Effect of salt (zinc chloride) on antioxidant activity of gallic acid

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ABSTRACT

The first 100s of reaction between gallic acid (GA) and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical follows the second order reaction. The addition of zinc (Zn) significantly enhanced the rate of reaction, such that the rate constant when GA:Zn is 1:1 was significantly higher, (P<0.05), 120 ± 12 M⁻¹s⁻¹ as compared to 92 ± 6 M⁻¹s⁻¹ when zinc was absent. However, the addition of excess zinc, GA:Zn 1:2, decreased the second order rate constant to 75 ± 9 M⁻¹s⁻¹. Therefore, the presence of catalytic amount of zinc enhanced gallic acid scavenging properties, while excess zinc inhibited the rate of reaction. The mechanism by which GA scavenge for DPPH radical was probably that of a two step process, electron transfer, followed by proton transfer.

INTRODUCTION

Gallic acid, as well as its derivatives has important antioxidant activities by displaying radical scavenging abilities. They are polyphenols, which scavenge for radicals by donating hydrogen to radical, while itself oxidizes to a quinone, which is stable. Other than stabilizing radicals, which are products of cell metabolism, phenolic compounds can also combine with copper and iron metal ions, which can induce the radical production via Fenton reaction. Hynes et al. (2001) showed that complexation between ferric ions and gallic acid can result in the oxidation of gallic acid to hydroxyquinone and reduction of ferric ions to ferrous ions.

However, some transition metal salts render protective effect to cells against harmful radicals. In a recent experiment, Kagay et al. (2002) demonstrated that zinc increased the hepatoprotective activity of epigallocatechin gallate in isolated rat hepatocytes. Zinc is an important metal, which is present in human bodies in large quantities, approximately 3 g in a 70 kg human and it is found in all six classes of enzymes important for physiological activities (Dunn, 1995). Zinc exhibits both hard and soft acid properties and is able to bind favourably to oxygen, nitrogen and sulphur compounds. For example, zinc plays a stabilizing role in metallothioneins by forming zinc-sulphur clusters. Other than structural role, zinc has a regulatory function in the body. Zinc is the active metal centre in the enzyme alcohol dehydrogenase and the primary function of the enzyme is to catalyse detoxification of alcohol to acetaldehyde.

Therefore, based on these known structural, as well as catalytic function of zinc, we aim to determine if zinc exhibits a similar effect on the reaction between GA and DPPH radical. The reaction between GA and DPPH can be interpreted as the oxidation of a phenol to a quinone, which is analogous to the oxidation of alcohol to a carbonyl, thus a similar catalytic mechanism by zinc could thus take place. From this experiment, we also aim to elucidate if gallic acid donate a hydrogen atom to DPPH radical via a direct hydrogen transfer mechanism, or via an electron transfer, followed by proton transfer mechanism.

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MATERIALS AND METHODS

Materials

1,1-diphenyl-2-picrylhydrazyl (DPPH) and 1,1-diphenyl-2-picrylhydrazine (DPPH₂) were purchased from Sigma. Gallic acid was obtained from Acros Organics. Zinc chloride, >98% was purchased from Fluka and methanol of HPLC grade was obtained from Merck. A stock solution of 2x10⁻⁴ M DPPH was prepared freshly. Gallic acid (GA) and zinc chloride (Zn) was made up to 0.06 M and diluted with methanol to make up the required ratio of GA and Zn mixture.

Spectral and kinetics measurements

UV-1601 spectrophotometer was used in the kinetic and spectral measurements. CPS-240A was used to set the cell temperature at 10°C. Spectral scan of DPPH, DPPH₂, GA and Zn mixture were carried out from 280-800 nm. A calibration curve for DPPH was made from 1x10⁻⁵ to 30x10⁻⁵ M at 515 nm. Thus the concentration of DPPH, C_{DPPH} is Abs_{515} = 12443 x (C_{DPPH}) – 0.0746 determined by linear regression, where, ε = 12443 M⁻¹ cm⁻¹.

Disposable cuvettes were filled with 3 ml of DPPH solution and placed in the spectrophotometer for 10 minutes at 10°C. After that, 10 µl of 0.012 M GA was added to make up a GA:DPPH 1:5 ratio. The rate of reaction was followed at Abs_{515} for 1200s and a plateau has not reached. A mixture of GA and Zn was prepared according to the ratio 1:0.5, 1:1 and 1:2, and five repeats was carried out.

The rate constant for the reaction was calculated based on the first and second order rate law given by Atkins (2000). Microsoft Excel 2000 was used to calculate the rate constant.

Statistics

Results were presented as means of rate constants ± SE. Student’s t-test was used to compare differences between means of rate constants. Differences with p < 0.1 were regarded as statistically significant.

RESULTS

When different concentration of Zn was added to GA in methanol, there was only slight hypsochromic shift, approximately 2 nm for GA+1Zn. GA+2Zn displayed the greatest drop in absorbance from 290 to 320 nm, as compared to the rest of the GA and Zn mixture.

When GA and Zn are added to 2x10⁻⁴ M DPPH at 10°C, both Abs_{515} and Abs_{324} changes with time. However, Zn alone did not cause a change (Fig 1). The various time dependent spectra for all three reaction conditions do not intercept with the initial DPPH spectrum at a common isosbestic point. However, these spectra have common isosbestic points with other spectra, such that they can be approximately grouped into 4,6 and 5 sets with common isosbestic points. It is evident from the presence of different isosbestic points in the course of reaction that there are different absorbing species, inclusive of DPPH, present in the reaction mixture.

![Fig 1 Change in Abs_{515} with time for reaction D+GA (---), D+GA+1Zn (-----), D+GA+2Zn (▲ ▲) and D+Zn(••).](image-url)
From the plot of Abs 515 against time for the various reactions (Fig 1), the initial 100s is marked by a sharp dip in absorbance. Thus the first 100s, is probably a simple reaction between DPPH, GA and Zn. Complex reactions then take place between intermediates which led to changes in isosbestic points. Therefore, only the first 100s of reaction time is considered in the determination of initial rate. Based on Atkins P.W. (2000), the rate law for first and second order reaction is used to calculate the rate constant.

### Table 1 Integrated rate law for first and second order reaction

<table>
<thead>
<tr>
<th>Order</th>
<th>Reaction</th>
<th>Rate Law</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D → P</td>
<td>( k_t = \ln[D]_0/([D]_0 - x) )</td>
<td>([D]_0 - [D] )</td>
</tr>
<tr>
<td>2</td>
<td>a) D + GA → P</td>
<td>( k_t = \ln[GA]_0([D]_0-x)/([D]_0([GA]_0-x) )</td>
<td>([D]_0-([GA]_0) )</td>
</tr>
<tr>
<td></td>
<td>b) 2D + GA → P</td>
<td>( K_t = \ln[GA]_0([([D]_0-2x)/[D]_0([GA]_0-x) )</td>
<td>([D]_0-2([GA]_0) )</td>
</tr>
</tbody>
</table>


A plot of time, time, \( t/ s \) against the logarithm equation is used to find the rate constant, \( k \), where \( k \) is the gradient of the graph. There was poor linear correlation (r² < 0.9) for rate constants based on first order and second order 2a rate law. Moreover, based on the 2a rate law the graph was only linear for 30-40s, thus indicating a more complex rate law is needed. A good linear correlation (r² > 0.9) is obtained for the second order reaction 2b. Therefore, the rate-determining step for the first 100s is probably via equation 2b.

### Table 2 Summary of rate constant, \( k \) for different rate equation. Results are expressed as average rate constant ± standard error. * indicates that the data are significantly different from D+GA rate constant based on student’s T-test (P<0.1).

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Rate constant, ( k )</th>
<th>D+GA</th>
<th>D+GA+0.5Zn</th>
<th>D+GA+1Zn</th>
<th>D+GA+2Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-100s</td>
<td>( k/ x 10^{-3} s^{-1} )</td>
<td>4.4 ± 0.1</td>
<td>4.7 ± 0.4</td>
<td>5.6 ± 0.1*</td>
<td>3.8 ± 0.3*</td>
</tr>
<tr>
<td>2a) D + GA → P</td>
<td>0-40s</td>
<td>K/ M⁻¹ s⁻¹</td>
<td>310 ± 30</td>
<td>260 ± 20</td>
<td>170 ± 42</td>
</tr>
<tr>
<td>2b) 2D + GA → P</td>
<td>0-100s</td>
<td>K/ M⁻¹ s⁻¹</td>
<td>92 ± 6</td>
<td>120 ± 20</td>
<td>120 ± 12*</td>
</tr>
</tbody>
</table>

### DISCUSSION

Gallic acid is able to decrease the concentration of DPPH radical by 65% after 20 mins (Fig 1). The addition of Zn did not significantly increase the amount of DPPH radical scavenged. However, it affected the initial rate of reaction depending on the amount of Zn added (Table 2). When GA:Zn 1:1 was added to 2x10⁻⁴ M DPPH, the initial rate of reaction increases significantly (P< 0.05) by 26%, while GA:Zn 1:0.5 increased the rate by 24% (P<0.1). However, GA:Zn 1:2, decreased the rate constant by 18% (P<0.1). Recently, Hynes et al. (2000) has shown that the complex formed between GA-Al (III) at pH 3.5 is mainly due to the neutral form of GA coordination to hydrated Al (III), pKa of GA is 3.8. Therefore, it is highly likely that GA-Zn complexation could have occurred. Zn can form complex with coordination number 4,5 and 6 (Dunn 1995). Therefore Zn is able to complex with methanol, GA and DPPH intermediates via charge dipole interactions between O→Zn and N→Zn respectively. Zn is not redox active and acts as a Lewis acid by accepting electron from O-H of GA. This coordination of Zn with
GA polarizes the bound group. DPPH radical thus have a higher electron affinity for the electron rich GA-Zn complex. The coordination of Zn to GA also increased the acidity of the proton. Therefore, Zn-GA complexation increases both electron and proton transfer.

Proposed mechanism:

### CONCLUSION

Based on the effect of Zn on the initial rate of reaction and more complex time dependent absorption spectra of DPPH, it can be postulated that gallic acid probably undergo an electron transfer reaction followed by a proton transfer to DPPH radical. The initial rate of reaction when GA+0.5 Zn and GA+1Zn is added is not significantly different as only catalytic amount of Zn is needed. The order of reaction with respect to Zn (II) is thus zero. The addition of excess Zn decreased the rate by coordinating to DPPH· thus stabilising the molecule and decreasing rate of reaction. Further research would include elucidation of the structure for the various intermediates and side products generated in the reaction, as well as calculation of rate constants for reaction after 100s by advance computer programme such as Igor Pro Version 4.0.

### REFERENCE


Hynes, M.J (2001), The kinetics and mechanisms of the reaction of iron (III) with gallic acid, gallic acid methyl ester and catechin, *J. Inorg. Biochem.*, 85, 131-142


### ACKNOWLEDGEMENT

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