Modified SOD for Cosmeceuticals

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Key words: transcriptional transactivator (Tat), protein transduction domain (PTD), aging, peptide, cosmeceuticals, superoxide dismutase (SOD), delivery, reactive oxygen species (ROS), human immunodeficiency virus (HIV)

Summary

A human Cu, Zn-superoxide dismutase (Cu, Zn-SOD) was fused with a Tat PTD of HIV-1 to produce a novel anti-aging ingredient, Tat-SOD for cosmeceuticals. Test of stability and evaluation of transduction efficacy and enzymatic activity suggest Tat-SOD is an effective active ingredient for anti-aging treatment.

Introduction

Recently, there has been considerable emphasis on delivery of drugs through the transdermal route. The stratum corneum provides the principal barrier to the percutaneous penetration of topically applied substances. It is therefore important to look for ways to enhance skin permeability.

The use of biological enzymes that are large size molecules generally has been resisted in cosmetic application because of their poor skin permeability and instability. It can be very difficult to delivery an active ingredient inside skin for cosmetic researcher but it must be the key problem to be solved.

Several special small size protein regions called protein transduction domains (PTDs) including carrier peptides derived from the transactivator of transcriptional (Tat) protein from immunodeficiency virus type-1 (HIV-1), have been developed for the delivery of exogenous effective protein molecules for example, enzyme, and cytokine for cosmetic use into living cells.

Although the mechanism of transduction across cell membrane is unknown, the entire HIV-1 Tat protein or parts of it have been shown to serve as carriers to direct uptake of heterologous proteins including ovalbumin, horseradish peroxides and beta-galactosidase[1-3].

The human epidermis provides the outermost barrier against the invasion of infectious agents, reactive electrophiles and free radicals. The surface of the skin is especially vulnerable to damaging free radicals generated by a number of physical and biological processes.

Antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) have been considered to have a beneficial effect against various diseases mediated by reactive oxygen species (ROS). SOD catalyze the reaction $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$. By thus scavenging O_2^- they serve to protect respiring cells against its deleterious reactivities.

Among the numerous pathways and mechanisms involved in the complex phenomenon of aging, recent findings designated oxidative processes as a major extrinsic cause.[4, 5] Keratinocyte membranes, as well as deep-sited cells like fibroblasts, are targets for oxidants released in the atmosphere (free radicals, peroxides, ozone) or even deposited on the skin. These so-called ROS, highly reactive intermediates derived from the atmospheric molecular oxygen (O_2), or even formed intrinsically in the skin deleterious results of a cascade of oxidative reactions, have received much attention.[6]

Once the ROS reach the biological membranes, they oxidize its various constituents, in particular, the phospholipids, and basic components of cell membranes. SOD is among the key cellular enzymes by which cells detoxify free radicals and protect themselves from oxidative damage.[7]

Since every biological macromolecule can serve as a target for the damaging action of the abundant oxygen radicals, interest has grown over the therapeutic potential of SOD. They have been considerable interest in the use of SOD because of its role in protection against human aging. A wide variety of SOD, conjugated or encapsulated, are available, including SOD conjugated with polyethylene glycol, lecithin and SOD encapsulated with liposome.[8, 9]

Cosmeceuticals should show the activity of active ingredients. Used in this sense, activity is closely related to thermodynamic activity of a compound within a delivery system. Any active substance administered to the skin need to be penetrated into the skin in sufficient amounts in order to have a proper cosmeceutical effect.

The potential for intracellular therapeutic use of proteins, peptides and oligonucleotides has been limited by the impermeable nature of the cell membrane to these compounds. A wide variety of methods have been proposed for the delivery of proteins and other macromolecules into the living cells for therapeutic uses, including microinjection, electroporation, liposomes.[10, 11] The molecular weight of SOD is so large that it can not penetrate the skin.

Recently Tat protein from HIV-1 was shown to enter cells when added exogenously.[12, 13] Tat protein can simply be added to medium at concentration as low as 1 nM, and biological responses can be detected.[13]

Tat-SOD is an enzyme derivative derived with conjugation SOD with Tat and the key of the efficacy is to increase the transepidermal penetration of the target protein, SOD.

The purpose of the present research was to create a novel fusion protein, Tat-SOD that has a skin penetration activity of Tat protein and a superoxide anion scavenging activity of SOD together. Another was to clarify Tat-SOD as an anti-aging active ingredient by estimating the enhancing activity of skin penetration and protective activity against oxidative stress from environments .[14, 15]

We had studied the stability, skin penetration efficacy and superoxide anion scavenging activity of Tat-SOD. In that study, Tat-SOD had a good stability, skin penetration efficacy regardless of high molecular weight of SOD and maintain the activity as same as SOD.

We suggest that Tat-SOD fusion protein is an effective active ingredient for anti-aging by a beneficial effect against various diseases mediated by ROS. This article also proposes the powerful method of delivery of large size protein into the skin for developing cosmeceuticals for anti-aging.

The aim of this article is to outline the production of Tat-SOD, and its efficacy of anti-aging agents in a point of activity, stability and permeability into the skin. In the this study, we describe the transduction of full length Tat-SOD fusion protein into HeLa cells and mouse skin and possibility of cosmetic usage and whether this transduced Tat-SOD has a protective effect against oxidative stress in the cells and skins.

Methods and Materials

Materials

Restriction endonucleases and T4 DNA ligase were purchased from Promega Co. Pfu polymerase was obtained from Stratagene. Oligonucleotides were synthesized from Gibco BRL custom primers. IPTG was obtained from Duchefa Co. Plasmid pET15b and E.coli strain BL21 (DE21) were from Novagen. Ni^{2+}-nitrilotriacetic acid Sepharose super flow was purchased from Qiagen.

A human Cu, Zn-SOD cDNA fragment was isolated using PCR technique from the λZap human placenta cDNA library and monoclonal antibody raised against human Cu, Zn-SOD was produced in laboratory. DMEM (Dulbecco's Modified Eagle's Medium), fetal bovine serum (FBS), streptomycin and penicillin were all purchased from Gibco BRL (Grand Island, NY, USA). Potassium phosphate, sodium chloride and sodium phosphate were obtained from Sigma Co. (USA). All other ingredients were reagent or cosmetic grade.

A solubilized state was prepared for stabilities in Malate (pH4, 5, 6) and Tris-HCl (pH7, 8) based buffer solution at various pH values and a skin care formulation (Formula 1, 2) containing 4.4㎎/㎖ of Tat-SOD was prepared for stability studies with surfactant or without surfactant.

Formula 1. Skin care formulation without surfactant (pH 7.5)	
Dipropylene glycol	7.0%
Metyl paraben	0.1
Sodium polyacrylate	0.008
Algin	0.005
EDTA	0.03
Tat-SOD	4.4㎎/㎖
Water (aqua) qs 100.00	

Skin Formula 2. Skin care formulation with surfactant (pH 7.5)	
Dipropylene glycol	7.0%
Metyl paraben	0.1
Octyldodeceth-16	0.1
PEG-60 Hydrogenated Castor Oil	0.25
Algin	0.005
EDTA	0.03
Tat-SOD	4.4㎎/㎖
Water (aqua) qs 100.00	

Preparation for Tat-SOD

The pTat-SOD protein expression vector was constructed to express the basic domain (amino acids 49-57) of HIV-1 Tat as a fusion with Cu, Zn-SOD as described previously.[15] First, two oligonucleotides were synthesized and annealed to generate a double-stranded oligonucleotide encoding nine amino acids from the basic domain of HIV-1 Tat. The double-stranded oligonucleotide was directly ligated into the digested pET15b in frame with the six histidine open-reading frame to generate the HisTat expression plasmid, pHisTat. Next, on the basis of the cDNA sequence of human Cu, Zn-SOD, human SOD1 cDNA was sub cloned into a pET15b and pTat expression vectors (Figure 1).

The host E. coli BL21 (DE3) was transformed with pSOD and pTat-SOD, and then the transformants were selected on a LB plate containing ampicillin. The selected colonies were cultured in a LB medium containing ampicillin at 37℃ with shaking. After the cells had grown, isopropyl-β-D-thiogalactoside (IPTG) was added, and the incubation was conducted. The cells were harvested, and a binding buffer was added and sonicated. The recombinant Tat-SOD was purified under denaturing and native conditions, respectively.

To denature Tat-SOD, harvested cells were disrupted by sonication in a binding buffer. After centrifugation, supernatants containing Tat-SOD were loaded onto a Na^{2+}-nitrilotriacetic acid Sepharose column. After the column was washed with a binding buffer and washing buffer, the fusion protein was eluted with an elution buffer. The fusion protein containing fractions were combined and the salts were removed using Sephadex G-15 column chromatography. The protein concentration in fractions was estimated by Bradford procedure using bovine serum albumin as a standard[16].

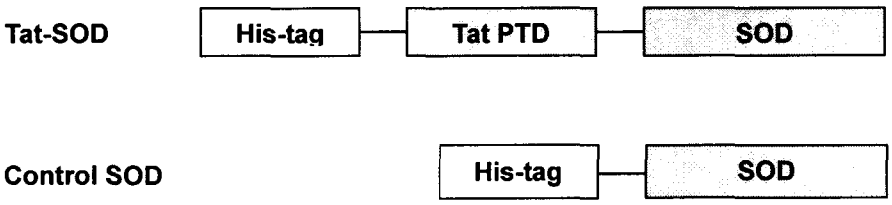

Figure 1. Diagram of expressed Tat-SOD and control SOD fusion protein.

Cell Culture and Transduction of Tat-SOD

The human fibroblast cells were cultured in a Dulbecco's Modified Eagle's Medium containing 20 mM HEPES/NaOH (pH 7.4), 5mM $NaHCO_3$, 10 % fetal bovine serum and antibiotics (100㎍/㎖ streptomycin, 100U/㎖ penicillin) at 37°C. For the transduction of Tat-SOD, the fibroblast cells were grown to confluence on a 6-well plate. And then, the culture medium was replaced with 1㎖ of fresh solution without a fetal-bovine serum. After fibroblast cells were treated with various concentrations of Tat-SOD, the cells were treated with trypsin-EDTA and washed with phosphate buffered saline (PBS). The cells were harvested for the preparation of cell extracts to perform a SOD enzyme assay and Western blot analysis.

For Western blotting, proteins in cell extracts were separated on a 15 % SDS-PAGE and the proteins were transferred onto a nitrocellulose membrane. The membranes were incubated with 5 % dry milk in PBS, and then with a rabbit polygonal antibody (Santa Cruz 1:500) for 1 h at room temperature, followed by incubation with goat anti-rabbit immunoglobulins (Sigma, dilution 1:10,000). The bound antibodies were then visualizes by enhanced chemiluminescence according to the manufacturer's instruction (ECL; Amersharm).

Immunohistochemistry

Mice were housed at constant temperature (23°C) and relative humidity (60%) with a fixed 12h light/dark cycle and free access to food and water. Animals were anesthetized with 3 % isoflurance in nitrogen and oxygen, 50 g control SOD, Tat-SOD fusion proteins and formula 1, 2 were topically applied onto the shaved area of the mice skin for various time intervals. Thereafter, frozen and sectioned tissues were prepared and fixed with 4 % paraformaldehyde for 10 min. For removal of nonspecific immunoreactivity, free-floating sections were first incubated with 0.3 % Triton X 100 and 10 % normal goat serum in PBS for 1 h at room temperature.

They were then incubated with a rabbit anti-histidine polyclonal antibody (Santa Cruz Bio 1:500) for 24 h, at room temperature. After washing three times for 10 min with PBS, the sections were incubated for 1 h with biotinylated goat anti-rabbit IgG (Vector Laboratories, USA. Dilution 1:200), then visualized with 3,3'-diaminobezidine (40㎎ DAB/0.045% H_2O_2 in 100㎖ PBS) mounted on gelatin-coated slides. The immunoreactions were observed under the Axioscope

microscope (Carl Zeiss, Germany).

MTT assay

The biological activity of transduced Tat-SOD was assessed by the cell viability of fibroblast cells treated with paraquat (methyl viologen), which is well known as an intracellular superoxide anion generator.[17] Cells were plated into 6-well trays at 70% confluence, and they were allowed to attach cells per well.

After the cells were treated with 0.1~2 μM denatured Tat-SOD and control SOD for 1 h, respectively, then the 5 mM paraquat was added to the culture medium for 12 hrs. Cell viability was estimated by with a colorimetric assay using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide).

Superoxide Anion Radical Scavenging Activity

The superoxide anion radical scavenging activity was performed using the method of Okamura et al.[18] with some modification. This assay is based on the removal rate of xanthine/xanthine oxidase-generated superoxide by measuring the reduction of nitro blue tetrazolium (NBT).

The sample solution (0.1㎎/㎖) in water was added to 1ml of a mixture of 0.1 mM xanthine and 0.2 mM NBT (Sigma) in 50 mM potassium phosphate buffer (pH 7.5) containing 0.05mM EDTA. Xanthine oxidase (0.1㎖) (Sigma; 0.8 unit/㎖) diluted in 50 mM phosphate buffer (pH 7.5) was added, and the resulting mixture was incubated at 37 °C for 20 min. Addition of 2 ml of 2.5 N HCl to the mixtures terminated the reaction, followed by increase of coloration of NBT, which was measured at 540 nm. The percent of removal rate by sample was calculated relative to the control.

Stability studies

To measure the temperature stabilities at various pH values, Tat-SOD was prepared in 50mM Malate buffer (pH 4, 5, 6) and 50mM Tris-Cl buffer (pH 7, 8). Also Tat-SOD was prepared in skin care formulation (Formula 1, 2) which have only difference to have a surfactant or not to judge the application possibilities in cosmetics.

Samples were stored at 25°C and 40°C for as long as approximately 30~40days. Periodically, 50㎕ aliquots of each sample were collected out and measured the radical scavenging activity rather than amount of residual SOD because the activity of SOD is more important key factor than the amount of it to design a cosmetic formulation.

Results and Discussion

Outline the preparation of Tat-SOD

Cu, Zn-SOD is one of the cell's primary defenses against oxygen-derived free radicals, and it is vital for maintaining a healthy balance between oxidants and antioxidants. Tat-SOD protein shown in Figure 1, originally developed in our collaborative laboratory[14]. A human Cu, Zn-superoxide dismutase (Cu, Zn-SOD) gene was fused with a gene fragment encoding the nine amino acid transactivator of transcription protein transduction domain (RKKRRQRRR, where R, K and Q are arginine, lysine and glutamine, respectively) of HIV-1 in a bacterial expression vector to *produce a gene in-frame Tat SOD fusion protein.*[14]

The recombinant SOD and Tat-SOD proteins have an estimated molecular mass of approximately 17 and 18 kDa, respectively. However, it was detected that recombinant fusion proteins migrate to bands with a higher molecular weight than those of the expected sizes on the SDS-PAGE, which is consistent with the previous reports.[19, 20] The purified products were further confirmed by Western blot analysis using a rabbit anti-histidine polyclonal (Figure 2). SOD was detected at the corresponding bands in SDS-PAGE, respectively (Figure 2).

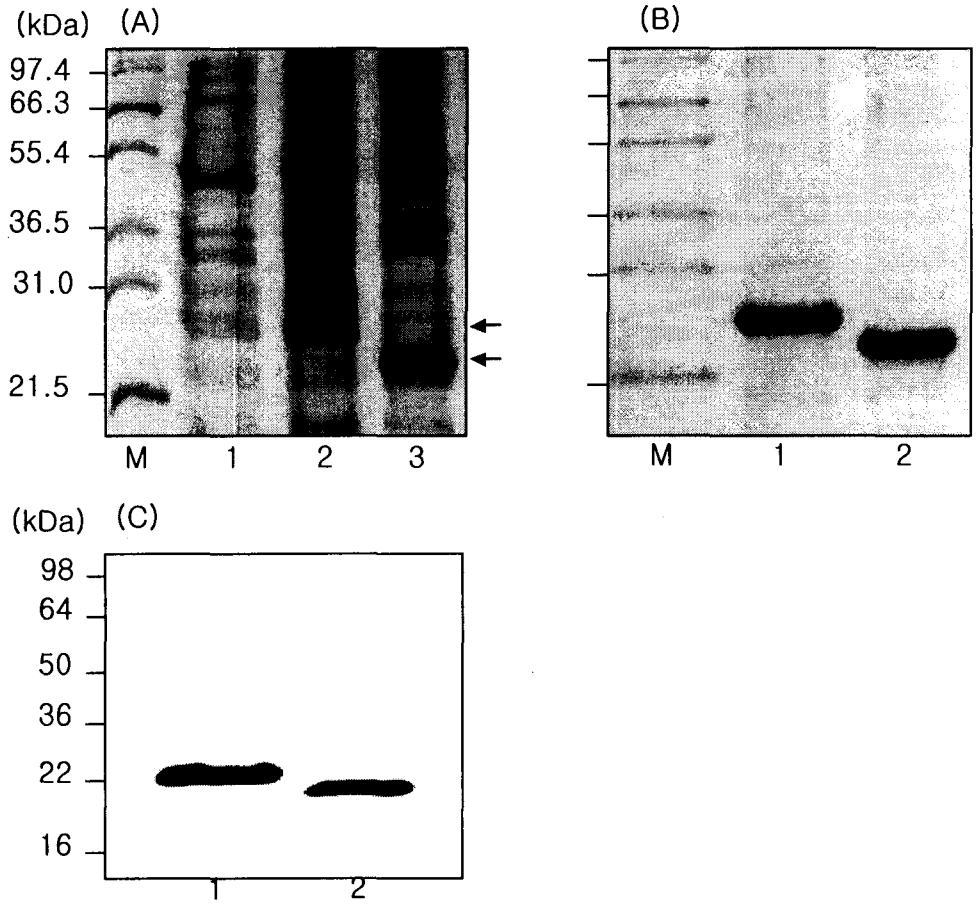

Figure 2. Expression and purification of Tat-SOD in E. coil

Protein extracts of cells (A) and purified fusion proteins (B) were analyzed by 15% SDS-PAGE

gel and subjected to Western blot analysis with a monoclonal antibody to human Cu, Zn-SOD (C) follows: extracts of bacteria containing expression vector pET (lane 1), pTat-SOD (lane 2), and pSOD (lane 3). Lanes in B and C are as follows: lane 1:Tat-SOD, lane 2: SOD.

Transduction of Tat-SOD into fibroblast cells

As shown in Figure 4, Tat-SOD was successfully delivered into the human fibroblast cells, whereas the control SOD was not delivered into the cells. More over, denatured Tat-SOD was more successfully delivered into the cells than native Tat-SOD (data not shown). These results indicate that protein unfolding is required for more efficient transduction of Tat-SOD into fibroblast cells.

The expressed and purified Tat-SOD fusion protein in *Escherichia coli* can enter HeLa cells in a time- and dose-dependent manner when added exogenously in a culture media.

Although the molecular mechanisms of transduction mediated by the HIV-1 Tat basic domain are poorly understood, an inherent characteristic of specific amino acid sequences/numbers such as arginine or lysine or some structural motifs like α-helix may be important in this process.[21] The positively charged moiety of this domain may interact with anionic components on the surface of the cell membrane.

The dose-dependency of the transduction of Tat-SOD fusion proteins was further analyzed. Various concentrations of denatured and native Tat-SOD proteins was added to the culture media of human fibroblast cells for 1 h, and the levels of transduced proteins were measured by Western blotting. The level of transduced proteins in the cultured fibroblast cells were increased concomitantly with the amounts of fusion treated in media, and the enzyme activities of SOD was increased in dose-dependent manner.

These results implicate that denatured Tat-SOD in fibroblast cells may be correctly refolded by the mechanism of molecular chaperone or by a spontaneous process. However, intracellular refolding mechanisms for transduced proteins wait to be elucidated in the future. Because denatured Tat-SOD proteins restores the enzyme activities of SOD after transduction, Tat-SOD has advantage over any other enzyme that has no enzymatic activities after denaturation condition as cosmetic ingredients.

Cellular Activity of Tat-SOD into fibroblast cells

To determine whether transduced Tat-SOD can play its biological role in the cells, we have tested the effect of transduced fusion proteins on cell-viability under oxidative stress. After the cells were exposured to 5 mM paraquat without Tat-SOD, only 35% of the fibroblast cells were viable, the viability was significantly increased when Tat-SOD was pretreated in a dose-dependent manner. The cell-viability of fibroblast cells pretreated with 0.1~2 μM Tat-SOD was increased by approximately 40~80% as compared with that of the control. These results indicate that

transduction of Tat-SOD was definitely effective against superoxide anion induced by paraquat in fibroblast cells.

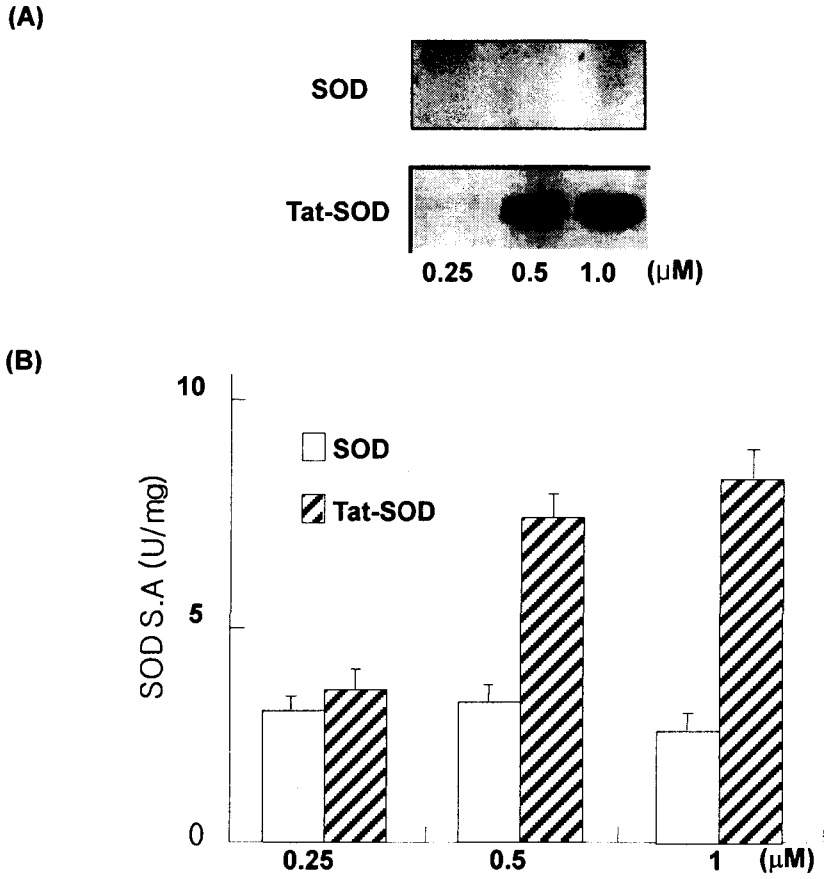

Figure 3. Transduction of Tat-SOD into cultured human fibroblast cells.

(A) 1 μM of Tat-SOD and control SOD were added to the culture media for 1 h. Lanes are as follows: lane1; cell extracts, lane 2; SOD, lane 3; Tat-SOD. (B) Fibroblast cells plated in a 6 well plate treated with Tat-SOD and control SOD at concentrations of 0.25~1μM for 1 h. The transduction activity of each protein was analyzed by measuring the level of the transduced proteins in the cells by Western blot and the specific enzyme activity (C). Each bar represents the mean ± S.E.M. obtained from five experiments.

Skin Penetration of Tat-SOD:

We evaluated the ability of the Tat-SOD fusion proteins to transduce into the skin. The fusion proteins were time dependent sprayed on mice skin and the degree of penetration of these fusion proteins into skin were analyzed by immunohistochemistry and enzymatic activities. As shown in Figure 4, the immunofluorescence signal in skin treated with Tat-SOD fusion proteins were significantly detected in the epidermis as well as the dermis and subcutaneous fat in skin tissue.

Unlike Tat-SOD and control SOD without PTD could not penetrate into the skin efficiently. The levels of enzyme activities in skin tissue were also significantly increased when treated when

treated with SOD fusion proteins (Figure 4). These results demonstrate that the SOD fusion proteins with HIV-1 Tat basic domain cannot only transduced into the cultured fibroblast cells but also penetrate into animal skin.

Analysis of skin sections treated with SOD fusion proteins revealed that the SOD fusion protein was distributed in dermis as well as epidermis of skin. A single application of Tat-SOD onto skin resulted in significant enhancement of the level of transduced proteins as well as the enzymatic activities. This result suggest that cell permeable SOD fusion proteins may have therapeutic potential against various skin disorders such as skin inflammation by ROS when applied topically.

Taken together, the present experimental results demonstrate that Tat-SOD in cosmetic formulation (Formula 1, 2) also penetrated into the skin with intact enzymatic activities regardless of exiting surfactants (Figure 5). The availability of cell-permeable antioxidant enzymes, such as SOD, catalase, may significantly contribute to the development of strategies aimed at controlling ROS generated under oxidative stress. In addition, this transduction of antioxidant enzyme may allow the design of cosmeceuticals of anti-aging.

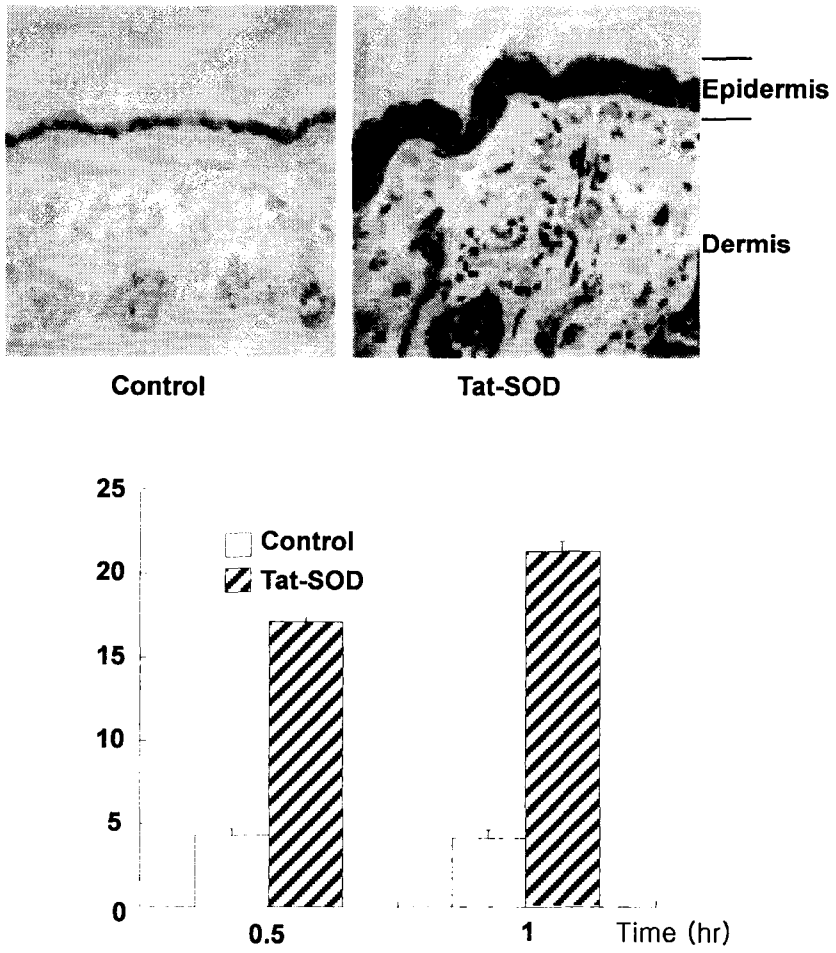

Figure 4. Histochemical analysis of mice skin transduced with Tat-SOD fusion proteins.
50 μg of SOD fusion proteins were topically applied onto the shaved area of the mice dorsal skin

for 30 min and 1 h, respectively. Frozen sections of skin tissues were immunostained with a rabbit anti-histidine IgG (1:500) as described in *Materials and Methods*. Top: The sections were visualized with 3,3'-diaminobenzidine and observed under the Axioscope microscope after 1hr. Bottom: Transduction efficiencies were analyzed by measuring specific enzyme activities of skin tissue. Each bar represents the mean ± S.E.M. obtained from three experiments.

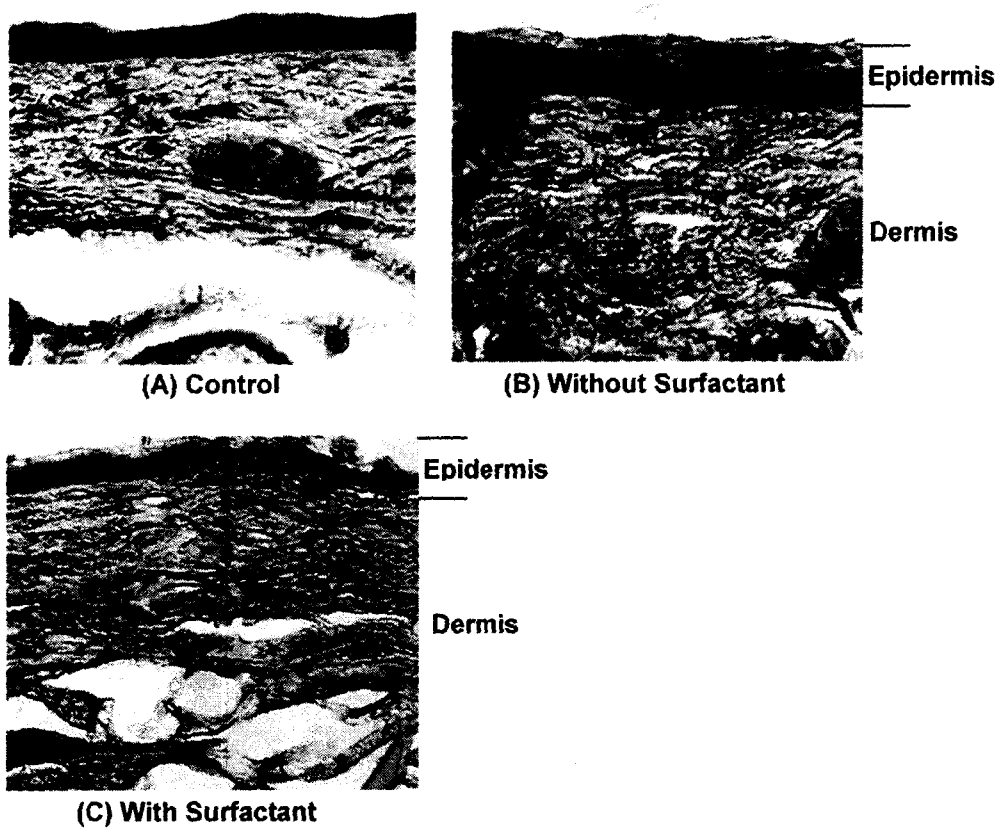

Figure 5. Histochemical analysis of mice skin transduced with Tat-SOD fusion proteins. Immunohistochemical analysis of animal skin transduced with Tat-SOD fusion proteins in formula 1,2 skin formulation. The sections were visualized with 3,3-diaminobenzidine and observed under the Axioscope microscope. (A). Skin treated with Formula 1 without Tat-SOD (B). Skin treated with Formula 1 containing Tat-SOD without surfactant (C). Skin treated with Formula 2 containing Tat-SOD with surfactant. The existence of SOD was detected as a brown area in the section. Counterstaining of the nuclei with hematoxylin was blue.

Stability Studies in Skin care formulation & in Aqueous Solutions

In order to measure the stability of Cu, Zn-SOD, it was prepared in aqueous buffered solution and in skin care formulation as a cosmetic application. Tat-SOD samples in aqueous solution were stored at 25°C and 40°C up to 41days and samples in skin were stored up to 30days. Because the

activity of Tat-SOD is more important than the residual amount of Tat-SOD to have an effect as an active ingredient into the skin, we measured activities of samples instead of the amount of residual Tat-SOD. Aliquots of each sample were pipetted out and measured superoxide anion radical scavenging activity as described in *Materials and Methods*. As shown in Figure 6 and Figure 7, the activity of Tat-SOD is more stable at higher pH (pH 7, 8) rather than lower pH (pH 4, 5).

The tendency is same in for experimental periods in accelerated conditions. From this point of view, cosmetic formulations using Tat-SOD as an active ingredient should have a pH range from 6 to 8 approximately and avoid low pH conditions. Figure 8 and 9 demonstrate the stabilities of Tat-SOD in skin care formulation at selected pH during 1month of storage at 25℃ and 40℃. The stabilities of Tat-SOD are also stable in skin care formulation condition regardless of exiting surfactant.

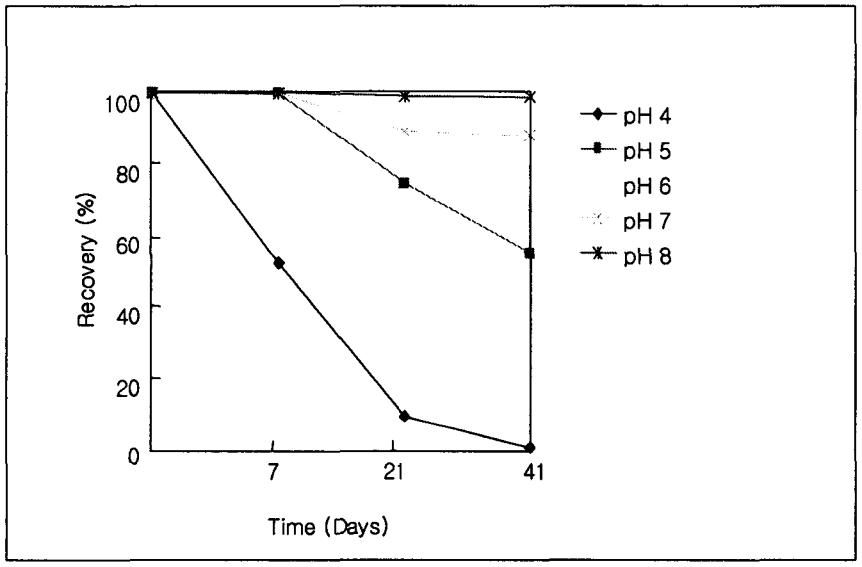

Figure 6. Recovery Percent of Tat-SOD in aqueous buffered solution stored at 25℃

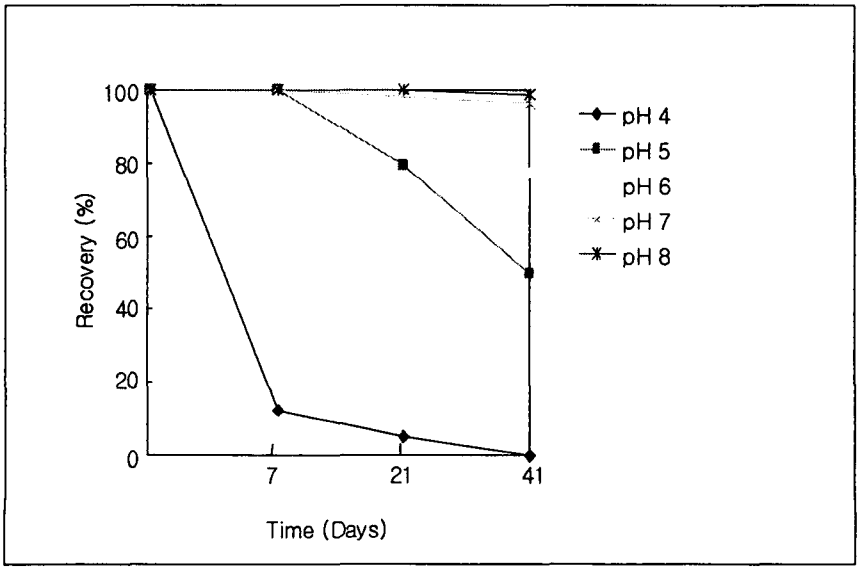

Figure 7. Recovery Percent of Tat-SOD in aqueous buffered solution stored at 40℃

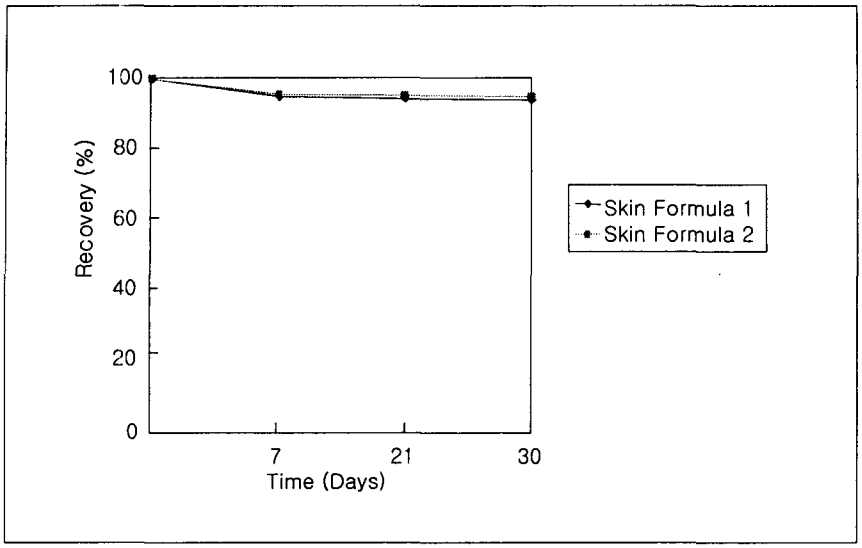

Figure 8. Recovery Percent of Tat-SOD in skin care formulation stored at 25℃

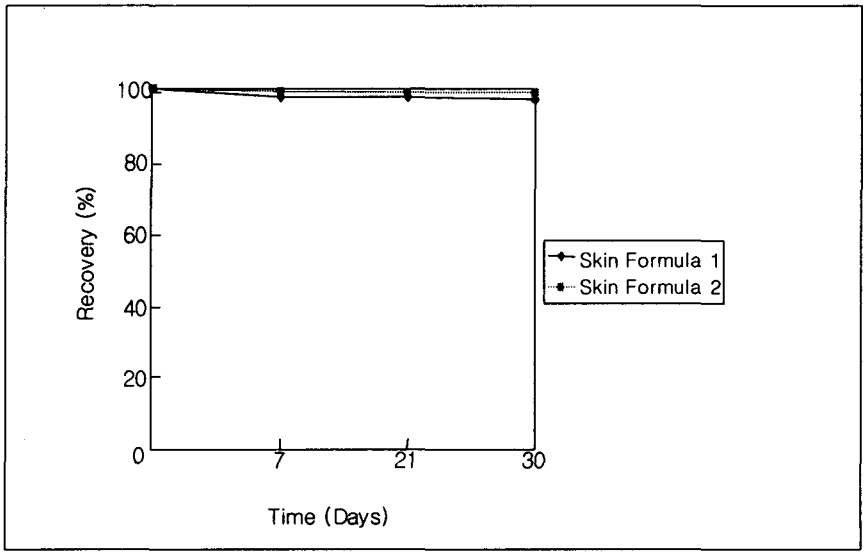

Figure 9. Recovery Percent of Tat-SOD in skin care formulation stored at 40℃

Conclusion

The stabilities of Tat-SOD was evaluated at two different temperatures (25℃ and 40℃), at five different pHs (pH4, 5, 6, 7, 8) and in skin care formulation at selected pH (pH 7.5). Tat-SOD has good stability at the tested conditions but it should be considered to test stability for more long-term storage.

In order to estimate the transduction efficiency of Tat-SOD, fusion protein were dose dependent added to the culture of fibroblast cells and the levels of transduced proteins into the cells were determined by Western blot analysis. Tat-SOD was successfully delivered into fibroblast cells, whereas control SOD was not.

Denatured Tat-SOD as well as native Tat-SOD was successfully delivered into fibroblast cells. This result implicates that denatured Tat-SOD in fibroblast cells may be correctly refolded by the mechanism of molecular chaperone or by a spontaneous process and Tat-SOD is more easily used as an active ingredient for cosmetic products. Even if Tat-SOD is denatured state in cosmetic formulation, Tat-SOD is refolded spontaneously and has superoxide anion radical scavenging activity after it penetrates into skin.

From the test of effect of transduced Tat-SOD on the cell-viability under oxidative stress, transduced Tat-SOD was very effective against superoxide anion in fibroblast cells.

For evaluation of the ability of the Tat-SOD to penetrate into skin, Tat-SOD in aqueous solution and in skin formulations (Formula 1, 2) was applied on mice skin and the degrees of penetration of these proteins into skin were analyzed by immunohistochemistry and enzymatic activities. The transduction signals were significantly detected in the epidermis as well as the dermis of the subcutaneous layer in skin tissue. Unlike Tat-SOD, control SOD could not penetrate into the skin efficiently. The level of SOD activity in skin treated with Tat-SOD was approximately a 3~4 fold higher than baseline of untreated skin and treated skin with control SOD. Tat-SOD in skin care formulation as well as aqueous solution has a skin penetration activity.

These results suggest that Tat-SOD would be an effective anti-aging agent for reducing the super oxide anions and may have therapeutic potential against various skin disorders such as skin inflammation, cancer mediated by ROS when applied topically.

It has demonstrated the ability of Tat-PTD to transduce high molecular weight molecules for example, proteins into cells and skins. Tat-SOD has a high potential to overcome the cosmetic applications of enzymes, their well-known instability and poor skin penetration capacity.

From now on, we plan to test the stability and activity of Tat-SOD in different cosmetic formulations, for example, skin, o/w emulsion, w/o emulsion *etc* for a long storage and screen the condition to stabilize it.

Discoveries in biotechnology have provided a novel penetrating system for cosmeceuticals. With its unique and powerful property, this Tat technology is at the cross-point of cosmeceutical development and delivery technology.

Tat-SOD effectively penetrates into the skin and shows powerful effects of detoxification of free radicals. Therefore, this Tat-technology will be applied to solve the adsorption problem of active ingredients of cosmeceuticals, i.e., anti-aging, anti-wrinkle and whitening cosmeceuticals.

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