Miwa et al; not published.

A51(05rev2)ti

Anti-aging properties ;
Enhancement of Collagen Synthesis

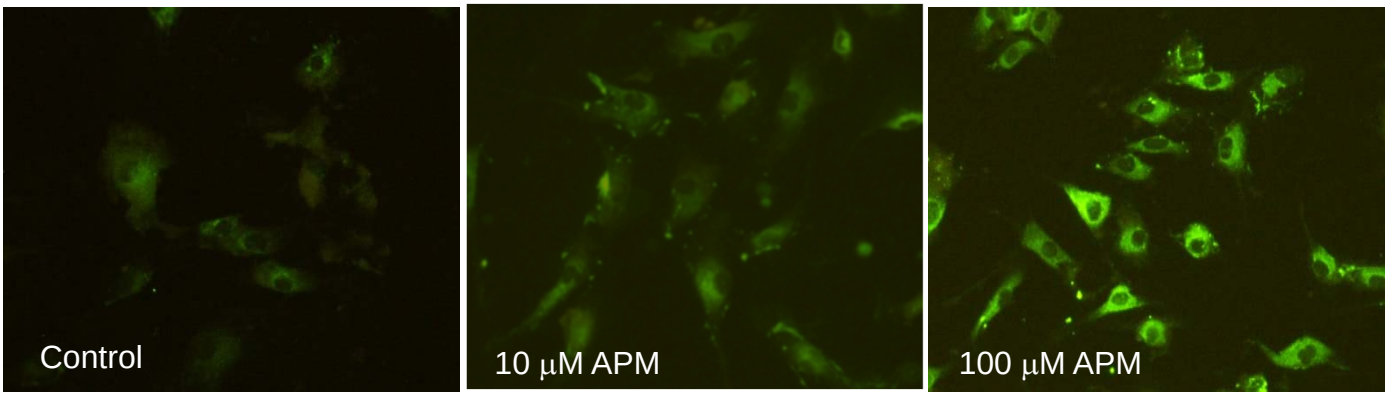


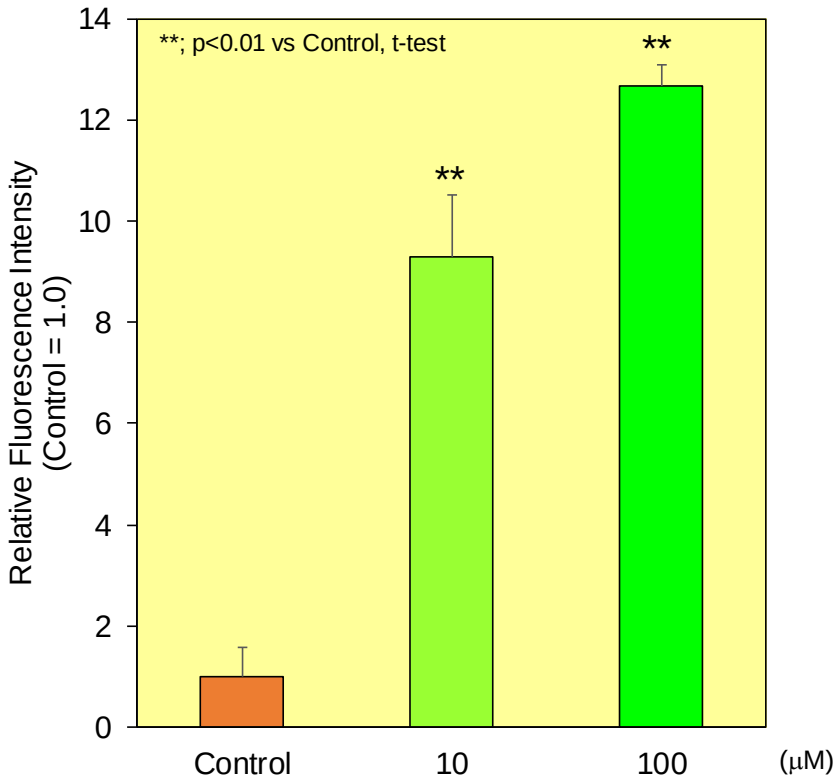
Enhancement of collagen synthesis by APM in human fibroblast.

Normal human fibroblast cell (NB1RGB) was cultivated with magnesium APM or AG at various concentrations for 72 hours. After the cultivation the type I collagen content in cultivation medium was determined by ELISA method. Addition of 3μM and 10μM APM significantly enhanced collagen synthesis in this condition.

A53/53tjp

Anti-aging properties ;
Enhancement of Collagen Synthesis



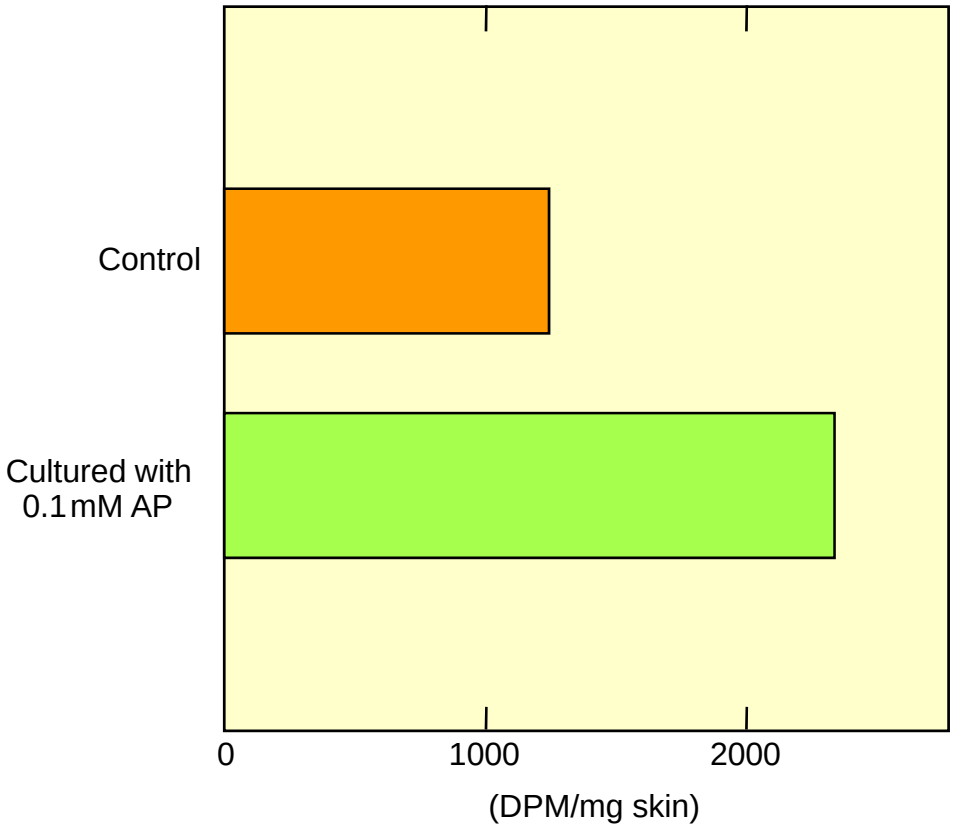


Collagen synthesis induced by APM is visualized by immunofluorescence staining.

The enhancement of collagen synthesis in human fibroblast (NB1RGB) was assessed by immunofluorescence staining with anti-type I collagen antibody. The cells were immuno-stained and photographed after 96 hrs-treatment with APM or vehicle (Control). The cell images were analyzed by detecting fluorescence intensity using Image-J software (NIH). The graph shows the relative fluorescence.

The results show APM significantly enhanced collagen synthesis in dose dependent way, which agrees with the results obtained by other studies.

Anti-aging properties of APM ;
Collagen Synthesis in the Skin



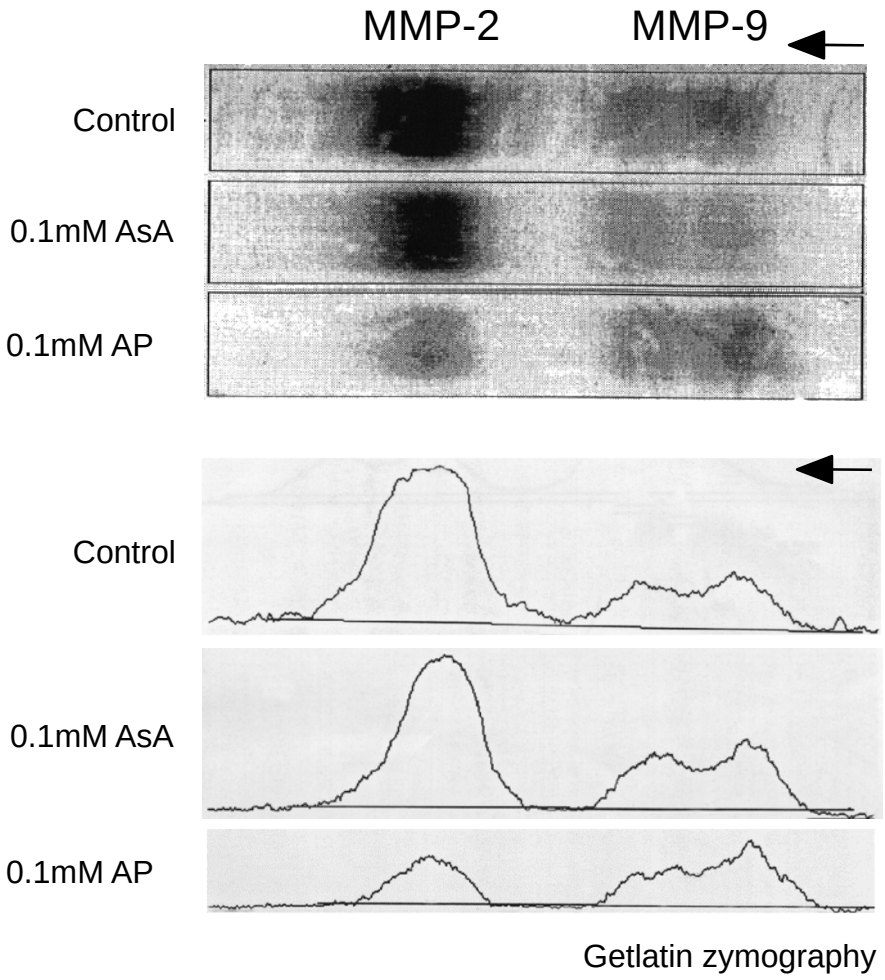
APM enhances the collagen synthesis in the skin.

The enhancement of collagen synthesis was more significant in experiments using human skin samples. Normal skin was taken from volunteers and cultivated in the medium containing none or 0.1 mM AP for two weeks. Collagen synthesis was measured by uptake of radioactive proline, which resulted in the significant increase by addition of AP in the medium.

Data obtained by Miwa et al; not published.

A52(06)ti

Anti-aging properties of APM ; Inhibition of MMP



Getlatin zymography

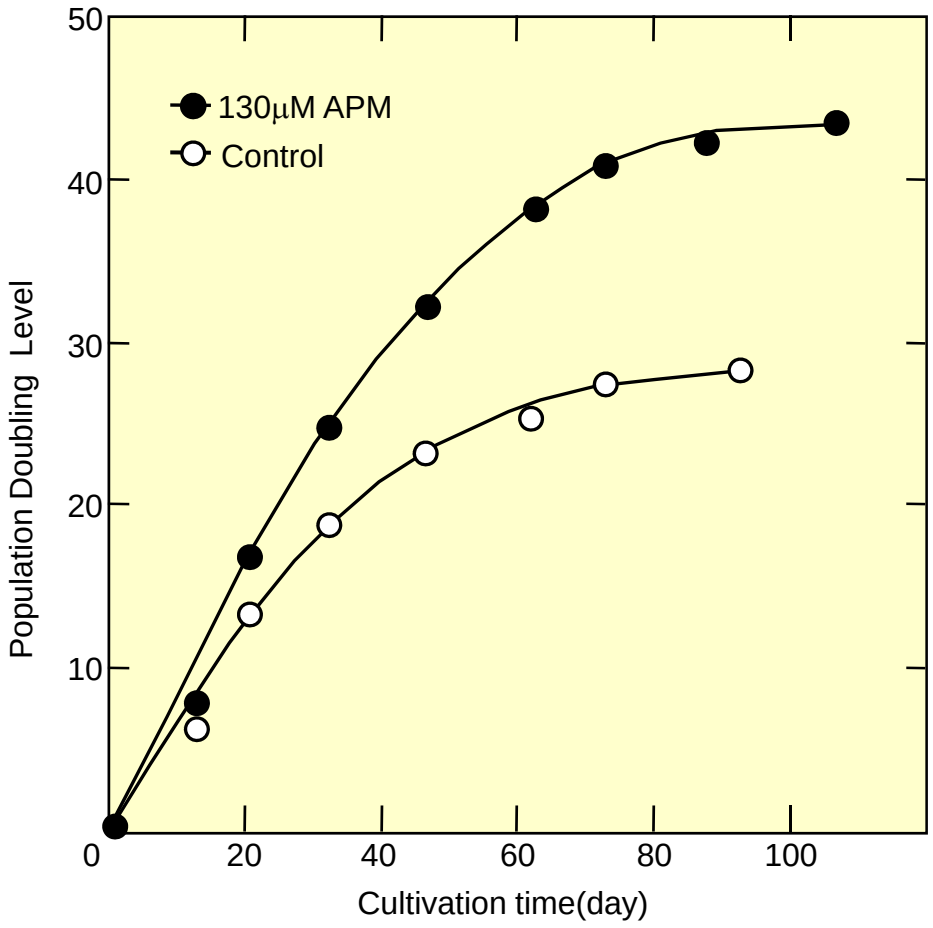
APM reduces matrix metalloprotease (MMP) activity.

Collagenase inhibition was examined using human skin preparations. Normal human skin was taken from volunteers and cultivated in the medium containing 0.1 mM AsA or AP for two weeks. Then the secreted activity of MMP-2 and MMP-9 which are responsible for degradation and reconstruction of three-dimensional collagen structure in skin was measured by gelatin zymography.
The addition of 0.1 mM AP during the cultivation reduced the MMP-2 and MMP-9 activity respectively.

Data obtained by Miwa et al; not published.

A55(08)ti

Anti-aging properties of APM ;
Deceleration of cell aging



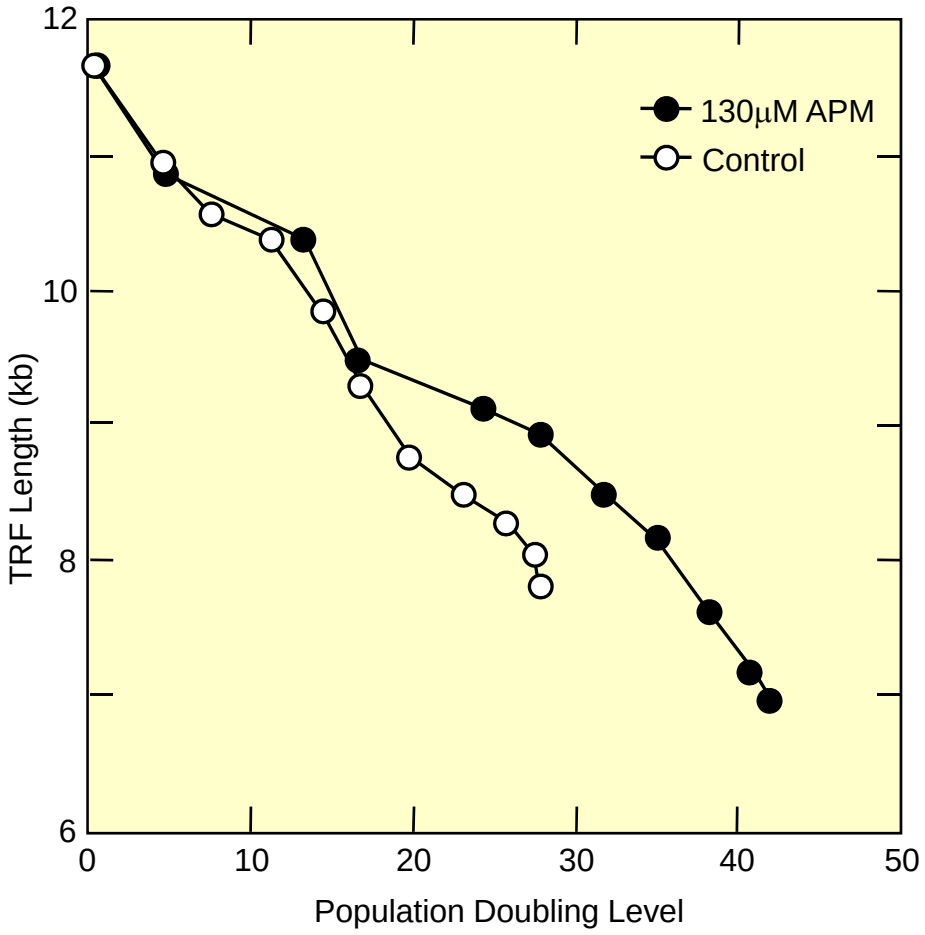
APM prolongs cell life.

Normal human umbilical vein endothelial cells (HUVEC) were kept subconfluent by transferring cells into fresh medium (2500 cells/ml) every 48-72 hours. At each transfer, 60-200 μM ascorbic acid (AsA) or APM were added to the fresh medium. Without APM cell growth stopped after twenty-seven (27) doublings as generally observed, while addition of APM improved the cell-dividing rate and increased the total doubling times to forty-one (41).
In presence of APM the cells were kept morphologically normal until the final doubling with an average cell size of 14.0 μm. Generally, the cell size becomes larger and larger as the cells ages, however, with APM it was not the case, which may suggest that APM keeps the cells in a “young and healthy” condition. Other experiments support that those effects are based on APM’s excellent radical-scavenging activity, and are quite likely related to the maintenance of telomere length.

Miho Sugimoto, Norio Nagao and Nobuhiko Miwa, Fragrance Journal, 3, 41-54 (1997)

A71ti

Anti-aging properties of APM ;
Inhibitory Effects on Telomere Shortening



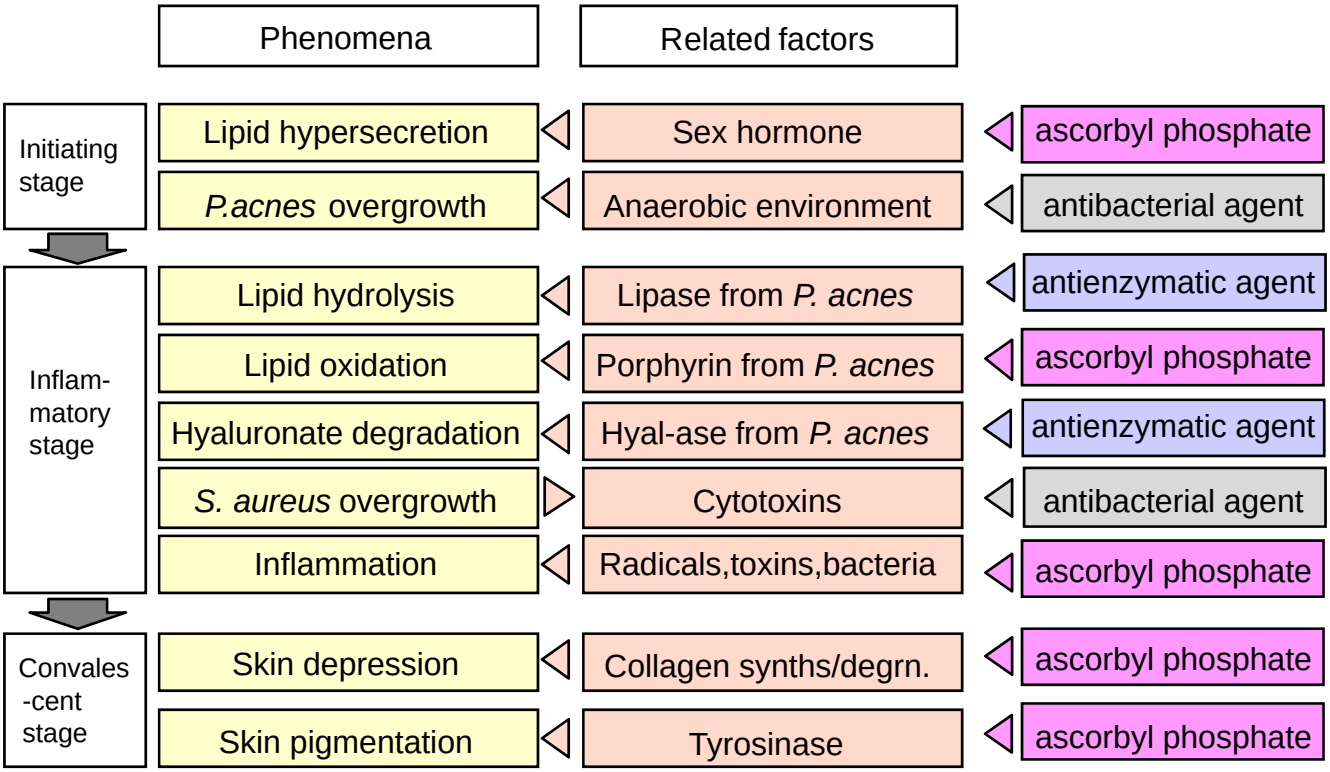
Telomere shortening is decelerated by the addition of APM in medium.

During the cultivation of HUVEC in the presence of 130 μM APM, the length of telomere was examined by Southern blotting method using 32P-labeled $(TTAGGG)_4$ oligonucleotide probe for terminal restriction fragments (TRFs). Telomeres in control cells shortened by 0.16-0.17 kilobases per PDL on the average during the cultivation. Telomere-shortening rate in APM-added cells was 0.09-0.10 kb/PDL, as slow as 52-62% of that of non-added cells. The slow-down was distinct at the stage later than PDL 15.

Kayo Furumoto, Eiji Inoue, Norio Nagao, Eiso Hiyama and Nobuhiko Miwa, *Life Science*, 63(11), 935-948 (1998)

A72ti

Anti-acne properties of APM ;
Causes of Acne Vulgaris



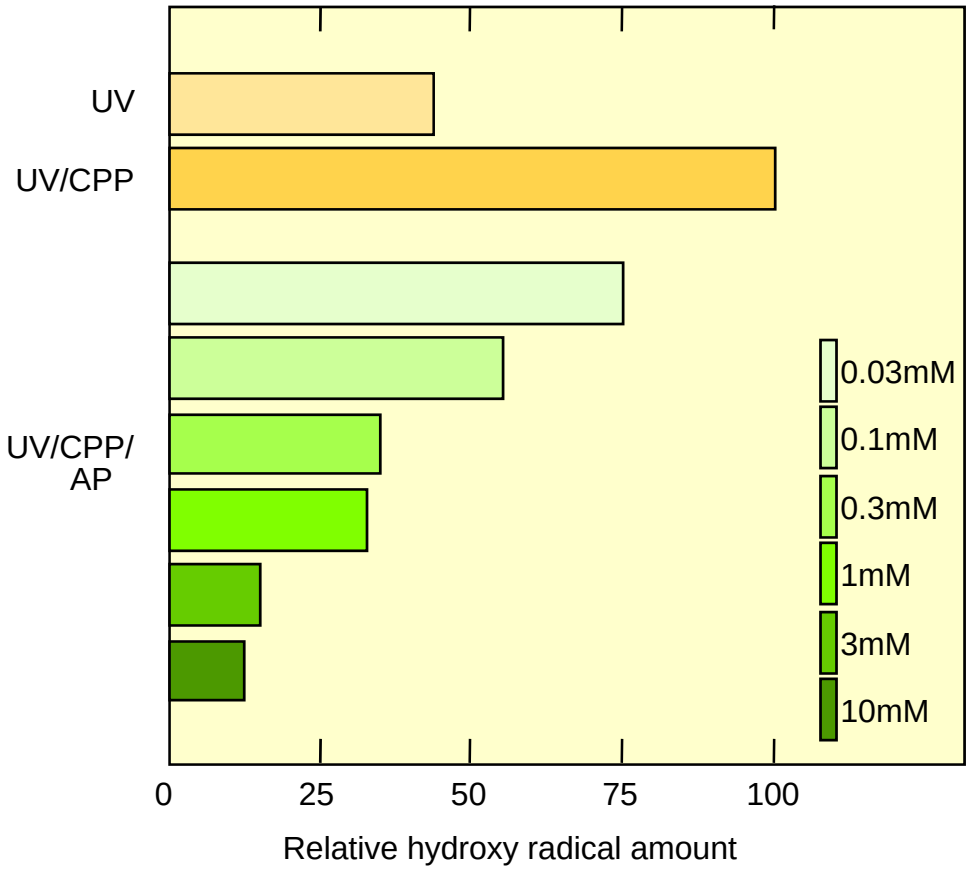
APM is effective for a complicated skin disease, Acne.

The common skin trouble, acne vulgaris is far from a simple disease. Currently, more than ten chemical, biochemical, physiological, and bacteriological factors are reported as at least a partial cause of acne. Among them, recent studies point out that the importance of the pathogenic bacteria, Propionibacterium acnes and the presence of several radical species. Targeting them is doubtlessly one of the most effective ways to develop an efficacious cosmetic for acne.
However, in some cases, the pigmentation or depression of the skin is a bigger concern for the patients than the inflammation or the infection of bacteria, since those problems usually last longer and more difficult to improve by daily care. Current anti-acne cosmetics are not fully capable of satisfying these demands.
Moreover, it must be noted that those complicated and independent events are occurring parallel at the same time: Some comedo are in their initiating stage, while some other are in their convalescent stage. This fact indicates that a single-functioning active is not enough efficacious for the acne treatment.

AP has outstanding effects on multiple events of acne. In initial stage and inflammation stage, AP's strong intracellular radical scavenging activity reflects on preventing lipid peroxidation and suppressing the inflammation. In the convalescent stage, AP inhibits the skin depression caused by collagen degrading enzymes and the skin pigmentation caused by tyrosinase. In many cases, the treatments of such scars are very important for the patients, since scars last for a long time. There is almost no acne care products to cover the convalescent stage so far.

A41(001)ti

Anti-acne properties of APM ;
Scavenging Radicals Caused by *P. acnes*



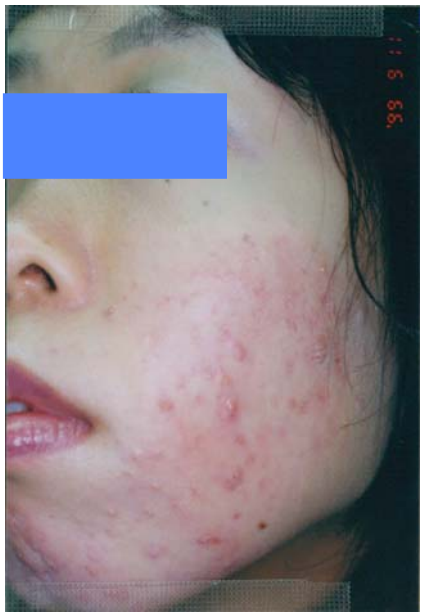
APM reduces the enhancement of radical generation caused by coproporphyrin.

Coproporphyrin (CPP), a kind of porphyrin secreted by *Propionibacterium acnes* on skin, is an important factor of acne. It enhances the hydroxyl radical generation upon UV-irradiation.
In this experiment 1mM of CPP was added to HEPES buffer (pH 7.2), which doubled the hydroxyl radical generation. The addition of AP in the buffer effectively reduced radicals.

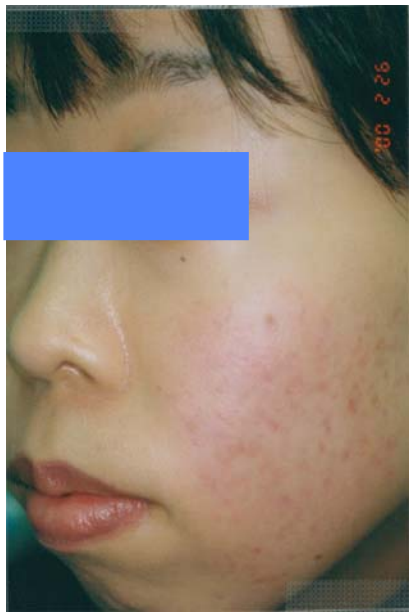
A34(04e2)ti

Anti-acne properties of APM ;
Clinical Studies (1)

Before treatment

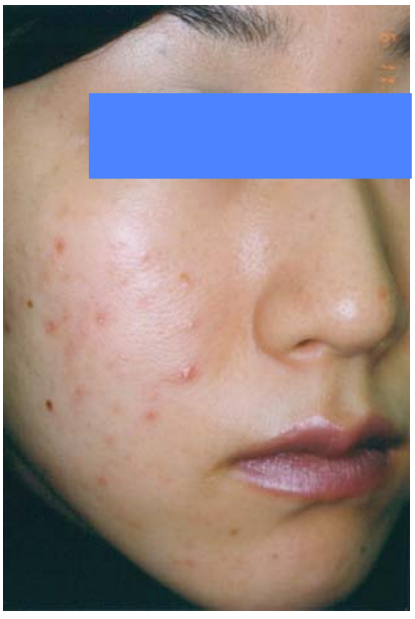


After treatment

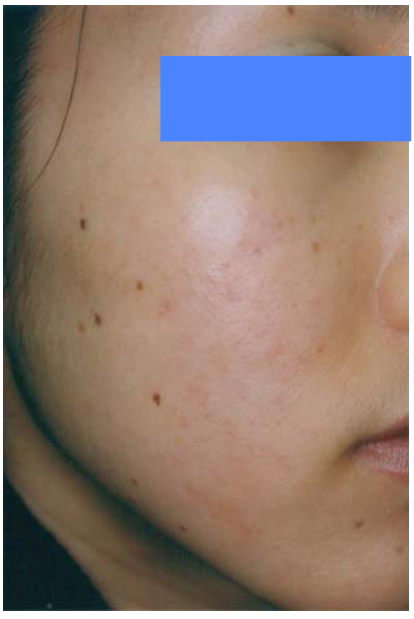


Medication: 5% AP (topical), Sep99-Feb00 (5months)

Before treatment



After treatment



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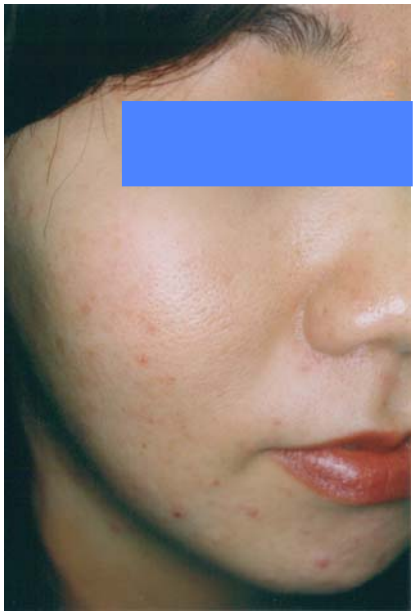
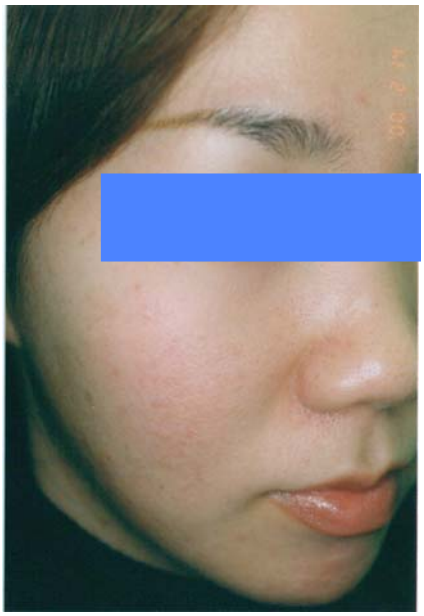
A0441(007/108)ti

Anti-acne properties of APM ; Clinical Studies (2)

Before treatment

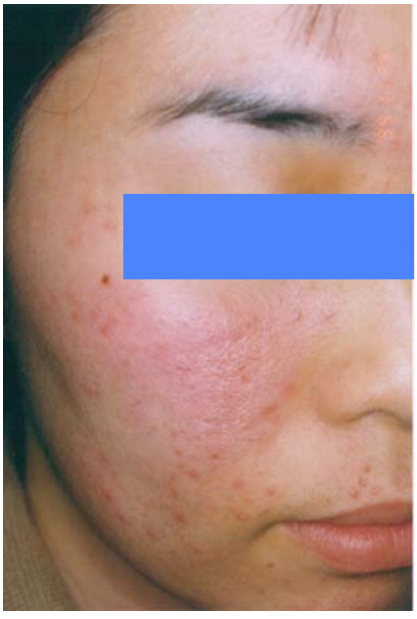


After treatment

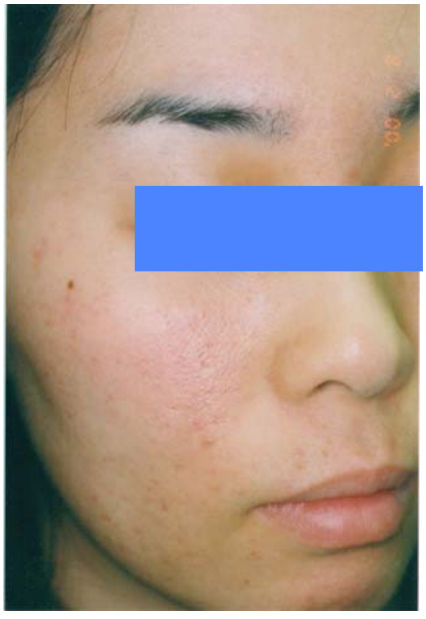


Medication: 5% AP (topical), Nov99-Feb00 (3months)

Before treatment



After treatment

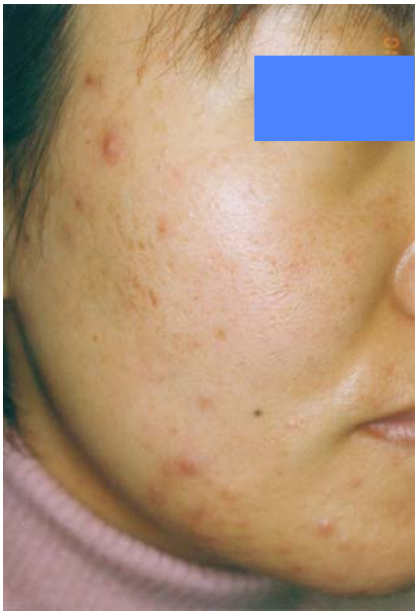


Medication: 5% AP (topical), Nov99-Feb00 (3months)

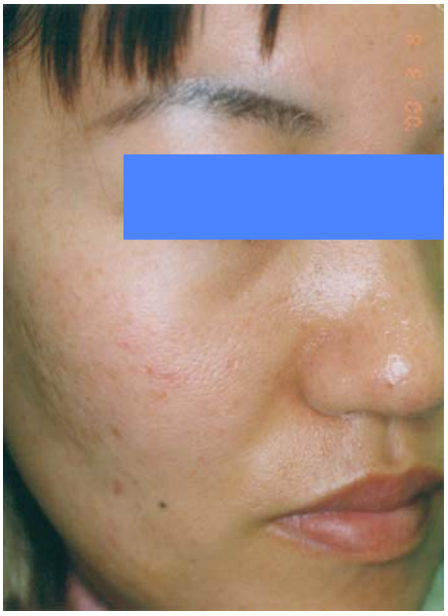
A0442(109/111)ti

Anti-acne properties of APM ;
Clinical Studies (3)

Before treatment

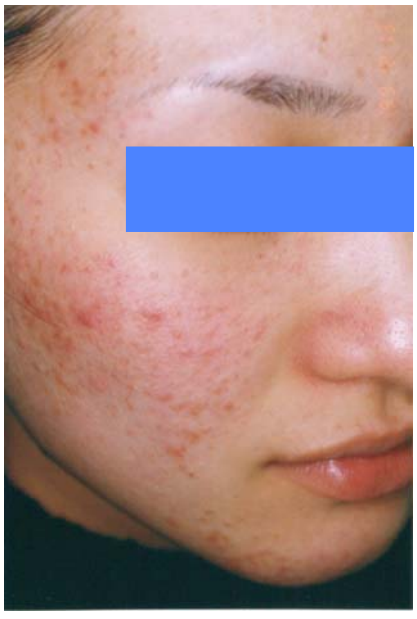


After treatment

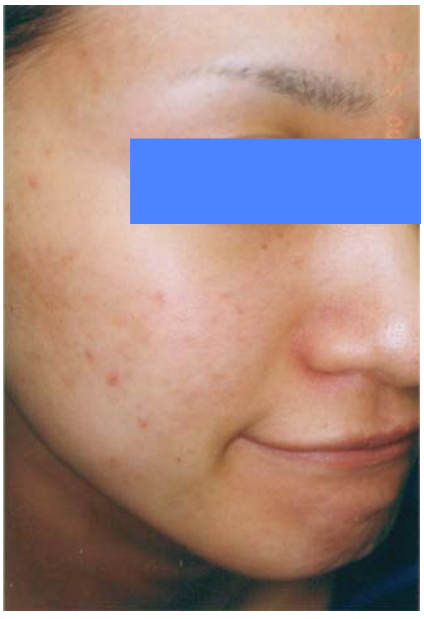


Medication: 5% AP (topical), Nov99-Mar00 (4months)

Before treatment



After treatment



Medication: 5% AP (topical), Dec99-May00 (5months)

A0443(112/213)ti

Anti-acne properties of APM ;
Summary of Clinical Studies

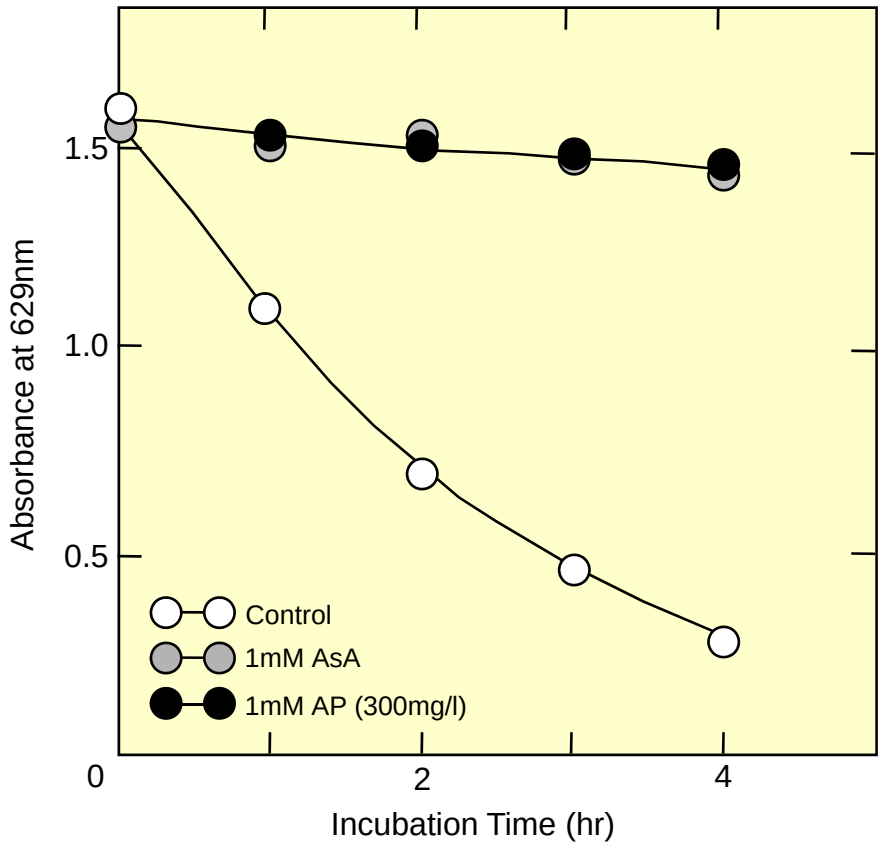
Treatment of acne Vulgaris

Medication		Efficacy(%)	# of patients
Topical	Oral		
2% AP	MC	57	12
3% AP	MC	88	25
5-7% AP	MC	93	40
6% sulfur	MC	44	27
None	MC	40	15

MC: minocycline 50 mg/day

A series of clinical studies was carried out at Ikeno Dermatoplasty Clinic (Tokyo). All the patients in these studies were human female aged 11-50 who had at least six inflammatory lesions. Efficacy was determined by scoring using Dr. Lookingbill's method after three months of treatment. Each patient received an oral administration of 50 mg minocycline as a basal medication.
The efficacy of AP was significant and was dose-dependent.

A45(44)ti

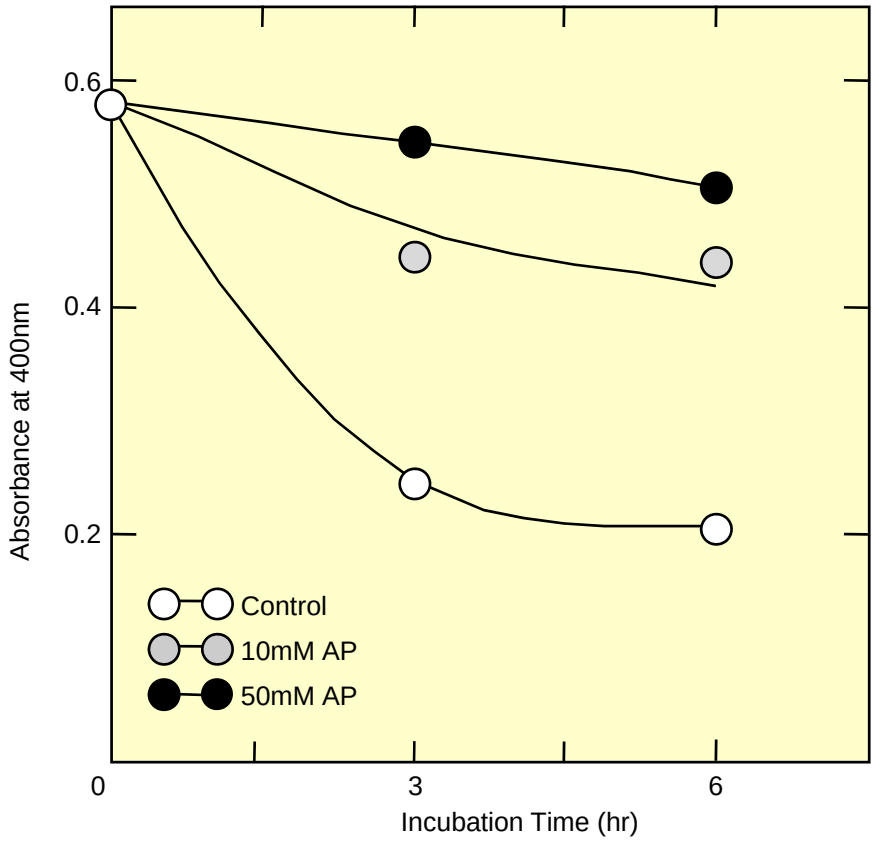


APM eliminates dissolving chlorine quickly.

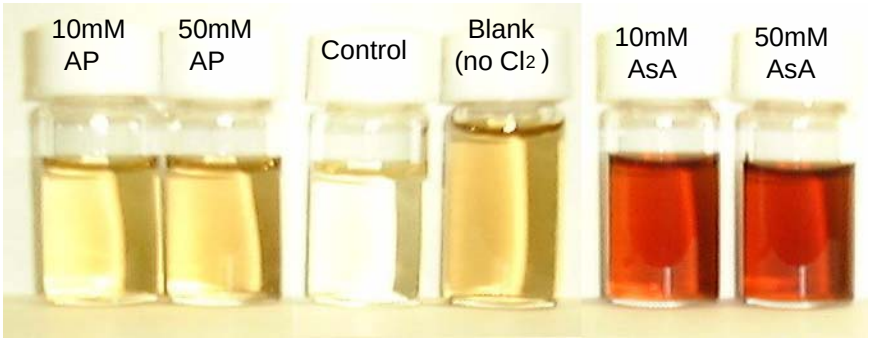
Though AP is stable to oxygen (O_2) in the atmospheric condition, it reacts with strong oxidizers such as free chlorine. Shown above is the protective effect of AP against a dye-decolorization caused by chlorine. An azo-dye, Brilliant blue FCF (Food Blue #1), was dissolved in distilled water and 25 ppm of sodium hypochlorite (NaClO) was added to the solution. The dye was gradually oxidized and lost its color. Upon the addition of 1 mM AP, the decolorization was prevented significantly. Along with the experiment, the hypochlorite concentration was determined periodically by titration method, which gave a result that hypochlorite was undetectable even immediately after the addition of AP: The elimination of hypochlorite with AP was quite quick. The effect of AP was comparable to that of ascorbate (AsA), a strong reductant.

A61(10)ti

Dechlorination properties of APM ;
Protection of Melanin against Chlorine



At 6hr:

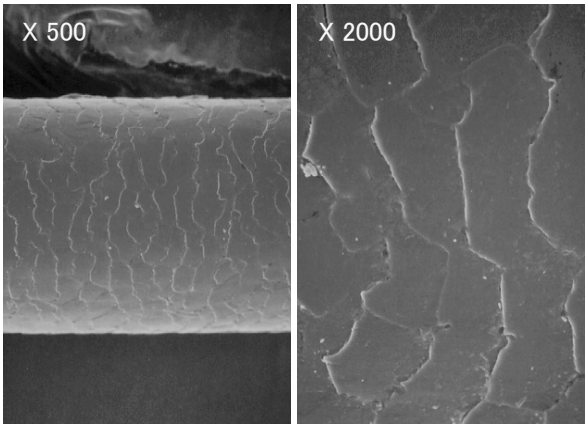


APM protects melanin from the damage caused by chlorine.

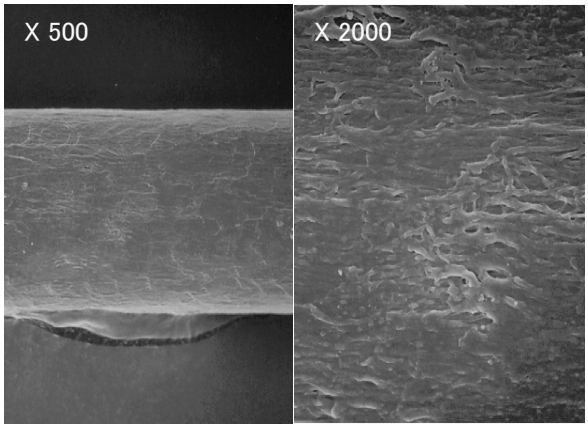
As a result of chlorine-elimination in aqueous solution, AP protects melanin, the brown hair pigment, from decolorization. Dissolved in 2N sodium hydroxide solution (at a concentration of 45 ppm), the brownish color of melanin was reduced quite quickly by the addition of 6.75 mM (480 ppm) chlorine (Cl_2). The addition of 50 mM (15 mg/ml) AP prevented the color-reduction remarkably. The effect of ascorbate (AsA) could not be evaluated in this experiment, since the addition of AsA significantly increased the color, possibly because of the oxidized AsA.

A62(11)ti

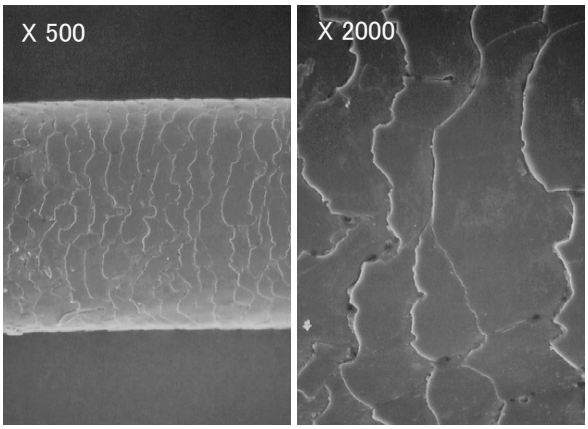
Dechlorination properties of APM ;
Protection of Hair Cuticle from Chlorine



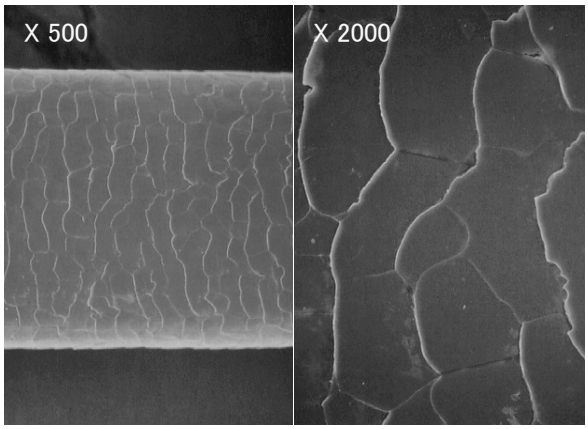
A. Normal hair



B. Treated with chlorine



C. Treated with AP and chlorine



D. Treated with hydrogen chloride

Protection of hair cuticles against chlorine-damage.

AP reduces free chlorine in aqueous solution and protects hair cuticles from the damage caused by oxidative chlorine.
Above shown is the electron microscopic observations of the hair surface chemically damaged by chlorine water. Normal hair (A; from female adult, 25 years old) was treated with water containing 480 ppm (6.8 mM) of chlorine for 30 minutes at room temperature (B). The structure of the cuticles was severely damaged by oxidation. On the contrary, the addition of 0.3 % (10 mM) AP during the treatment prevented cuticle damages significantly (C). In this experiment, the chlorine solution was neutralized by AP (pH 2 to 6), however, the protective effect does not owe to the pH-neutralization, since there was no damages observed by the treatment with acidic 1N hydrogen chloride (D).

A63(12)ti