Photochemical stability of lipoic acid and its impact on skin ageing

Photochemical Stability of Lipoic acid and Its Impact on Skin Aging

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Abstract

It is well known that α-lipoic acid (LA) functions as an essential cofactor of the mitochondrial multienzyme complex and thus plays an important role in energy metabolism. Currently, it is attracting attention as a nutritional supplement because of its unique antioxidant properties and broad spectra of cellular functions. Skin protection from photodamage and aging is one of the functional applications of LA. Medical and cosmetic application has been widely realized in the world. However, LA has a unique structure bearing a distorted five membered 1, 2-dithiolane ring, making it quite vulnerable to UV radiation. In the present article, we briefly reviewed skin aging from the viewpoint of oxidative stress and sun exposure, and discussed related the photochemical properties of LA. We further discuss the effect of LA on cellular signaling and its adequate applications to treat skin aging caused by oxidation. Data presented in this review suggest that LA is a powerful anti-aging agent under the appropriate usage.

Abbreviations: LA lipoic acid; DHLA dihydrolipoic acid; ROS reactive oxygen species

Introduction

α-Lipoic acid (LA) functions as a cofactor of the mitochondrial multienzyme complex and thus plays a key role in energy metabolism. LA has attracted attention as a medicine and nutritional supplement because of its unique antioxidant properties [1-3]. LA is also used in cosmetics where it is smoothly decomposed to afford many products, often for topical applications [4, 5].

LA has a distorted five membered dithiolane ring chromophore. This characteristic dithiolane ring structure is responsible for its antioxidant activity; however, the structure is unstable because it can absorb light around 333 nm. In contrast, linear disulfide amino acids such as cysteine which does not absorb light at wavelengths > 300 nm is stable under ultraviolet (UV) irradiation [4-6]. LA is also quite vulnerable to thermolysis, partially because of its low melting point [6]. As LA shows diverse actions depending on its cellular concentration [7], it is therefore difficult to reproduce the expected action of LA *in vivo*. For instance, in photoaging, exposure of the skin to UV radiation results in inflammatory reactions, DNA damage, immunosuppression, and skin cancer. Photoaging always comprises a risk of photocarcinogenesis. There appears to be

a delicate balance between the deleterious effects of cytokines leading to photoaging and their beneficial effects in ameliorating UV irradiation-caused damage [8]. Modulation of cytokine networks and signaling pathways by LA may contribute to the induction of skin cancer, thus its mechanism of action needs to be studied extensively before being adopted for widespread use. In this article, we review the photochemical reaction of LA and its potential applications as an anti-aging skin treatment.

Skin aging

To establish the long term and safe use of LA, it is quite important to fully characterize its chemical behavior. Although LA is known to function as a potent antioxidant, it is also reported that LA itself is subjected to photochemical reactions under certain conditions. When the photochemical reaction is carried out in the presence of a photosensitizer (e.g. methylene blue) to produce singlet oxygen, LA readily reacts with the singlet oxygen to produce S-O, and S-O_2 compounds [9] (**Fig. 1**).

Fig. 1

The formation of these oxidized products suggests that a direct reaction occurred between LA and singlet oxygen, and thus indicates that LA is also a quencher of singlet oxygen.

In recent decades, society as a whole has significantly aged as medicine has advanced. Many people are concerned about aging, especially the appearance of skin aging. Cosmetic therapies for skin show incredible improvement in both surgical and medical treatments [10]. Although the mechanism of aging is still under discussion, there have been two major theories regarding the process. Aging is thought to be a multifaceted process characterized by genetic and epigenetic changes in the genome [11]. Genetic aging is affected by age-dependent changes in certain chromosomes. Accumulation of genomic mutations occurring during the process of DNA repair with age remains the leading cause of genomic instability. In contrast, epigenetic aging depends on environmental stresses such as UV rays, temperature and infection.

These theories also apply more specifically to skin aging however it is differently defined as having two processes, chronological aging and sun exposure-related aging

[12]. Chronic exposure to UV radiation causes diverse changes in skin composition and various direct and indirect effects. Wrinkles, pigmentation, and atrophy are clinically characterized. Histological alteration is specified by degenerative elastic fibers in the dermis, called actinic elastosis. The aging caused by chronic sun exposure is termed photoaging, which results from intrinsic as well as extrinsic processes [13].

Oxidative stress in skin aging

Reactive oxygen species (ROS) produced in various situations play a critical role in the aging process. Skin is constantly exposed to stress, including sunlight and oxygen in the air. Although the action spectrum for photoaging has not been determined, UVA and UVB rays are the major factors which directly or indirectly damage the skin. UVB photons, having higher energy (1000 times more) than UVA photons, tend to damage skin even in a short-time exposure [14]. Previous studies demonstrated that UVB induces DNA damage such as production of pyrimidine dimers and other photoproducts (6-4) determined at the sequence level [15], and also generates 8-hydroxydeoxyguanosine (8-OHdG) production through ROS in mouse keratinocytes

[16]. UVB plays the main role in sunburn and photocarcinogenesis following sun exposure. In contrast, UVA, having longer wavelengths and 10-fold greater abundance than UVB rays, can penetrate into the deep dermis to cause skin aging [14]. Large amounts of UVA exposure also damage DNA with a similar mechanism to UVB [17]. Fifty percent of UV-induced damage is presumably due to the formation of free radicals [18]. Zhang *et al.* characterized ROS induced by UV irradiation by using a set of enzymatic and chemical scavengers, and demonstrated that hydrogen peroxide (H_2O_2) and hydroxyl radicals (HO) as well as singlet oxygen ($^1O_2$) are important in UV-induced 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxodGuo) formation following UVA or UVB irradiation [19]. Herrling *et al.* directly detected ROS in human skin generated during UV irradiation by using electron spin resonance (ESR) imaging, and showed that UVB acts primarily in the epidermis while UVA is present in the epidermis and dermis [20].

ROS, which are generated by UV exposure in skin [21, 22], reduces physiological antioxidant levels such as α–tocopherol (VE), ubiquinol-10 (CoQH2-10), ascorbic acid (VC), and glutathione (GSH) in the epidermis and dermis [23], and thus impairs the cellular redox system. Overall, these data insist that antioxidant protection is promising

against UV-induced aging.

Medicinal and cosmetic applications of LA

GSH is an important water-soluble endogenous antioxidant and plays a significant role in skin aging as the reduced thiol (GSH) and oxidized disulfide (GSSG) couple because of their potent activity in cellular redox regulation [24]; thus various clinical trials are ongoing to study the impact of increasing the cellular GSH level. The redox property is linked to many physiological processes such as the detoxification of xenobiotics, modulation of signal transduction, prostaglandin metabolism, regulation of immune response, and enzyme activities. Cysteine availability is known as the rate-limiting factor in GSH synthesis [24-26]. LA is taken up rapidly by the cell and then is reduced to dihydrolipoic acid (DHLA). The DHLA is secreted to the extracellular medium and successively reduces cystine to cysteine. Cysteine, as a precursor of GSH biosynthesis, is taken up into the cells about 10 times faster than cystine and thus enhances GSH biosynthesis. Han *et al.* revealed that treatment with 100 μM of LA achieved a 45.8% increase of cellular GSH levels, and correlated to the

increase of cellular GSH and DHLA in a T-lymphocyte cell line [25]. They also demonstrated that LA (racemic mixture) increases GSH levels in human peripheral blood lymphocytes using flow cytometric analysis [26]. Studies with human cells have provided insights into the mechanism. Sen *et al.* evaluated the effect of LA on human lymphocyte cell lines at various concentrations and found that a low concentration (no more than 100 μM) of LA is highly effective for increasing both cellular GSH and non-GSH thiols [7]. They also reported that a high concentration (2 or 5 mM) of LA induces apoptosis owing to its fatty acid structure [7], indicating that careful usage of LA is required for clinical applications.

In addition to this indirect function of LA in improving cellular anti-oxidant potential, there are reports of the free radical scavenging effects of LA both *in vitro* and *in vivo* [6]. For example, Anzai *et al.* studied the protective effect of LA against ionizing radiation in mice. After the administration of LA (200 mg/kg), the mice were subjected to X-ray exposure (4Gy or 6Gy) and several biochemical parameters were evaluated such as lipid peroxidation, protein carbonyl and total SH content. The administration of LA significantly decreased the formation of malondialdehyde (MDA) in all tissues

examined, especially in the brain, whereas measurement of the oxidized protein marker did not show clear evidence for an effect by LA. The authors argued that the observed inhibitory effect of lipid oxidation was mainly due to the free radical quenching effect of LA. At the same time, the X–ray induced decrease of non-protein SH was protected in the presence of LA and thus LA protected radiation induced protein oxidation as previously observed in other experimental systems [27, 28].

Because of its potent antioxidant potential, LA has been studied for its medicinal and cosmetic benefits to skin. Fuchs *et al.* found that oral administration of DHLA improved atopic dermatitis, but intradermal injection of R or S-LA did not [29]. Podda *et al.* demonstrated that topically applied 5% LA solution could penetrate into the dermis [22]. Many recent studies have focused on using LA in a topical format because of the ease in controlling its localization and concentration. Venkatraman *et al.* successfully treated inflammatory skin conditions with oral and topical administration of LA-based PPAR-gamma agonists in a mouse model of allergic contact dermatitis [30]. Beitner *et al.* reported that treatment with a cream containing 5% LA improved clinical characteristics related to photoaging of facial skin [31]. The objective method of

assessment showed a significant improvement in skin roughness and brightness after 12 weeks of treatment. In a randomized, double-blind, placebo-controlled study, efficacy of oral treatment with a tablet containing LA (100 mg) and other antioxidants on skin aging was verified [32]. Forty women with aging symptoms of the skin were involved in the study and results indicated that they showed a significant improvement in skin quality measured as decreases in wrinkles and roughness using both objective and subjective parameters after 6 months' treatment with the tablets (two per day) compared with the placebo.

LA also acts a protective agent against UVB-induced damage. Rijnkels *et al.* demonstrated that a single topical application of LA (0.5 μmol/cm^2) on pig skin can reduce UVB-radiation-induced oxidative stress and lipid peroxidation thereby reducing apoptosis [33]. In contrast, Lin *et al.* reported that topical application of 5% LA is ineffective at suppressing UV-induced sunburn cells as evaluated by apoptotic markers in keratinocytes [34]. As mentioned above, LA shows diverse actions in various applications. Although some of the actions (e.g. anti-apoptosis) still need to be clarified, there are many studies supporting the fact that LA works as a potent antioxidant to

prevent or improve skin aging. Further efforts are needed to develop advantageous applications of LA together with improving our basic understanding of the mechanism of action including the differential activity of the enantiomers of LA, as generally a racemic mixture of LA has been used in most previous studies. Selective usage of R-LA over S-LA could provide improved therapeutic benefits [35].

Photochemical reactivity of LA

Although LA has attracted much attention as a potential antioxidant, it is not particularly stable because of its low melting point and the distorted dithiolane ring which absorbs light around 330 nm. For this reason, LA is easily decomposed by UV irradiation [4, 5]. The photodecomposition of LA was first studied in relation to its photosynthetic pathway [36]. Then the chemical reactivity of LA and its photochemical behavior were examined using spectroscopic analyses [37]. In connection with the earlier studies of Calvin *et al.* [36], the photochemical reaction of LA has also been carried out in the presence of vitamin B_1 [38], riboflavin [39], and aldehydes [40, 41]. The formation of the photocoupling products produced from the reaction of LA with

vitamin B1 [38] can be explained from the metabolic role of LA (**Fig. 2**).

Fig. 2

The photochemical reaction of LA itself was also carried out in various solvents and the formation of many polymerized products was reported [4, 5]. Recent studies by Matsugo *et al.*, however, revealed that the photodegradation of LA by UVA generates DHLA [42]. The formation of DHLA from the reduction of LA needs two electrons thus it does not readily occur unless a certain reducing reagent is available in the reaction system. The formation of DHLA will be also highly dependent on other reactive conditions such as irradiation, solvent type, and temperature if the disproportionate reaction disrupts the formation of DHLA. Bucher *et al.* reported the results from the laser flash photolysis of LA, and discussed the mechanism based on a quantitative energy calculation [43], in that the formation of a dithiyl radical and other radical species such as carbon-centered radicals were expected and actually observed in the reaction. The irradiation of UVB light induces the regeneration of LA, and it was

suggested that homolytic cleavage of LA to produce highly reactive dithiyl radicals and the following hydrogen abstraction leads to DHLA formation. This reaction is heavily dependent on the amount of initially produced dithiyl radicals and the presence of hydrogen to be abstracted by the dithiyl radicals. When LA is exposed to UVB in the presence of thiol compounds such as cysteine and homocysteine, the decomposition of LA is retarded and instead an increased recovery of LA is obtained [44, 45] (**Fig. 3**). While the UVB irradiation of LA in the presence of methionine did not increase LA regeneration, a mechanism was proposed which involved the intervention of thiyl radical formation and the essential presence of thiol compounds in the reaction.

Fig. 3

Based on the photochemical properties of LA, a ring-opening polymerization of LA [46] and copolymerization of LA with 1,2-dithiane [47] have been examined. Photochemical studies of the polymer produced in the thermal reaction of LA with and without 1,2-dithiane revealed that both polymers consisted of an interlocked structure.

Cellular signaling related to the skin anti-aging effect of LA (Fig. 4)

The roles of ROS in signal transduction induced by stimuli have been evaluated and there have been much evidence regarding ROS involvement in the pathways [48, 49]. Schmidt *et al.* have shown direct evidence of ROS involvement with the transcription factor nuclear factor kappa B (NF-κB) and its activation of cell lines overexpressing the H_2O_2-degrading enzyme catalase [48]. Huang *et al.* performed a more direct method, ESR, and demonstrated that high dose (60 J/m^2) of UVC generates H_2O_2 [49]. They also have shown that H_2O_2 itself dose-dependently phosphorylated protein kinase B (Akt) in a similar manner to UV rays. The basic mechanisms of LA function in cellular signaling pathways are not fully understood yet. Antioxidants contribute to modulating signaling pathways *via* changes of *in vivo* redox conditions. Whether LA modulates signaling pathways by scavenging ROS as an antioxidant or by direct inhibition of a signal transduction like an enzyme is difficult to determine. The molecular action of LA has been studied in several human cell lines. Saliou *et al.* reported that LA treatment suppresses UVR-induced NF-κB activation through down-regulation of

mitogen-activated protein kinase (MAPK) activation in human immortalized keratinocytes, HaCaT cells [50]. Similarly, the inhibitory effect of LA on p44/42 MAP kinase phosphorylation is observed in human fibroblasts and adipocytes [51, 52]. Those reports where LA is utilized as a scavenger strongly suggest that MAPK is activated *via* ROS, indicating a signal mediator for NF-κB activation however details of the signaling modifications remain to be clarified. Therefore, we recently demonstrated that UVB and UVC activate an important stress sensitive transcription factor, signal transducer and activator of transcription 3 (Stat3), and the activation is directly through ROS and DNA damage in human keratinocytes and fibroblasts by using cells with xeroderma pigmentosum [53]. In this study, LA inhibited the ROS-mediated Stat3 phosphorylation, but did not affect the Stat3 phosphorylation induced by DNA damage. The actions of LA may occur differentially depending on the cell type and the cellular LA concentration as previously reported [25]. It is still challenging to achieve the expected action of LA *in vivo*. Avoiding the UV-related skin inflammatory damage by LA, the benefit is always accompanied with risks such as skin cancer. The deleterious effects of cytokines leading to photoaging are not quite distinct with their beneficial effects in

ameliorating UV irradiation-caused damage [6]. The anti-aging action brought about by LA treatment may contribute to the induction of skin cancer. In fact, there are no reports of the inhibitory effect of LA on photocarcinogenesis. Careful application of LA for skin aging is desirable to avoid unwanted adverse effects. Further accumulation of basic data is necessary for long and safe use of LA.

Fig. 4

Conclusions

Our results show that LA/DHLA functions as a potent antioxidant and thus is an attractive target of study for applications as anti-aging nutraceuticals and skin preservation agents. To our knowledge, 100 μM of LA appears to be high enough to gain the expected effect while an extremely high concentration should be avoided to limit undesirable side effects. Topical application of LA is quite practical and safe because the concentration can be controlled in the target area and systemic side effects can be avoided. However, LA is not only sensitive to the light because of its

characteristic dithiolane structure, but is also quite vulnerable to thermal stimuli and easily decomposed to afford a polymeric material. Thus it is important to find approaches to improve the thermal and photochemical stability of LA such as using a matrix like chitosan [54]. The inclusion of LA in the large molecule such ass chitosan stabilizes the vulnerable features of LA and this methodology may be used for the drug delivery system (DDS) application. As described in this article, the photochemical properties of LA in the presence of a certain thiol compound allows regeneration of LA under photolysis conditions, thereby suggesting another approach for developing improved applications of LA function.

References

[1] Scott BC, Aruoma OI, Evans PJ, O'Neil C, Van der Viet A, Cross CE, Tritschler T. Halliwell B (1994). Lipoic and dihydrolipoic acids as antioxidants. A critical evaluation. Free Rad Res Commun 20; 119-133.

[2] Moini H, Packer, L, Sans HE (2002). Antioxidant and prooxidant activities of alpha-lipoic acid and dihydrolipoic acid. Toxicol Appl Pharmacol 182; 84-90.

[3] Packer L, Cadenas E (2011). Lipoc acid energy metabolism and redox regulation and cell signaling. J Clin Biochem Nutr 48; 26-32.

[4] Brown PR, Edwards JO (1969). Effect of solvent on the photolysis of α-lipoic acid. J Org Chem 34: 3131-3135.

[5] Brown PR, Edwards JO (1969). The use of thin-layer chromatography in following product formation in the photolysis of α-lipoic acid. J Chromatography 43; 515-518.

[6] Packer L, Witt EH, Tritschler HJ (1995). Alpha-lipoic acid as a biological antioxidant. Free Rad Biol Med 19: 227-250.

[7] Sen CK, Roy S, Han D, Packer L (1997). Regulation of cellular thiols in human lymphocytes by alpha-lipoic acid: a flow cytometric analysis. Free Radic Biol Med

22:1241-1257.

[8] Kondo S (2000). The roles of cytokines in photoaging. J Dermatol Sci 23 Suppl 1: S30-36.

[9] Stary FE, Jindai SL, Murray RW (1975). Oxidation of α-lipoic acid. J Org Chem 40: 58-62.

[10] Tierney EP, Hanke CW (2010). Recent advances in combination treatments for photoaging: review of the literature. Dermatol Surg 36: 829-840.

[11] Gonzalo S (2010). Epigenetic alterations in aging. J Appl Physiol 109: 586-597.

[12] Uitto J, Fazio MJ, Olsen DR (1989). Molecular mechanisms of cutaneous aging. Age-associated connective tissue alterations in the dermis. J Am Acad Dermatol 21: 614-622.

[13] Rabe JH, Mamelak AJ, McElgunn PJ, Morison WL, Sauder DN (2006). Photoaging: mechanisms and repair. J Am Acad Dermatol 55:1-19.

[14] Yaar M, Gilchrest BA (2007). Photoageing: mechanism, prevention and therapy. Br J Dermatol 157: 874-887.

[15] Mitchell DL, Jen J, Cleaver JE (1992). Sequence specificity of cyclobutane pyrimidine dimers in DNA treated with solar (ultraviolet B) radiation. Nucleic Acids Res 20:225-229.

[16] Beehler BC, Przybyszewski J, Box HB, Kulesz-Martin MF (1992). Formation of 8-hydroxydeoxyguanosine within DNA of mouse keratinocytes exposed in culture to UVB and H_2O_2. Carcinogenesis 13:2003-2007.

[17] Applegate LA, Scaletta C, Panizzon R, Niggli H, Frenk E (1999). In vivo induction of pyrimidine dimers in human skin by UVA radiation: initiation of cell damage and/or intercellular communication? Int J Mol Med. 3:467-472.

[18] Bernstein EF, Brown DB, Schwartz MD, Kaidbey K, Ksenzenko SM (2004). The polyhydroxy acid gluconolactone protects against ultraviolet radiation in an in vitro model of cutaneous photoaging. Dermatol Surg 30:189-195; discussion 196.

[19] Zhang X, Rosenstein BS, Wang Y, Lebwohl M, Wei H (1997). Identification of possible reactive oxygen species involved in ultraviolet radiation-induced oxidative DNA damage. Free Radic Biol Med 23:980-985.

[20] Herrling T, Fuchs J, Rehberg J, Groth N (2003). UV-induced free radicals in the skin detected by ESR spectroscopy and imaging using nitroxides. Free Radic Biol Med 35: 59-67.

[21]. Yamamoto Y (2001). Role of active oxygen species and active oxygen species and antioxidants in photoaging. J Dermatol Sci 27, Suppl 1: S1-4.

[22] Shindo Y, Witt E, Han D, Packer L (1994). Dose-response effects of acute ultraviolet irradiation on antioxidants and molecular markers of oxidation in murine epidermis and dermis. J Invest Dermatol 102: 470-475.

[23] Hanada K, Sawamura D, Tamai K, Hashimoto I, Kobayashi S. (1997). Photoprotective effect of esterified glutathione against ultraviolet B-induced sunburn cell formation in the hairless mice J Invest Dermatol 108: 727-730.

[24] Podda M, Rallis M, Traber MG, Packer L, Maibach HI (1996). Kinetic study of cutaneous and subcutaneous distribution following topical application of [7,8-14C] rac-alpha-lipoic acid onto hairless. Biochem Pharmacol 52: 627-633.

[25] Han D, Tritschler HJ & Packer L (1995). Alpha-lipoic acid increases intracellular glutathione in a human T-lymphocyte Jurkat cell line. Biochem Biophys Res Commun

207: 258-264.

[26] Han D, Handelman G, Marcocci L, Sen CK, Roy S, Kobuchi H, Tritschler HJ, Flohe L & Packer L (1997). Lipoic acid increases de novo synthesis of cellular glutathione by improving cystine utilization. Biofactors 6: 321-338.

[27] Manda K, Ueno M, Moritake T, Anzai K (2006). Alpha-lipoic acid attenuates x-radiation induced oxidative stress in mice. Cell Biol Toxicol 23: 29-37.

[28] Manda K, Ueno M, Moritake T, Anzai K (2007). Radiation-induced cognitive dysfunction and cerebellar oxidative stress in mice: protective effect of alpha lipoic acid. Behav Brain Res 177: 7-14.

[29] Fuchs J, Milbradt R (1994). Antioxidant inhibition of skin inflammation induced by reactive oxidants: evaluation of the redox couple dihydrolipoate/lipoate. Skin Pharmacol 7: 278-284.

[30] Venkatraman MS, Chittiboyina A, Meingassner J, Ho CI, Varani J, Ellis CN, Avery MA, Pershadsingh HA, Kurtz TW, Benson SC (2004). Alpha-Lipoic acid-based PPARgamma agonists for treating inflammatory skin diseases. Arch Dermatol Res 296: 97-104.

[31] Beitner H (2003). Randomized, placebo-controlled, double blind study on the clinical efficacy of a cream containing 5% alpha-lipoic acid related to photoageing of facial skin. Br J Dermatol 149: 841-849.

[32] Thom E (2005). A randomized double-blind, placebo-controlled study on the clinical efficacy of oral treatment with Dermavite on ageing symptoms of the skin. J. Int. Med. Res 33: 267-272.

[33] Rijnkels JM, Moison RM, Podda E, van Henegouwen GM (2003). Photoprotection by antioxidants against UVB-radiation-induced damage in pig skin organ culture. Radiat Res 159: 210-217.

[34] Lin JY, Lin FH, Burch JA, Selim MA, Monteiro-Riviere NA, Grichnik JM, Pinnell SR (2004). Alpha-lipoic acid is ineffective as a topical antioxidant for photoprotection of skin. J Invest Dermatol 123: 996-998.

[35] Seaton TA, Jenner P, Marsden CD (1996). The isomers of thioctic acid alter C-deoxyglucose incorporation in rat basal ganglia. Biochem Pharmacol 51:983-98.

[36] Barltrop JA, Hayes PM, Calvin M (1954). The chemistry of 1,2-dithiane (trimethylene disulfide) as a model for the primary quantum conversion act in photosynthesis. J Am Chem Soc 76; 4348-4367.

[37] Wagmer AF, Walton E, Boxer GE, Pauss MP, Holly FW, Folkers K (1956). Properties and derivatives of a-lipoic acid. J Am Chem Soc 78; 5079-5081.

[38] Ohishi N (1967). Photochemical reaction product of hydroxylethylamine with lipoic acid. J Vitaminol 13; 80-88.

[39] Lu CX, Bucher G, Sander W (2004). Photoinduced interactions between oxidized and reduced lipoic acid and riboflavin. ChemPhysChem 5; 47-56.

[40] Takagi M, Goto S, Matsuda T (1976). Photoreaction of lipoic acid and related organic disulfides: reductive acylation with aldehydes. J Chem Soc Chem Commun; 92-93.

[41] Takagi M (1979). Photochemical preparation of acyl lipoic acid. Methods in Enzymol 62; 145-146.

[42] Matsugo S, Han D, Tritschler HJ, Packer L (1996). Decomposition of α-lipoic acid derivatives by photoirradaition – Formation of dihydrolipoic acid from α-lipoic acid – Biochem Mol Biol Int 38: 51-59.

[43] Bucher G, Lu C, Sander W (2005). The photochemistry of lipoic acid: Photoionization and observation of a triplet excited states of disulfide. Chemphyschem. 6: 2607-2618.

[44] Wada N, Wakami H, Konishi T, Matsugo S (2009). The degradation and regeneration of α-lipoic acid under the irradiation of UV light in the existence of homocysteine. J Clin Biochem. Nutr 44: 218-222.

[45] Wada N, Wakami H, Konishi T, Matsugo S (2008). The effect of biothiol on UV irradiated α-lipoic acid. Biofactors 34: 285-292.

[46] Kisanuki A, Kimpara Y, Oikado Y, Kado N, Matsumoto M, Endo K (2008). Ring-opening polymerization of lipoic acid and characterization of the polymer. J Poly Sci Part A Polym Chem 48; 5247-5253.

[47] Endo K, Yamanaka T (2006). Copolymerization of lipoic acid with 1,2-dithiane and characterization of the copolymer as an interlocked cyclic polymer. Macromolecules 39; 4038-4043.

[48] Schmidt KN, Amstad P, Cerutti P, Baeuerle PA (1995). The roles of hydrogen peroxide and superoxide as messengers in the activation of transcription factor NF-kappa B. Chem Biol. 2:13-22.

[49] Huang C, Li J, Ding M, Leonard SS, Wang L, Castranova V, Vallyathan V, Shi X (2001). UV Induces phosphorylation of protein kinase B (Akt) at Ser-473 and Thr-308 in mouse epidermal Cl 41 cells through hydrogen peroxide. J Biol Chem 276: 40234-40240.

[50] Saliou C, Kitazawa M, McLaughlin L, Yang JP, Lodge JK, Tetsuka T, Iwasaki K, Cillard J, Okamoto T, Packer L (1999). Antioxidants modulate acute solar ultraviolet radiation-induced NF-kappa-B activation in a human keratinocyte cell line. Free Radic Biol Med 26: 174-183.

[51] Shi SS, Day RM, Halpner AD, Blumberg JB, Suzuki YJ (1999). Homocysteine and alpha-lipoic acid regulate p44/42 MAP kinase phosphorylation in NIH/3T3 cells. Antioxid Redox Signal 1:123-128.

[52] Cho KJ, Moon HE, Moini H, Packer L, Yoon DY, Chung AS (2003). Alpha-lipoic acid inhibits adipocyte differentiation by regulating pro-adipogenic transcription factors via mitogen-activated protein kinase pathways. J Biol Chem 278: 34823-34833.

[53] Bito T, Sumita N, Masaki T, Shirakawa T, Ueda M, Yoshiki R, Tokura Y, Nishigori C (2010). Ultraviolet light induces Stat3 activation in human keratinocytes and fibroblasts through reactive oxygen species and DNA damage. Exp Dermatol 19: 654-660.

[54] Kofuji K, Nakamura M, Isobe, T, Murata Y, Kawashima S (2008). Stabilization of α-lipoic acid by complex formation with chitosan. Food Chem 109: 167-171.

Figure Captions

Figure 1. Photosensitized oxygenation of LA

Photosensitized oxygenation was carried out using methyl lipoate and methylene blue as the photosensitizer in chloroform or methanol. The reaction procedure was monitored by examining the amount of O_2 absorption. The yields of thiosulfinates and thiosulfonates were dependent on the solvent. Namely, the yields of thiosulfinates and thiosulfonates were 64 % and 25.7 % in chloroform and 75.4 % and 15.4 % in methanol respectively.

Figure 2. Photochemical reaction of LA with hydroxylethylthiamine

The photoirradiation of lipoic acid in the presence of hydroxyethylamine (Vitamin B_1 derivative) afforded the product where the 8-position sulfur atom was coupled with the methylene position of the side chain of hydroxyethylamine.

Figure 3. Photochemical reaction of LA in the presence of cysteine or homocysteine

In the presence of biothiols such as cysteine and homocysteine, an increase in the

formation of DHLA (**5**) was obtained which is readily oxidized to lipoic acid (**1**). The enhanced recovery yield of **1** was observed in the presence of biothiols.

Figure 4. UV-induced signaling pathways related to Stat3 and likely action points of LA

UV activates extracellular signals, membrane receptors such as epidermal growth factor receptor (EGFR) and cytokine receptors (e.g. interleukin-6 receptor), and then the receptor activation leads to intracellular signaling through janus kinase (JAK), Ras, Raf, MAPK/ERK kinase (MEK), and mitogen-activated protein kinases (MAPK) such as extracellular signal-regulated kinase (ERK). UV can also directly up-regulate intracellular signals, transcription factors such as nuclear factor-κB (NF-κB) and signal transducer and activator of transcription 3 (Stat3) through the generation of reactive oxygen species (ROS) or DNA damage, which triggers phosphorylation of those transcription factors. These signal cascades are stimulated directly with physical energy of UV radiation, or indirectly with ROS or DNA damage generated by UV radiation. ROS play a central role as a signal mediator of the each signaling pathway; thus

α-lipoic acid (LA) may modulate the indicated steps *via* its antioxidant activity.

Fig. 1

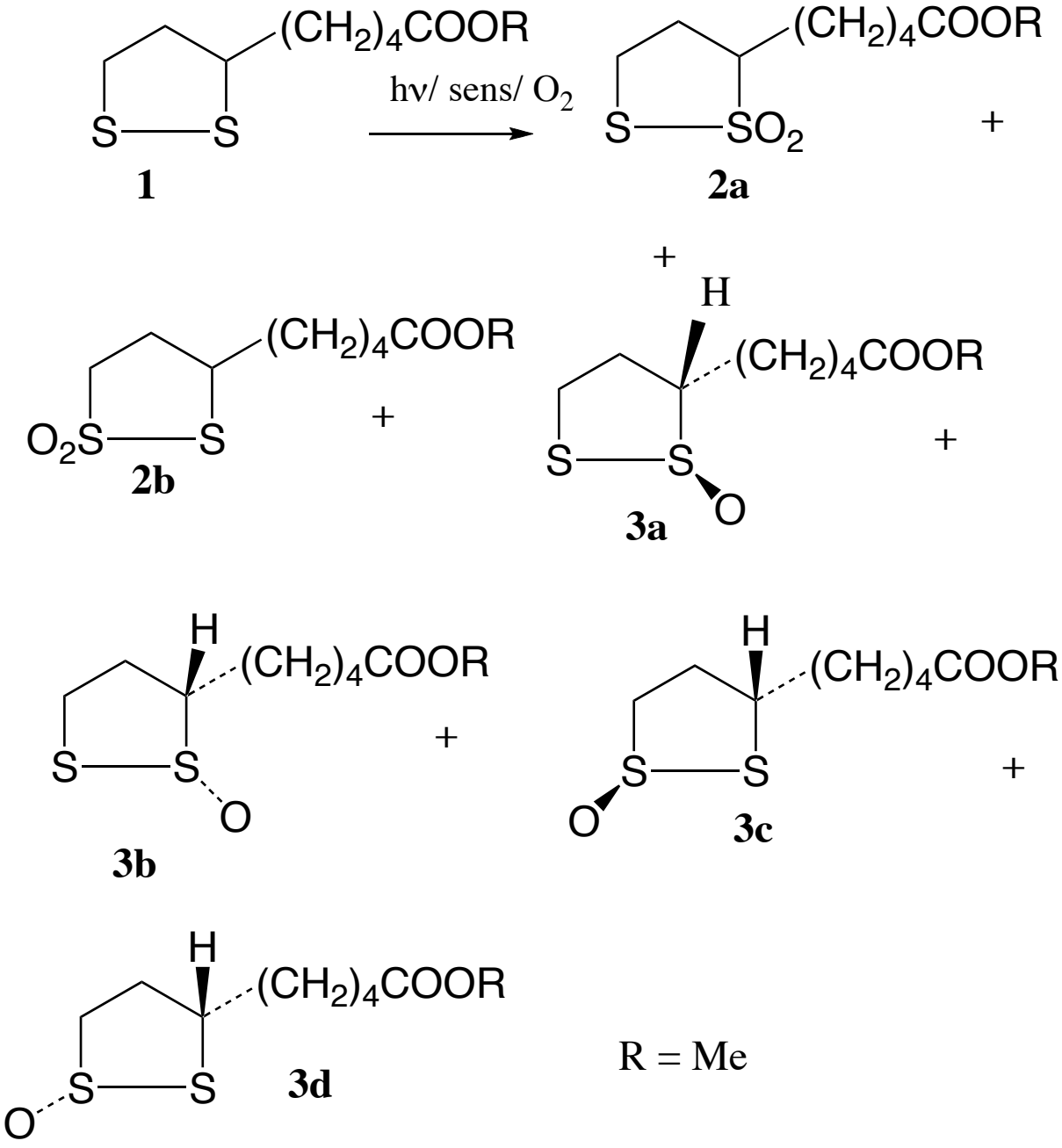
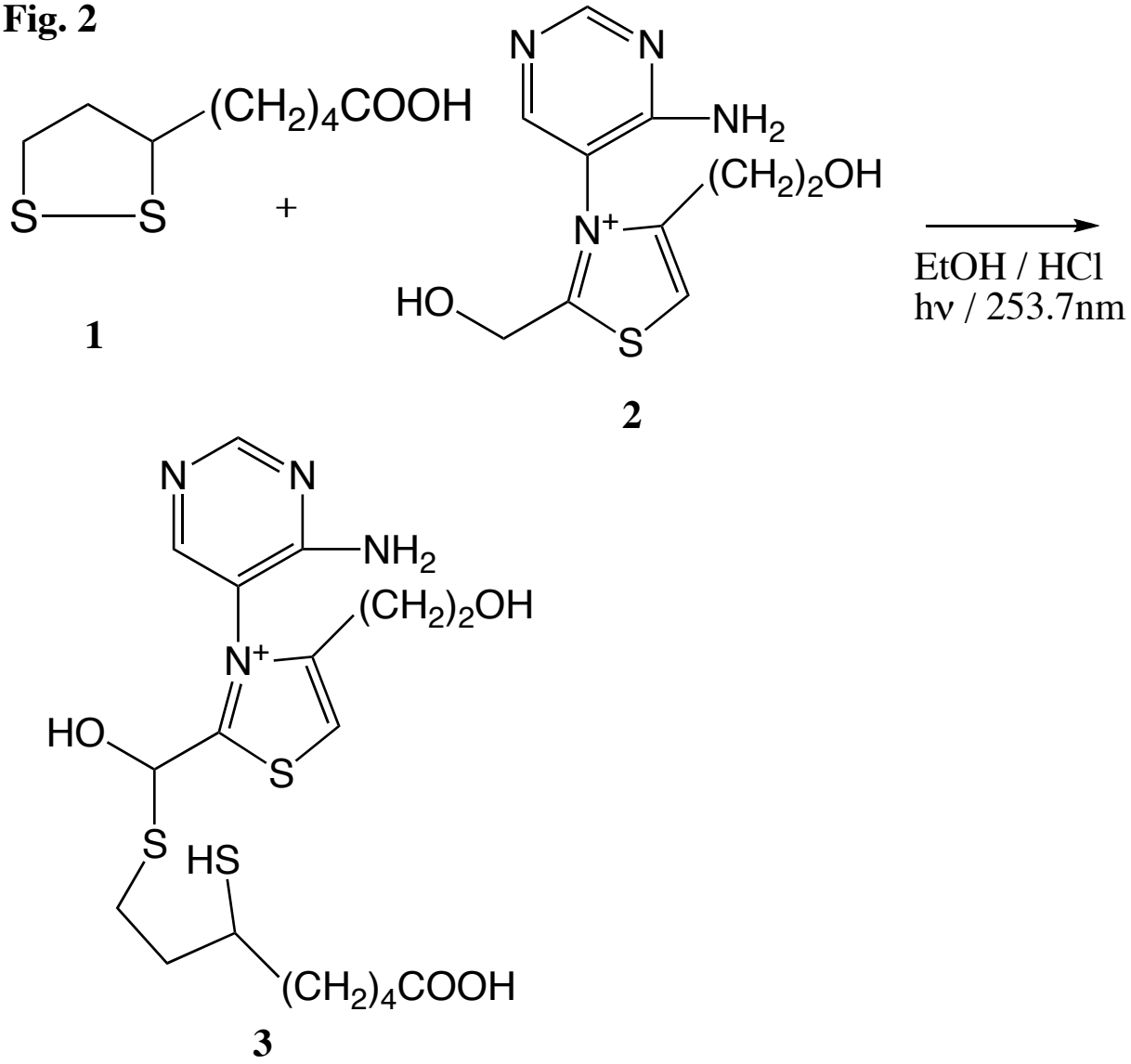
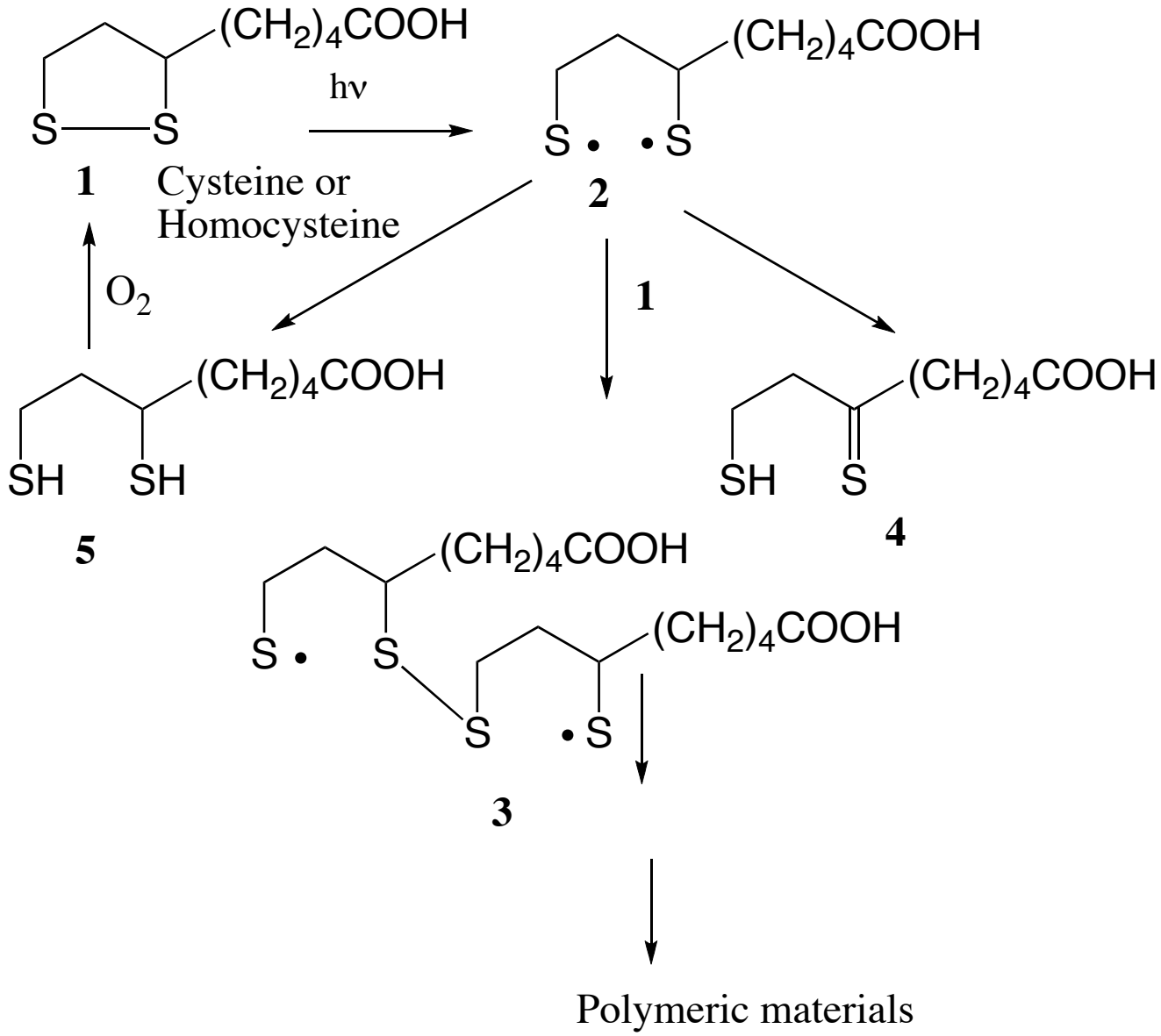


Fig. 2



1 **2** **3**

Fig.3


(CH2)4COOH
hν
S
S
1
Cysteine or
Homocysteine
(CH2)4COOH
S •
• S
2
O2
1
(CH2)4COOH
SH
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5
(CH2)4COOH
SH
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(CH2)4COOH
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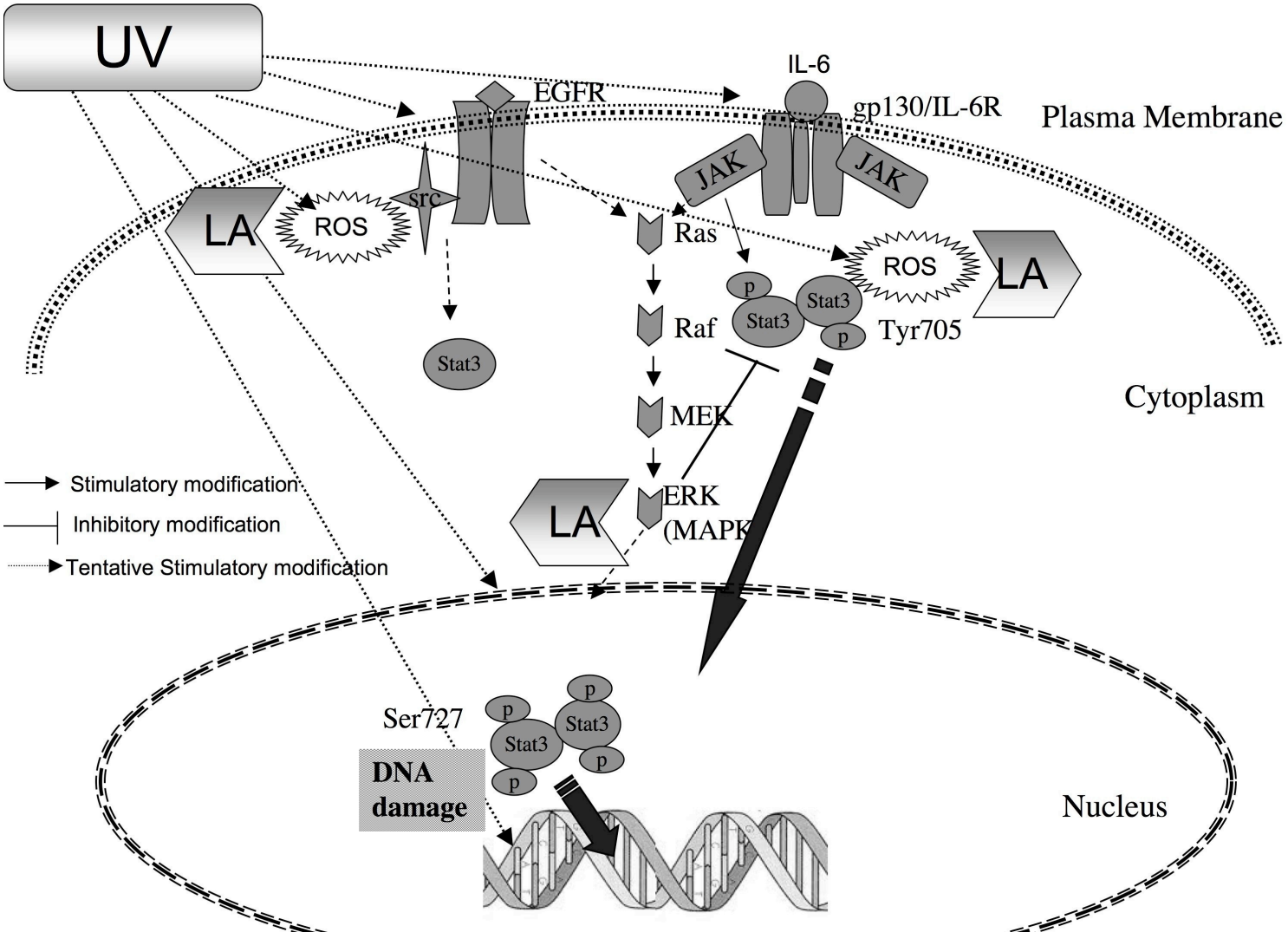
S
(CH2)4COOH
S
• S
3
Polymeric materials


UV
IL-6
EGFR
gp130/IL-6R
Plasma Membrane
JAK
JAK
src
LA
ROS
Ras
ROS
LA
p
Stat3
Stat3
p
Tyr705
Raf
Stat3
Cytoplasm
MEK
LA
ERK
(MAPK
Stimulatory modification
Inhibitory modification
Tentative Stimulatory modification
p
Stat3
Stat3
p
p
Ser727
DNA
damage
Nucleus