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Short communication

Stability of [6]-gingerol and [6]-shogaol in simulated gastric and intestinal fluids

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Abstract

The degradation kinetics of [6]-gingerol and [6]-shogaol were investigated in simulated gastric (pH 1) and intestinal (pH 7.4) fluids at 37 °C. Degradation products were quantitatively determined by HPLC (Lichrospher 60 RP select B column, 5 μm, 125 mm × 4 mm; mobile phase: methanol–water–acetic acid (60:39:1 v/v); flow rate: 0.6 ml/min; detection UV: 280 nm). In simulated gastric fluid (SGF) [6]-gingerol and [6]-shogaol underwent first-order reversible dehydration and hydration reactions to form [6]-shogaol and [6]-gingerol, respectively. The degradation was catalyzed by hydrogen ions and reached equilibrium at approximately 200 h. In simulated intestinal fluid (SIF) both [6]-gingerol and [6]-shogaol showed insignificant interconversion between one another.

Addition of amino acids glycine, 3-amino propionic acid (β-alanine) and γ-amino butyric acid (GABA), and ammonium acetate at a range of concentrations of 0.05–0.5 mM had no effect on the rate of degradation of [6]-shogaol in SGF and 0.1 M HCl solution. However, at exceedingly high concentration (0.5 M) of ammonium acetate and glycine, significant amounts of [6]-shogaol ammonia and glycine adducts were detected.

The degradation profile of [6]-gingerol and [6]-shogaol under simulated physiological conditions reported in this study will provide insight into the stability of these compounds when administered orally.

Keywords: [6]-Gingerol; [6]-Shogaol; SGF; SIF; Stability; Reversible degradation

1. Introduction

Ginger (*Zinger officinale*, Roscoe) has been used worldwide not only as food but also as medicine. It has long been used for the treatment of many gastrointestinal disorders and is often promoted as an effective anti-emetic herb. However, the active constituents and their mechanism of action remain to be determined. Gingerols, the pungent principles in the rhizome of ginger, have been reported to have anti-emetic, analgesic, antipyretic, anti-inflammatory, chemopreventive, and antioxidant properties [1–3]. Gingerols possess the labile β-hydroxy keto functional group, which makes them susceptible to transformation to less-pungent compounds such as shogaols and zingerone at elevated temperature [1,4]. Shogaols are not present in fresh ginger and are normally formed by the dehydration of gingerols during processing or storage [5,6].

The stability of [6]-gingerol and [6]-shogaol is of significance and relevance as these substances are generally considered to be the main active constituents in ginger medicinal products. Our previous study of stability of [6]-gingerol and [6]-shogaol in aqueous solutions showed that [6]-gingerol degraded to form [6]-shogaol and *vice versa* [7]. At 100 °C and pH 1, both compounds rapidly degraded and reached equilibrium within 2 h [7]. However, to enable development of medicinal products of reliable quality and efficacy the recognized instability of these constituents has not been adequately defined under ambient and biologically relevant conditions. There is no available data to envisage the degradation profile of gingerol *in vivo* when administered orally. In the gastrointestinal tract, gingerols are exposed, in addition to acid, to a digestive juice, containing components such as pepsin in stomach, and pancreatin in small intestine. Exposure of gingerols to these conditions could lead to considerable acid and/or enzyme-catalyzed decomposition. The unusual feature of interconversion between gingerols and shogaols and also the metabolically labile nature of [6]-shogaol forms the basis of the present study to determine the stability of [6]-gingerol and [6]-shogaol in simulated gastric and intestinal

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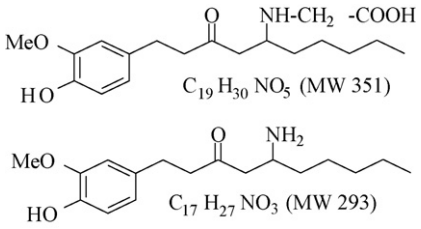

Fig. 1. The proposed chemical structures for addition products formed from the reaction of [6]-shogaol with glycine and ammonium acetate.

fluids at 37 °C. The effects of added pepsin in SGF and pancreatin in SIF on the degradation profile of the test compounds were also investigated. Reaction of metabolically labile [6]-shogaol with biologically relevant nucleophiles such as amino acids was investigated to identify if this was significant in terms of its stability or the formation of any adducts that, if formed, would need to be investigated for safety.

2. Materials and methods

2.1. Materials

[6]-Gingerol and [6]-shogaol were synthetically prepared in our laboratory according to published methods [8]. Methanol was HPLC grade. Pepsin, pancreatin, 3-aminopropionic acid (β-alanine, glycine), γ-aminobutyric acid (GABA) were purchased from Sigma–Aldrich (Australia). Ammonium acetate was AR grade and purchased from APS Chemicals (Australia). Other chemicals and solvents were of analytical grade. Simulated gastric (SGF) and intestinal fluids (SIF) were freshly prepared on the day of experiment according to the United States Pharmacopoeia specification [9].

2.2. Methods

2.2.1. High-performance liquid chromatography (HPLC) analysis

Chromatographic separation and quantitative analysis were performed on a Beckman System Gold high-performance liquid chromatography system as previously described [7].

2.2.2. Kinetic studies of [6]-gingerol and [6]-shogaol in simulated gastric and intestinal fluids

Degradation of [6]-gingerol and [6]-shogaol were investigated in simulated gastric and intestinal fluids, respectively in a similar manner to that previously described [7]. Briefly, to pre-incubated media of either simulated gastric or intestinal fluids (19 ml) containing DMSO (0.7 ml) was added a solution (0.3 ml) of [6]-gingerol or [6]-shogaol in DMSO to give a final concentration of the compounds of 15 μg/ml and DMSO of 5% (v/v). DMSO was required to solubilise the compounds under the experimental conditions. The resulting mixtures were incubated at 37 °C on a thermostat orbital mixer (Ratek Instruments, Australia). Samples (0.5 ml) were taken at specified time intervals and analyzed by HPLC. Standard curves were constructed over the range of 5–50 μg/ml for both [6]-gingerol and [6]-shogaol. Media consisting of 0.1 M HCl (pH 1) and phosphate buffer (pH 7.4) were used as controls.

2.2.3. Kinetic study of [6]-shogaol in media containing amino acids or ammonium acetate

Amino acids including glycine, 3-aminopropionic acid (β-alanine) and γ-aminobutyric acid (GABA), representing three different types α-, β-and γ-amino acids, which are building blocks of many proteins in our body, were chosen to examine the reactivity of shogaol to form a covalent bond with these amino acids under simulated physiological conditions as well as an artificial situation. The concentration of the amino acids and ammonium acetate chosen in this study was based on their average normal plasma concentrations. For example glycine concentration in normal healthy subjects is an average of 240 μM, whilst ammonia concentration is approximately 90 μM [10,11]. The amino acids and ammonium acetate were, respectively incubated with [6]-shogaol (54 μM) in a range of concentration from 50 to 500 μM at 37 °C in 0.1 M HCl and simulated gastric fluid, respectively. Samples (0.5 ml) were taken at time intervals and analyzed by HPLC as described above.

2.2.4. Data analysis

Kinetic data was fitted with a Boltzmann sigmoidal equation using GraphPad Prism 4 software. The correlation coefficient (r^2) was used as an indicator of goodness of fit of the equation to experimental data. Results were expressed as the mean ± S.D. ($n = 3$). Analysis of variance of two or more groups of data was performed using ANOVA, with probability values less than 0.05 considered as statistically significant.

3. Results

3.1. Degradation of [6]-gingerol in simulated gastric fluid and 0.1 M HCl solution

In SGF at 37 °C [6]-gingerol underwent reversible degradation to form its dehydrated product [6]-shogaol (Fig. 3a).

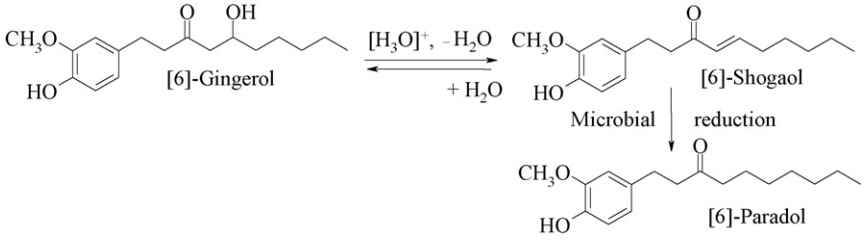

Fig. 2. Proposed mechanism of degradation of gingerol and shogaol in simulated gastric and intestinal fluid.

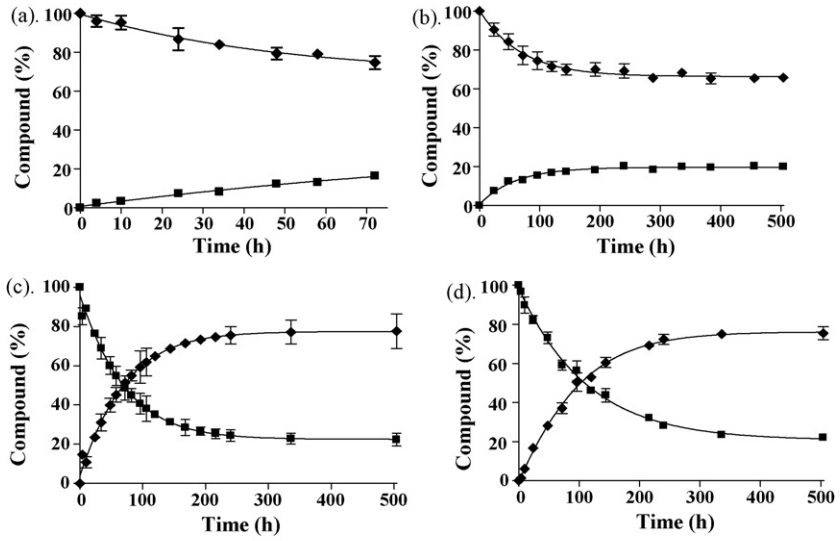

Fig. 3. Degradation kinetics of [6]-gingerol and [6]-shogaol in simulated gastric fluid (a and c) and 0.1 M HCl (b and d). The amount of [6]-gingerol (♦) and [6]-shogaol (■) at time (t) were determined by HPLC as described previously [7]. Data is presented as the mean ± S.D. ($n = 3$) of three independent determinations each performed in duplicate, and fitted with Boltzmann sigmoidal equation using GraphPad Prism 4 software. For all curves $r^2 \geq 0.98$ was observed.

This degradation was insignificantly (<2%) within a 1-h period. However, over a prolonged incubation the degradation of [6]-gingerol and the formation of [6]-shogaol reached equilibrium at approximately 200 h. This resulted in an equilibrium mixture of [6]-gingerol to [6]-shogaol with a ratio of 70:30 as shown in Fig. 3a. The kinetics for the degradation of gingerol is expressed as shown in the following equation:

$$A \underset{K_r}{\overset{K_f}{\rightleftharpoons}} B \tag{1}$$

$$\frac{-\mathrm{d}[A]}{\mathrm{d}t} = K_f[A] - K_r[B] \tag{2}$$

The net change in the concentration in the forward and reverse direction is given by Eq. (2), where K_f and K_r are the first-order rate constants for forward and reverse reactions, respectively. At time zero, when $A = A_0$ and $B = 0$; at equilibrium when $A = A_{eq}$ and $B = B_{eq}$, where A_0 and A are the concentrations of [6]-gingerol at time zero and time t, respectively, and A_{eq} and B_{eq} are the concentrations of [6]-gingerol and [6]-shogaol, respectively at equilibrium. The reversible first-order degradation of gingerol is, therefore, expressed according to Eq. (3) as shown below.

$$\mathrm{Log}\frac{A_0 - A_{eq}}{A - A_{eq}} = \frac{K_f + K_r}{2.303} \times t \tag{3}$$

$$K = \frac{B_{eq}}{A_{eq}} = \frac{K_f}{K_r} \tag{4}$$

At equilibrium, the rate constant (K) is given by Eq. (4), and there was approximately 70% of gingerol (A_{eq}) remaining and 30% of shogaol (B_{eq}) formed in solution (Fig. 3a). The equilibrium constant (K) was calculated according to Eq. (4) as 0.43 ± 0.01. Thus the rate constants for the forward (K_f) and reverse (K_r) reactions of [6]-gingerol were respectively calculated as $K_f = 0.0050 \pm 0.0005\ \mathrm{h}^{-1}$, $K_r = 0.012 \pm 0.002\ \mathrm{h}^{-1}$. Data derived from linear slope of Fig. 3a and expressed according to Eq. (3) revealed first-order reversible degradation of [6]-gingerol. There was no significant difference in the rate of degradation of [6]-gingerol in the presence (SGF) and absence of pepsin (0.1 M HCl) as shown in Fig. 3a and b and Table 1.

3.2. Degradation of [6]-shogaol in simulated gastric fluid and 0.1 M HCl solution

Under similar conditions to that of [6]-gingerol in SGF [6]-shogaol showed approximately 2% degradation within the first hour of incubation but, in contrast to [6]-gingerol, displayed significant degradation after 48 h with the formation of [6]-gingerol as its major degradation product. The degradation reached equilibrium at approximately 200 h, at which time approximately 80% of [6]-shogaol was converted to [6]-gingerol (Fig. 3c). Thus

Table 1
Rate of reaction for the forward (K_1) and reverse (K_2) reactions of [6]-gingerol and [6]-shogaol at 37 °C in 0.1 M HCl and simulated gastric fluid

Medium	[6]-Gingerol		[6]-Shogaol	
	K_1 (h^{-1})	K_2 (h^{-1})	K_1 (h^{-1})	K_2 (h^{-1})
SGF	0.0053 ± 0.0005	0.013 ± 0.002	0.012 ± 0.001	0.0033 ± 0.0006
0.1 M HCl	0.0042 ± 0.001	0.012 ± 0.002	0.011 ± 0.004	0.0027 ± 0.001

K_1 and K_2 were calculated as described in the data analysis section.

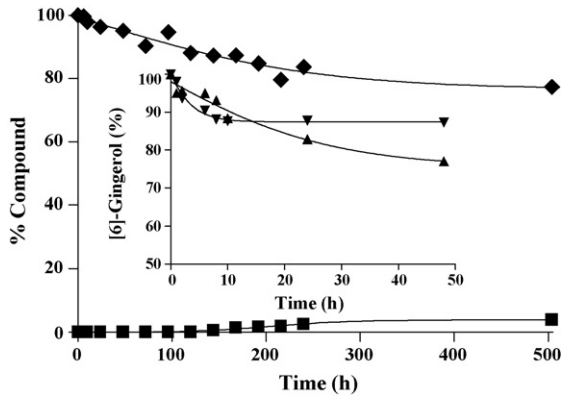

Fig. 4. Degradation kinetics of [6]-gingerol in simulated intestinal fluid (inset) and phosphate buffer. The amount of [6]-gingerol (♦) and [6]-shogaol (■) at time (t) were determined by HPLC as described previously [7]. Data is presented as the mean ± S.D. ($n = 3$) of three independent determinations each performed in duplicate. For all curves $r^2 \geq 0.98$ was observed. Inset graph: [6]-gingerol in SIF (▼) and phosphate buffer (▲) within 48 h.

the forward and reverse rate constants were calculated for [6]-shogaol as $K_f = 0.015 \pm 0.001\ h^{-1}$, $K_r = 0.0038 \pm 0.0006\ h^{-1}$, respectively. Similarly expression of the data according to Eq. (3) as described above revealed first-order reversible degradation of [6]-shogaol. There was no significant difference in the rate of degradation of [6]-shogaol in the presence and absence of pepsin as shown in Fig. 3c and d and Table 1.

3.3. Degradation of [6]-gingerol in simulated intestinal fluid and phosphate buffer

In SIF there was approximately 10% of gingerol degraded over 48 h incubation (Fig. 4, insert). Within the first hour of incubation, there was no change in gingerol concentration. Interestingly, there appeared to be little or no formation of [6]-shogaol under these conditions as a negligible amount of [6]-shogaol was detected over the 48-h period. Similar observation was made for [6]-gingerol in phosphate buffer (pH 7.4) over a 48-h incubation period with no significant amount of [6]-shogaol formed (Fig. 4). Notably after 48 h incubation of [6]-gingerol in SIF fluid, there appeared significant growth of microorganisms in the medium, thus extended incubation data of [6]-gingerol in SIF is not available. This contamination, however, did not occur in phosphate buffer even after a prolonged incubation time (21 days), where approximately 20% of gingerol degraded. This degradation of gingerol, however, did not result in formation of [6]-shogaol as shown in Fig. 4.

3.4. Degradation of [6]-shogaol in simulated intestinal fluid and phosphate buffer

In freshly prepared SIF it was observed that there was no change in [6]-shogaol within the first hour of incubation. However, [6]-shogaol was completely degraded within 24 h (Fig. 5a). The degraded product was later verified by liquid chromatography–mass spectrometry (LC–MS) and nuclear magnetic resonance (^{1}H NMR) (data not shown) as 1-(4-hydroxy-3-methoxyphenyl)decan-3-one (i.e. [6]-paradol). The identity of [6]-paradol was confirmed by comparison with an authentic standard. Notably, there was no [6]-gingerol formed under these conditions. Interestingly, this transformation was not observed in phosphate buffer in the absence of added pancreatin as shown in Fig. 5b. Extended incubation of [6]-shogaol in phosphate buffer to 500 h (i.e. 21 days) resulted in approximately 40% of [6]-shogaol being degraded, while only 20% was accounted for as [6]-gingerol that had formed, and neither reaction reached equilibrium after 21 days of incubation (Fig. 5b).

3.5. Kinetic study of [6]-shogaol in aqueous solution in presence of amino acids

[6]-Shogaol contains a conjugated ketone group which may potentially react with nucleophilic groups in protein such as amino acids to form adducts as demonstrated by addition reactions of [6]-shogaol with water [7], methanol or ethanol [12]. This study was designed to investigate the reactivity of [6]-shogaol toward different types of amino acids, as described above, as well as ammonium acetate in 0.1 M HCl (pH 1), phosphate buffer (pH 7.4) and simulated gastric fluid (pH 1), respectively at 37 °C. Under these conditions [6]-shogaol showed no reaction towards the amino acids or ammonium acetate (0.05–0.5 mM). There was no degraded product other than its hydrated derivative [6]-gingerol. Equilibrium was reached at about 96 h at which time 80% of [6]-gingerol was formed whilst 20% of [6]-shogaol remained in the solution

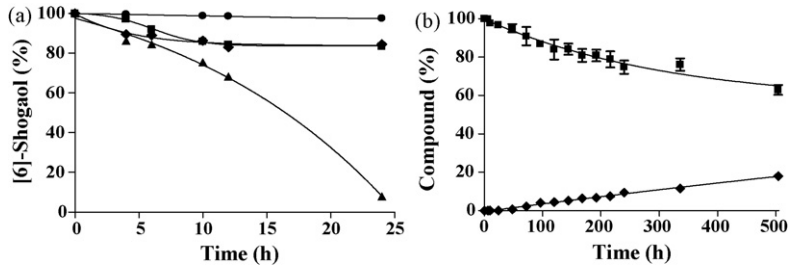

Fig. 5. Degradation kinetics of [6]-shogaol in simulated intestinal fluid (a) and phosphate buffer (b) at 37 °C. (a) [6]-Shogaol in SIF in the presence (●) and absence (▲) of penicillin–streptomycin; and in the presence of sodium azide (♦), and in phosphate buffer (pH 7.4) (■). (b) [6]-Shogaol (■) and [6]-gingerol (♦) in phosphate buffer. The amount of [6]-shogaol at time (t) were determined by HPLC. Data is presented as the average of at least two independent determinations each performed in duplicate. For all curves $r^2 \geq 0.96$ was observed.

(data not shown). However, at exceedingly high concentrations (0.5 M) of ammonium acetate or glycine, [6]-shogaol was found to react with ammonium ion and glycine respectively in 0.1 M HCl (pH 1) solution to form the corresponding addition products as shown in Fig. 1. Molecular mass of the proposed adduct products were determined by LC–MS analysis, which showed an $[M+1]^+$ ion at *m*/*z* 352 for the molecule of molecular weight 351 formed by the addition of glycine molecule across the double bond of [6]-shogaol (Fig. 1). Similarly an $[M+1]^+$ ion at *m*/*z* 294 was observed for the molecule of molecular weight 293 formed by the addition of ammonia across the double bond of [6]-shogaol (Fig. 1).

4. Discussion

The present study determined the degradation kinetics of [6]-gingerol and [6]-shogaol *in vitro* using simulated gastric and intestinal conditions and provided insight into the degradation profile for these compounds under similar conditions *in vivo*. [6]-Gingerol and [6]-shogaol undergo reversible dehydration and hydration reactions respectively under acidic conditions similar to that in the stomach, to form an equilibrium mixture of [6]-gingerol and [6]-shogaol. This degradation mechanism appeared to be catalyzed by acid as the presence of the digestive enzyme pepsin had neither effect on rate nor the overall degradation reaction for both compounds (Fig. 3). [6]-Gingerol was shown to have greater stability than [6]-shogaol in acidic conditions even after 21 days of incubation approximately 30% of gingerol had degraded compared with 80% of [6]-shogaol decomposed. Prolonged incubation of the compounds was carried out to determine their degradation kinetics. However, both [6]-gingerol and [6]-shogaol remained unchanged within the first hour incubation period.

At pH 7.4 these compounds may ionize to some extent through the phenolic hydroxyl group ($pK_a \sim 10$), however, no major degradation products were identified under these conditions. Notably, this degradation occurred at an insignificant rate compared with that observed under acidic conditions. The unexpected conversion of [6]-shogaol to [6]-paradol can be explained by microbial reductive enzymes, a situation similar to that occurring in the gastrointestinal (GI) system where a rich source of bacteria and microorganisms resides. Therefore one would expect that [6]-shogaol would readily reduce to form the saturated ketone [6]-paradol in the GI tract when administered orally. A schematic degradation of [6]-gingerol is thus proposed to depict its fate in the gastrointestinal system when administered orally (Fig. 2). In consideration of possible therapeutic consequences it is noted that [6]-paradol has been shown to retain some of the pharmacological properties of the parent unsaturated compound [6]-shogaol [13].

It is known that biotransformation of drugs in biological systems consist of reactions such as, oxidation, reduction, hydrolysis and/or conjugation of a drug or its metabolites leading to activation or inactivation of drug or forming metabolites with different pharmacological activity to that of the parent drug. The transformation of drugs may also occur through chemical reactions as with compounds with keto groups which are known to be susceptible to nucleophilic attack. Thus, the α,β-unsaturated ketone function of shogaol present in this study is a good candidate to examine this type of reaction. In this study and our previous report [7], shogaol has been shown to react with water to form gingerol, and an earlier report showed that shogaol reacted with ethanol and methanol to form ethylated or methylated gingerol [12]. In light of nucleophilic additions to shogaol, adduct formation with ammonia and amino acids was investigated and found to occur only when high concentrations of ammonium acetate or glycine were present. Therefore under physiological condition it is unlikely that shogaol will react with biological nucleophiles such as amino acids.

5. Conclusions

This study has established the degradation profile for [6]-gingerol and [6]-shogaol in a simulated physiological situation. Under the conditions described both compounds were relatively stable within a 1-h period. However, both [6]-gingerol and [6]-shogaol displayed first-order degradation kinetics to dehydrate to form [6]-shogaol and hydrate to form [6]-gingerol, respectively as major degradation products after a prolonged incubation in acidic environments.

Overall, this study further revealed the fate and mechanism of degradation of [6]-gingerol and [6]-shogaol under simulated physiological conditions, which in turn will provide insight into stability of these compounds when administered orally. Definition of their degradation profiles will enable the development of more effective oral dose pharmaceutical formulations for these compounds.

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