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SHORT COMMUNICATION

Antioxidant activity and Neurite outgrowth-enhancing activity of scorbamic acid and a red pigment derived from ascorbic acid

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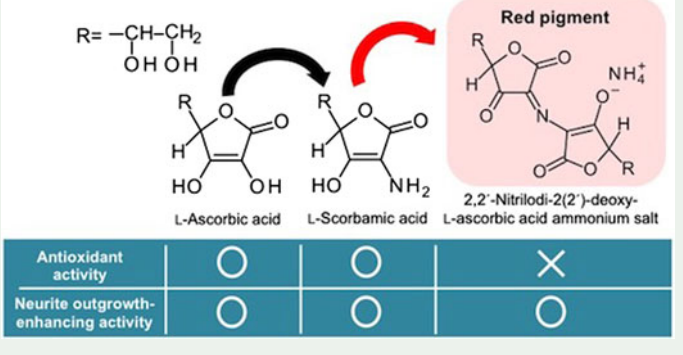
ABSTRACT

L-Ascorbic acid (AA), known as vitamin C, can form browning products by a non-enzymatic process during storage and the browning products cause deterioration of agricultural products. In the browning reaction, a red pigment, 2,2′-nitrilodi-2(2′)-deoxy-L-ascorbic acid ammonium salt (NDA), is generated from AA *via* L-scorbamic acid (SCA) as an intermediate. However, the biological activities of SCA and NDA have not yet been clarified. In this study, we assayed the antioxidant activities of SCA and NDA using ABTS radical cation and their neurite outgrowth-enhancing activities in PC12 cells. SCA showed stronger radical-scavenging activity than that of AA, while NDA hardly showed any activity. SCA and NDA enhanced the neurite outgrowth induced by dibutyryl cyclic AMP after their incorporation into cells in the same manner as that of AA. The results indicated that SCA has antioxidant activity and that SCA and NDA have neurite outgrowth-enhancing activity.



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1. Introduction

L-Ascorbic acid (AA, Scheme 1), known as vitamin C, has diverse physiological functions including a collagen synthesis effect (Murad et al. 1981), an antioxidative effect

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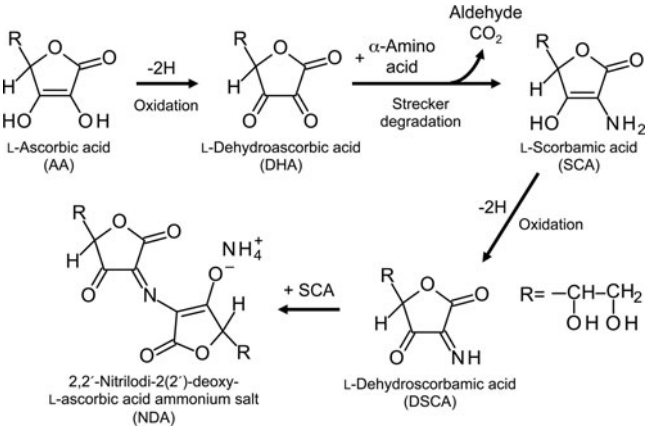

Scheme 1. Possible formation mechanism of a red pigment derived from AA.

(Satish and Dilipkumar 2015) and a neurite outgrowth-enhancing effect (Zhou et al. 2003). AA is naturally contained in *Citrus* fruits and vegetables such as lemons and peppers. On the other hand, it has been reported that AA can cause deterioration of agricultural products during storage by a non-enzymatic process (Kurata and Sakurai 1967; Kurata et al. 1973). One of the mechanisms of deterioration of agricultural products by AA is the formation of a red pigment *via* L-scorbamic acid (SCA) (Scheme 1). In one of the possible formation mechanisms of the red pigment, a carbonyl group of L-dehydroascorbic acid (DHA), the oxidized form of AA, firstly reacts with the amino group of an α-amino acid involving Strecker degradation, and this reaction leads to the formation of SCA. Next, SCA is oxidized to L-dehydroscorbamic acid (DSCA) and the reaction of DSCA and SCA finally leads to the formation of a red pigment, 2,2′-nitrilodi-2(2′)-deoxy-L-ascorbic acid ammonium salt (NDA). NDA is involved in further browning reactions and it has been found in dried cabbage (Ranganna and Setty 1968). However, the biological activities of SCA and NDA have not yet been clarified.

In this study, we investigated the antioxidant activity of SCA and NDA by using ABTS radical cation, which is one of the model radicals. We also evaluated the anti-degranulation effects and the neurite outgrowth-enhancing effects of SCA and NDA by using two cell-based biological assays.

2. Results and discussion

AA is well known to have potent antioxidant activity, and it scavenges reactive oxygen species and free radicals, which cause various kinds of illness. However, antioxidant activities of SCA and NDA have not been reported yet. We kinetically and stoichiometrically assessed the antioxidant activities of these compounds by using an ABTS radical cation-scavenging assay (Takebayashi et al. 2003; Walker and Everette 2009). The radical-scavenging activities of SCA, NDA and AA were in the order of SCA $>$ AA$\gg$NDA against ABTS radical cation (Figure 1). The ABTS radical-scavenging reactions of SCA and AA were rapid and accomplished within 5 min, while the scavenging reaction of NDA against ABTS radical cation was slow and continued over a period of 120 min. After 120 min, one mole of SCA and one mole of AA scavenged about 2.4 mole and

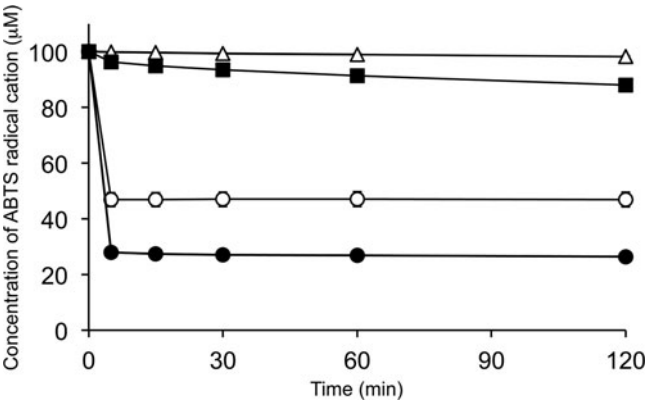

Figure 1. Time courses of ABTS radical cation-scavenging activities of AA, SCA and NDA. AA (○), SCA (●) and NDA (■) (each 30 μM) or control (Δ) and ABTS radical cation were incubated at room temperature in citrate buffer (50 mM, pH 6). Change in the remaining radicals were determined at indicated times. Each value is the mean ± SD of three separate experiments.

about 2 mole of ABTS radical cation, respectively, but one mole of NDA only scavenged about 0.2 mole of ABTS radical cation. NDA can be generated by oxidation of SCA in this assay, but it is thought that NDA did not contribute to the strong radical-scavenging activity of SCA since NDA hardly showed any radical-scavenging activity against ABTS radical cation. The results indicated that SCA showed greater antioxidant activity than that of AA, while NDA hardly showed any antioxidant activity.

To examine the anti-allergic effects of SCA and NDA, we evaluated the anti-degranulation activities of SCA and NDA in RBL-2H3 cells. As a result, SCA, NDA and AA did not show any anti-degranulation activity (Figure 2a). We also evaluated the effects of SCA and NDA on neurite outgrowth induced by dibutyryl adenosin 3′,5′-cyclic monophosphate (Bt_2cAMP) in PC12 cells as a model to study neuronal differentiation (Greene and Tischler 1976). The effects of SCA and NDA on Bt_2cAMP-induced neurite outgrowth were compared with the effect of AA because it has been reported that AA enhances neurite outgrowth induced by Bt_2cAMP in PC12 cells (Zhou et al. 2003). In the presence of SCA, NDA and AA at concentrations of 3 μM, the percentages of neurite-bearing cells induced by Bt_2cAMP were higher than that percentage with only Bt_2cAMP (Figure 2b). The neurite-enhancing activity profiles of SCA, NDA and AA were similar at all assayed concentrations. We also examined the effects of SCA, NDA and AA at concentrations of 3 μM on Bt_2cAMP-induced neurite outgrowth in the presence of two transporter inhibitors, DIDS and cyt B. It has been reported that DIDS, which is one of the anion transporter inhibitors, inhibits incorporation of AA into cells *via* sodium-dependent vitamin C transporter (SVCT) (May and Qu 2015) and that cyt B, which is one of the glucose transporter (GLUT) inhibitors, inhibits incorporation of DHA, the oxidized form of AA, into cells *via* GLUT (García-Krauss et al. 2016). DIDS decreased the percentage of neurite-bearing cells with SCA, NDA and AA in a dose-dependent manner (Figure S1a), while cyt B did not decrease the percentage of neurite-bearing cells with these compounds (Figure S1b). These results suggested that SCA and NDA were incorporated into cells not *via* GLUT but *via* SVCT in the same manner as AA to enhance Bt_2cAMP-induced neurite outgrowth. NDA had almost no

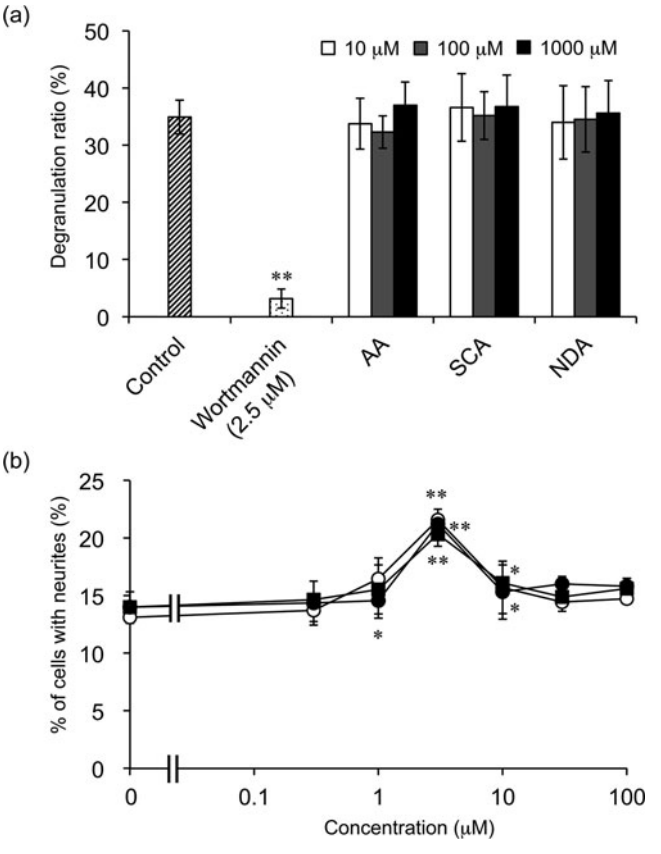

Figure 2. Biological assays of AA, SCA and NDA. (a) Anti-degranulation effects of AA, SCA and NDA in RBL-2H3 cells. Each value is the mean ± SD of three separate experiments. $^{*}p < 0.05$ compared with control. (b) Effects of AA (○), SCA (●) and NDA (■) on neurite outgrowth induced by Bt_2cAMP in PC12 cells. Each value is the mean ± SD of three separate experiments. $^{**}p < 0.01$, $^{*}p < 0.05$ compared with 0.5 mM Bt_2cAMP-treated cultures without samples.

radical-scavenging activity against ABTS radical cation (Figure 1), but it enhanced Bt_2cAMP-induced neurite outgrowth as did SCA and AA. The results suggested that the enhancing effects of SCA, NDA and AA on neurite outgrowth in PC12 cells are due not to their simple antioxidant activities but to their unique chemical structures. Therefore, SCA and NDA were considered to show the neurite outgrowth-enhancing activity in PC12 cells induced by Bt_2cAMP after incorporation of SCA and NDA into cells in the same manner as that of AA.

We found that SCA showed stronger antioxidant activity than that of AA and that SCA and NDA showed neurite outgrowth-enhancing activities as did AA, suggesting that SCA and NDA are beneficial for health and that these compounds would be useful ingredients.

3. Conclusion

In this study, we investigated the unknown biological activities of SCA and NDA using multiple assays. In a radical-scavenging assay, SCA showed stronger radical-scavenging

activity against ABTS radical cation than did AA, while NDA hardly showed any radical-scavenging activity. In biological assays, SCA and NDA as well as AA enhanced Bt_2cAMP-induced neurite outgrowth in PC12 cells, while SCA and NDA as well as AA did not show anti-degranulation activities in RBL-2H3 cells. Therefore, we found that SCA has antioxidant activity and that SCA and NDA have neurite outgrowth-enhancing activities.

Disclosure statement

No potential conflict of interest was reported by the authors.

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