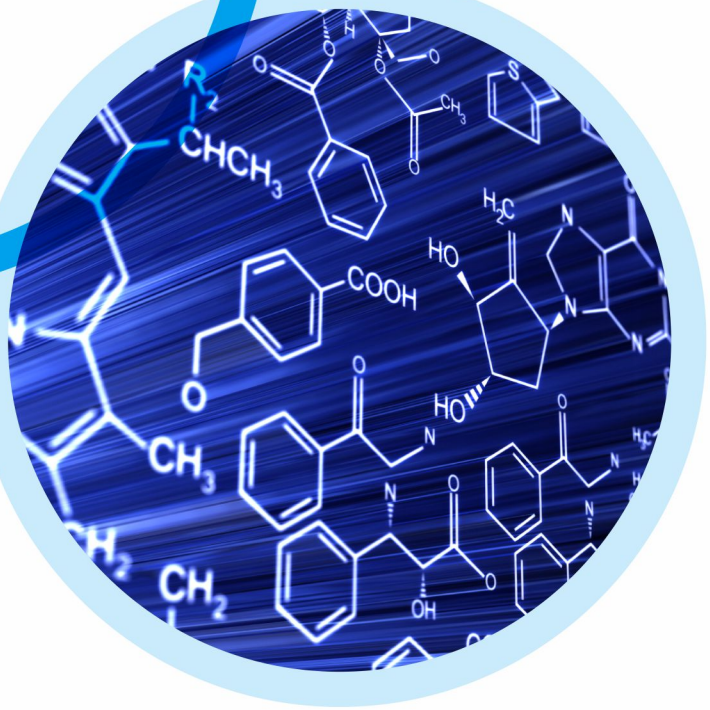

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Research Article
Production Of 2-O-Alpha-D-Glucopyranosyl-L-Ascorbic Acid by Transglycosylation Using Dextransucrase From *Leuconostoc mesenteroides*

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Abstract

Background and Objective: Ascorbic acid is one of the antioxidants active in several biological systems, which are the main oxygenated components and free radicals used mainly in pharmaceutical applications. However, it is highly unstable at temperature and light, losing the beneficial effects when exposed during use. This study had as main objective to improve the instability of ascorbic acid, directing the application of the new compound to the use of the topic. **Materials and Methods:** For this, an enzymatic transglycosylation of ascorbic acid was performed using sucrose as a donor in the reaction. Transglycosylation was catalyzed by purified dextransucrase after production by a bacterial lineage of *Leuconostoc mesenteroides* FT045B and which catalyzes the acceptable reaction to the reaction capable of training the α-glycosides ascorbic acid. In this research, the transglycosylation reaction was conducted using the dextransucrase enzyme FT045B, used by the *Leukonostoc mesenteroids* FT045B and characterized by thin-layer chromatography and mass spectrometry. **Results:** The production of AA-2G is carried out enzymatically. The thin layer chromatography plate after eluting the sample under UV light revealed the compound AA-2G. The peaks of the mass spectrum of the sample represented specifics characteristics of AA-2G. **Conclusion:** From the microbial enzymatic transglycosylation reaction of ascorbic acid using sucrose was possible to synthesize AA-2G with different stability characteristics.

Key words: 2-o-alpha-d-glucopyranosyl-l-ascorbic acid, dextrasucrase, *Leuconostoc mesenteroides*, enzyme, transglycosylation reaction, bacteria, L-ascorbic acid

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

L-Ascorbic Acid (AA), known as vitamin C, is an organic compound used as an antioxidant food additive that can modify functional biological properties for applications in anti-aging and immune regulation.

AA is highly unstable when exposed to variations in temperature and light and loses its beneficial effects when exposed in applications. The inactive form of vitamin C occurs in its conversion to dehydroascorbic acid. Many products on the market contain AA in solution which have low stability, being degraded in a few days[1,2].

A modified compounds have been developed to increase their stability maintaining an ideal biological activity due to their excellent antioxidant properties[3] and thus assisting in the formation of collagen, iron absorption and in the synthesis of carnitine[4,5]. There are AA derivatives that have attracted interest from pharmaceutical food industries such as 2-o-alpha-d-glucopyranosyl-l-ascorbic acid (AA-2G) which has many advantages such as absorption by different biological systems[6,7].

AA-2G is easily processed by enzymes such as α-glycosidase, which make L-ascorbic acid available, making it active *in vitro* and *in vivo*. Thus, it often stimulates the physiological effects of L-ascorbic acid in medical products.

Dextransucrase is an alternative trans-glucosyltransferase well known and used to modify a variety of bioactive substances. The transglycosylation reaction can be catalyzed by high purity dextransucrase.

The strategy used in this study to modify the instability of the new ascorbic acid, directing it to an application in topical use, was the use of the enzymatic transglycosylation reaction produced by the bacteria *Leuconostoc mesenteroides* FT045B using one of the most straightforward and most economical donors, sucrose that catalyzes acceptor reaction, generating α-glycosides ascorbic acid.

MATERIALS AND METHODS

Study area: The study was carried out at Rio Claro Biosciences Institute, Department of Biochemistry and Microbiology, Industrial Microbiology Lab, São Paulo State University, Brazil from January-December, 2012.

Transglycosylation reaction for AA-2G production: Total 20 mL of purified FT045B dextransucrase was added to 80 mL of the substrate solution composed of 1.25% ascorbic acid and 250 mM sucrose, prepared in 20 mM pyridine/acetate buffer (pH 5.2). A vacuum pump removed the air for 20 min and the solution was kept for 24 hrs at 23°C under vacuum, with light agitation. A 7 µL aliquot of the solution was applied 15 mm from the base of the CCD plate (Polygram sel G / UV), using a micropipette.

The application sizes were kept between 2 and 3 mm by adding 1 µL at a time, drying in intervals with cold air. The plate was irrigated to the top at a temperature between 20-22°C using 2-propanol/1-butanol/water 12: 3: 4 (v/v/v) in two ascents. The resulting compound bound to ascorbic acid can be visualized under UV light after separation.

Characterization of the structure of compound AA-2G by mass spectrometry: Total 80 applications were performed using mass analysis. About 1 µL at a time, using a micropipette, drying at intervals with cold air on CCD plates and 7 µL of the solution resulting from the transglycosylation reaction were applied with the use of a micropipette drying at intervals with cold air. The plate was irrigated to the top at a temperature of 20-22°C using 2-propanol/1-butanol/water 12:3:4 (v/v/v) in two ascents (sample A) in three ascents (sample B) and four ascents (sample C). The bands or spots corresponding to the sample were scraped from the plate, after visualization and marking under UV light, gathered in a single bottle. The extraction of the compound of interest was carried out by 3 consecutive washes with 800 µL of methanol under agitation, followed by total drying of the solvent under vacuum. The dry sample was solubilized in 200 mL of 10 mM ammonium acetate buffer, containing 50% (v/v) acetonitrile (MALLINCKRODT) and filtered through a MillexÒ-HV syringe filter, with a 45 m PVDF membrane (MILLIPORE). The mass spectra were obtained in a mass spectrometer equipped with an ionization source of the "electrospray" type (ESI) and a hybrid mass analyzer formed by the combination of "ion-trap" and "Time-of-Flight" (IT-TOF SHIMADZU). The analyzes were performed in positive (ESI +) and continuous mode. Throughout the experiment, the LCMS solution software (SHIMADZU) was used to control data acquisition and analysis. The temperature of the CDL and the interface was at 200°C, the needle voltage at 4.5 kV and the voltage at the cone at 3.5 V. The drying gas flow (Nitrogen) was 100 L/h and the gas flow nebulizer (Nitrogen) of 1.5 L/h. Scans (mass spectrometer) were performed using a range of m/z 50-1000 and a resolution of approximately 15000.

RESULTS AND DISCUSSION

The thin layer chromatography plate after eluting the sample under UV light to reveal the separate compounds is shown in Fig. 1. The arrow indicates the presence of compound AA-2G.

This first exposure of the CCD plate to ultraviolet-UV light was carried out to confirm the reliable products under this condition, checking if there was a connection of ascorbic acid to the glucose units. Thin-Layer Chromatography (TLC) has been widely used for the food analysis for the analysis of phenolic such as components of red wine[8].

The confirmation of the production of the compound of interest by exposing the CCD plate to UV, a larger quantity of this compound would be necessary for identification by mass spectrometry. In this regard, the application of a more significant amount of sample in CCD plates for later extraction of the compound after separation was necessary.

The ascorbic acid compound 2-glycoside can be produced from several enzymes such as α-glycosidase[9], cyclodextrin glycanotransferase[10] and sucrose phosphorylase[11]. The formation of AA-2G by a glucansucrase reported in 2010, via production catalyzed by the glucansucrase EG001 of *Leuconostoc lactis* EG001[12]. Glycosylation using glucansucrases studied improved the function of several bioactive compounds as in the production of catechin glycoside (+)- by *Streptococcus mutans* glycosyltransferase-D[13]; salicin and phenolic glycosides by *L. mesenteroides* glucanosucrase[14]; glycoside luteolin[15]; quercetin and arbutin glycoside[16].

The spectrum represented in Fig. 2 shows the m/z peaks obtained for sample A of AA-2G. Several peaks not corresponding to the sample in question were observed. This sample has several components in addition to AA-2G, whose corresponding peaks are those of m/z 177.028 (belonging to the glucose residue) and the peak of m/z 361.048 that corresponds to AA-2G contains an atom of sodium ($[M+Na]^+$). The identification of glycoside compounds from tobacco was performed by m/z 177 of the loss of glucose unit[17]. The remaining peaks, which do not refer to AA-2G, appear due to CCD has separated the sample in only 2 ascents, which did not allow efficient separation of the compounds present in the reaction.

The presence of the peak of m/z 177.029 was observed in the mass spectrum of sample B of AA-2G (Fig. 3), which corresponds to the glucose residue ion in the form $[M+H]^+$. In the determination of Ascorbic Acid in Food the peaks of low weight molecular ion [M+H]+176.13 were presented[18]. The other ions formed from m/z 339.067, 356.093 and 361.048

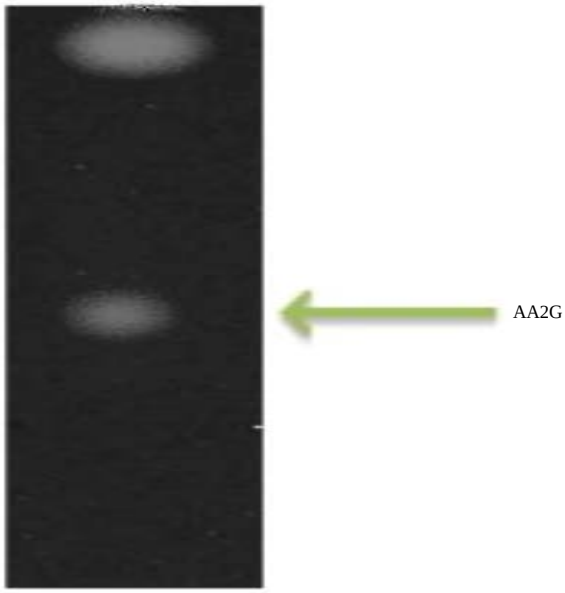

Fig. 1: CCD plate after elution, compound AA-2G is indicated with the arrow

correspond to AA-2G ions in the forms $[M+H]^+$, $[M+NH_4]^+$ and $[M+Na]^+$, respectively. It is also possible to observe the presence of other ions such as the m/z 527.071 that correspond to other unidentified components present in the extracted band due to this sample having undergone only 3 ascents in solvents in the CCD plate, which made it impossible to separate the components of the reaction.

The mass spectrum of AA-2G sample C is represented in Fig. 4. The peaks of m/z 177.029 observed corresponding to the glucose residue ion in the form $[M+H]^+$ and the ions referring to the AA-2G-C in monoprotonated forms (m/z 339.068; $[M+H]^+$), containing $NH4^+$ (m/z 356.093; $[M+NH_4]^+$) and sodium (m/z 361.048; $[M+Na]^+$). An analysis of water-soluble vitamins by mass spectrometry, the base presented a peak of ascorbic acid in m/z 116 ion, which accounted for loss from the molecular ion m/z 176[19].

The production of AA-2G is carried out enzymatically, such as the transglycosylation of ascorbic acid to ascorbic acid 2-glycosidic by recombinant *Bifidobacterium longum* saccharase-phosphorylase[11] which after analysis of the produced compound and AA-2G standard by LC-MS/MS the obtained spectrum showed a peak at 339 m/z of the protonated molecule ($[M+H]^+$) in addition to the molecular ion at 177 m/z. Zhang *et al.*[20] also reported the characterization of AA-2G by mass spectrophotometry, however after enzymatic transformation of 2-O-α-D-glucopyranosyl-L-ascorbic acid using recombinant *E. coli* α-cyclodextrin glucanotranferase.

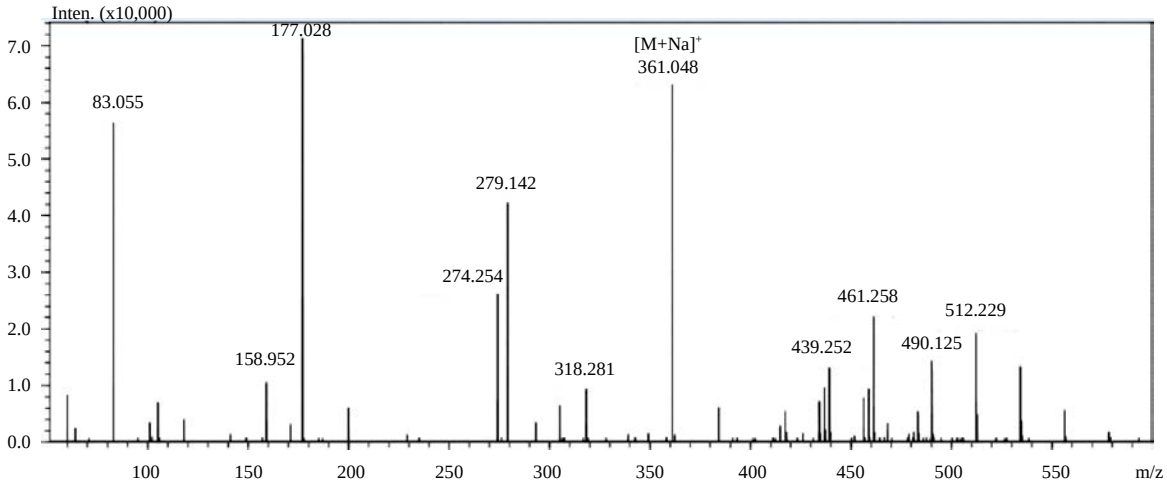

Fig. 2: Mass spectrum ESI-MS, in positive mode, from sample A of AA-2G

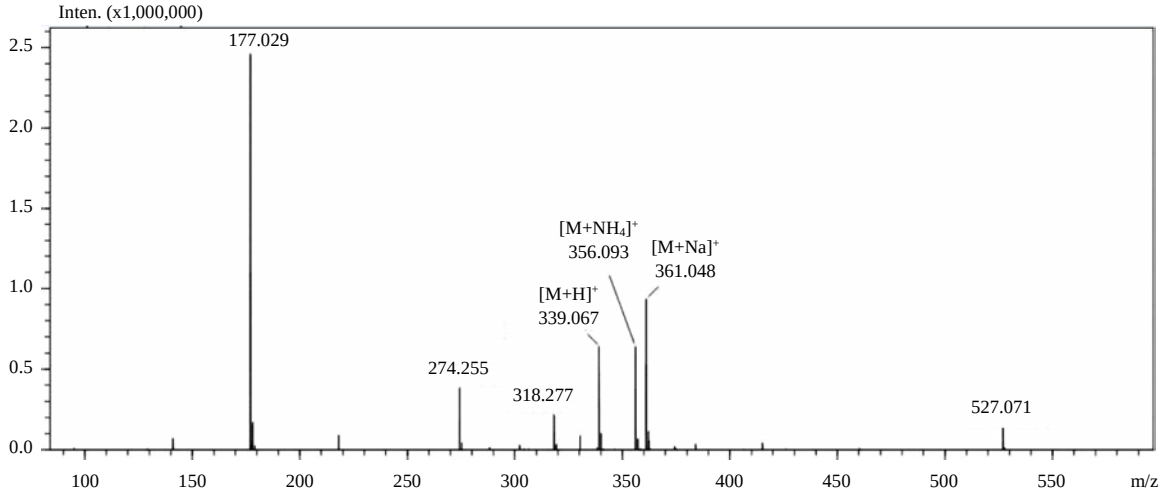

Fig. 3: ESI-MS mass spectrum, in positive mode, of sample B of AA-2G

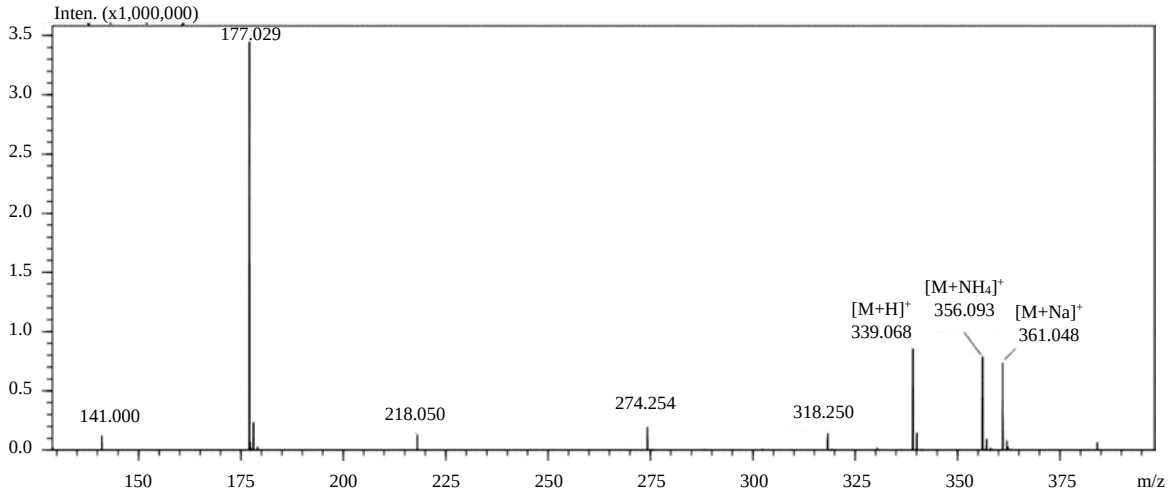

Fig. 4: ESI-MS mass spectrum, in positive mode, of sample C of AA-2G

LC-MS/MS analysis of the standard compound revealed the protonated molecule $[M+H]^+$ at 339 m/z and its molecular ions at 177. 141 and 95 m/z.

CONCLUSION

Ascorbic acid is one of the most potent antioxidants active in biological systems capable of removing active oxygenated compounds and free radicals from a biological system; however highly unstable to heat and light was produced in this study using a strategy capable of making it more stable.

The strategy used was the study of microbial enzymatic transglycosylation reaction of ascorbic acid using one of the simplest and cheapest donors, sucrose, which made the compound formed with specific characteristics for the challenges presented by the ascorbic acid molecule. The transglycosylation reaction was catalyzed by the purified dextransucrase, which was produced by the bacteria Leuconostoc mesenteroides FT045B, which catalyzes the acceptor reaction, generating AA-2G characterized by spectrometric methods.

SIGNIFICANCE STATEMENT

This study discovers a modified ascorbic acid, the AA-2G, which was synthesized by Dextransucrase enzyme from *Leuconostoc mesenteroides*.

This study will help the researcher to uncover the clinical application of this compound that may have the same bioactivity of AA *in vivo*; however, this derivative may prolong the benefits of AA, thus promoting the production of antibodies and collagen acting in tissue repair with high stability. Being that many researchers were not able to explore.

Thus a new theory on the application of ascorbic acid from the production of AA-2G may arrive.

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