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Does resveratrol retain its antioxidative properties in wine? Redox behaviour of resveratrol in the presence of Cu(II) and tebuconazole

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ABSTRACT

Resveratrol is antioxidant naturally occurring in wine grapes. It is thought to have a preventive biological activity against number of diseases. However, it has been recently shown that in the presence of metal ions, such as Cu^{2+}, resveratrol forms oxidative radicals. Cu^{2+} is usually present in wine due to former usage of bluestone in vineyards. Fungicide tebuconazole has substituted bluestone and is presently one of the most widely used agrochemicals in wine industry; wine thus may contain traces of tebuconazole. Here, we study the ternary system of resveratrol, Cu^{2+}, and tebuconazole experimentally and theoretically (using mass spectrometry, antioxidant capacity assay and quantum-chemical calculations) to model the redox behaviour of resveratrol in wine. We show that tebuconazole prevents formation of oxidative resveratrol radicals (induced by Cu^{2+} reaction with resveratrol) via preferential Cu^{2+} capture and protection of the binding sites of resveratrol. This positive effect of tebuconazole has not been observed before.

1. Introduction

Resveratrol (Res) belongs to the family of polyphenol (polyhydroxide) compounds. It is thought to have a strong biological activity against a number of diseases, such as cancer or cardiovascular diseases (Baur et al., 2006; Bradamante, Barenghi, & Villa, 2004; Jang et al., 1997; Quideau, Deffieux, & Pouysegu, 2012; Smoliga, Baur, & Hausenblas, 2011). The positive healing effect of Res is based on its antioxidant properties, i.e., ability to quench free radicals. Its most common natural resource are wine grapes; esp. red wine contains up to 14 mg L^{-1} of Res (Stervbo, Vang, & Bonnesen, 2007). Wine is also rich in copper cations (milligrams per litter, depending on the variety of the grape) (Clark, Wilkes, & Scollary, 2015) because of contamination of vineyard soils by copper cations due to historical antifungal spraying of vineyards with bluestone (copper sulphate).

It has been shown recently that in the presence of Cu^{2+} ions Res forms radicals with strong oxidative activity and a Cu^{2+}-Res complex (Fig. 1; see below), which can intercalate DNA (Hadi et al., 2010; Tamboli, Defant, Mancini, & Tosi, 2011; Tamboli et al., 2012). Wine is a complex mixture containing many natural and artificial chemicals. One of them, tebuconazole (Teb) is heavily used for protection of grapes against fungal diseases and can be present also in the final wine products (Alister et al., 2014). It is a reactive agrochemical with high affinity to metal cations (Jaklová Dytrtová, Jakl, Schröder, Čadková, & Komárek, 2011), and can thus be expected to strongly interact with Cu^{2+} and even with resveratrol. The purpose of this study was to investigate the ternary system of resveratrol, tebuconazole, and Cu^{2+} to mimic the redox behaviour of resveratrol in wine. Mixtures of resveratrol with Cu^{2+}, resveratrol with tebuconazole, and resveratrol with Cu^{2+} and tebuconazole were studied by means of electrospray ionization mass spectrometry to understand basic reactivity of these systems and structures of created products. Density functional calculations were used to quantify the interactions among studied molecules. The change in the antioxidant capacity of Res in solution was measured in relation to the individual interactions and time.

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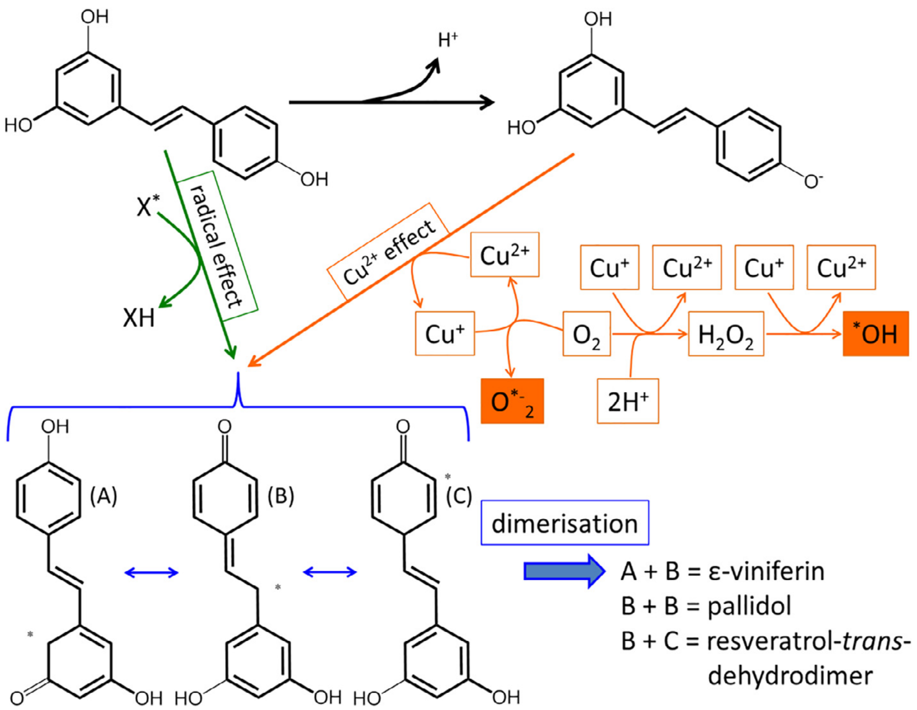

Fig. 1. Summary of the known actions of resveratrol (Res) alone (black arrows) and resveratrol in the presence of Cu^{2+} (orange arrows). The blue arrow indicates dimerization of deprotonated resveratrol radicals on the left. Based on general knowledge of Fenton reaction (Takaya et al., 2005; Tamboli et al., 2012). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Experimental

2.1. Mass spectrometry studies

The mass-spectrometry studies were performed using ion-trap mass spectrometer (ESI-MS; Finnigan LCQ Advantage, ThermoFinnigan, USA) fitted with an electrospray ion source operated in the positive-ion and negative-ion modes. The exact masses were measured using a QToF mass spectrometer (SYNAPT G2, Waters, UK). The sample solutions were continuously fed ($0.6\,mL\,h^{-1}$) into the ion source through a silica capillary. Nitrogen was used as the nebulizer gas. Operating conditions of the ion trap were set as follows: spray voltage of 4.0 kV, capillary voltage of 0–10 V, tube lens offset of −5 to 10 V, heated capillary temperature of 200 °C, and sheath-gas flow rate as well as auxiliary-gas flow rate of 10–50 arbitrary units. For the QToF the conditions were set as follows: capillary voltage of 3.0 kV, sampling and extraction cones of 28 V and 2 V, source and desolvation temperatures of 100 and 350 °C, cone and desolvation gas of 50 and $450\,L\,h^{-1}$. Stability of the complexes was experimentally determined in an ion trap using collision-induced dissociation (CID) experiments of mass selected ions by comparing appearance energies (*AE*, $kJ\,mol^{-1}$) of daughter ions for fragmentation of Res, Res/Cu, Teb/Res/Cu parent ions (Zins, Pepe, & Schröder, 2010).

The sample solutions were prepared at desired concentrations using resveratrol and tebuconazole (Sigma-Aldrich, Czech Republic) stock solution ($10^{-2}\,mol\,L^{-1}$) in ethanol (Maneko, Czech Republic) and $CuCl_2$ (Sigma-Aldrich, Czech Republic) stock solution ($10^{-2}\,mol\,L^{-1}$) in pure water ($> 18\,M\Omega$). The experiments were mostly realized for an equimolar mixture of Res, Teb, and $CuCl_2$ ($5 \cdot 10^{-5}\,mol\,L^{-1}$) in methanol/water (1:1).

2.2. EC/ESI-MS

For mimicking of Res and Teb interactions with copper an electrochemical device (EC) coupled online with ESI-MS (EC/ESI-MS) depicted in Reference Jaklová Dytrtová, Jakl, Navrátil, Cvačka, and Pačes (2016) was used. The sample solutions were continuously ($0.45\,mL\,h^{-1}$) fed into the EC through a silica capillary. The operating potential inserted to the copper working electrode was +700 mV, the operating time was 120 s. During this time the mass spectra detecting the reaction products were online recorded. Parameters of ESI-MS were the same as above. The sample solution consisted of equimolar mixtures (both $5 \cdot 10^{-5}\,mol\,L^{-1}$) of Res and Teb in methanol/electrolyte ($25\,mmol\,L^{-1}$ ammonium acetate) mixture (1:1).

2.3. Theoretical calculations

Molecular structures were optimized with Gaussian 09 code (Frisch et al., 2009) using the B3LYP density functional (Becke, 1988) and the 6-311G* basis set (Jakl, Straka, Jaklová Dytrtová, & Roithová, 2014).

Different mutual orientations of molecules in complexes were calculated to assure the global minima. Only the lowest energy isomers found are discussed. Frequency analysis was performed for each structure to assure the minimum and to determine the zero-point energy correction. The theoretical bonding energies (BDE) refer to the energies at 0 K, i.e., the sum of electronic energy and zero-point energy.

2.4. Antioxidant capacity assay

The Free Radical Scavenging by the use of the DPPH radical (2,2-diphenyl-1-picrylhydrazyl), and the Ferric Reducing Antioxidant Potential assays (FRAP) were used for the antioxidant capacity determination, both accordingly to Tupec et al. (Tupec et al., 2017), and Dudonne et al. (Dudonné, Vitrac, Coutière, Woillez, & Mérillon, 2009). The detailed procedures are described in SI. The experiments were realized for an equimolar mixture of Res, Teb, and $CuCl_2$ ($100\,\mu mol\,L^{-1}$ for FRAP, $800\,\mu mol\,L^{-1}$ for DPPH), or Res, Teb, $[(CH_3CN)_4Cu]PF_6$ ($2.5\,mmol\,L^{-1}$ for DPPH). For FRAP, Teb and Cu^{2+} solutions were also tested in $1–10\,mmol\,L^{-1}$ concentrations but they did not cause any changes in absorbance, thus they were considered of no antioxidant capacity. All the samples were measured at least 4-times. The data were analysed by One Way ANOVA (Bonferroni method); the differences were considered statistically significant at $P \leq 0.05$.

3. Results and discussion

The behaviour of Res in the presence of Cu^{2+} was studied previously by Tamboli *et al.* (Tamboli et al., 2012). We were able to