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Drug solubilization effect of lauroyl-L-glutamate

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This article proposes a new technique for the solubilization of poorly soluble drugs using lauroyl-L-glutamate, which is one of the amino acid detergents, with additional small additives. Lauroyl-L-glutamate was highly effective in solubilizing long-chain alkyl gallates, *e.g.* dodecyl gallate. Furthermore, lauroyl-L-glutamate and small additives, particularly arginine, acted to increase the solubility of alkyl gallates. The synergistic effect was not observed by sodium dodecyl sulphate with arginine. The solubilizing system can be applied to other drugs because of the low toxicity of both lauroyl-L-glutamate and arginine.

Keywords: alkyl gallates/arginine/detergent/lauroyl-L-glutamate/solubility.

Abbreviations: Arg, arginine hydrochloride; CMC, critical micelle concentration; Gdn, guanidine hydrochloride; Gly, glycine; Lys, lysine hydrochloride; NaCl, sodium chloride; SDS, sodium dodecyl sulphate.

Poor aqueous solubility of small molecules hampers their screening and development as pharmaceutical drugs (*1*, *2*). Although various detergents and organic solvents have been successfully used to enhance their aqueous solubilities (*3*–*16*), these compounds are toxic to cells and tissues to a varying degree or are insufficiently effective; thus, limiting their applications in both *in vitro* and *in vivo* experiments.

Thus, we have directed our focus on *N*-acyl amino acid detergent that has been used in various applications due to their detergent properties and weak toxicities (*17*–*27*). For example, *N*-acyl amino acid detergents find applications in food, cosmetic and healthcare products as emulsifying and anti-microbial agents. Among them, lauroyl-L-glutamate has recently been used to solubilize membrane proteins and proteins trapped in inclusion bodies (*28*, *29*). In these studies, it was apparent that the binding of this detergent to proteins was reversible; therefore, lauroyl-L-glutamate may be a biocompatible solubilizing agent for drug substances such as small molecules and proteins. However, its effect on the solubility of small molecules has never been reported.

We have recently shown that arginine significantly increases the solubility of organic compounds, especially aromatic compounds, which have low aqueous solubilities (*30*–*34*). This ability of arginine to enhance the solubility of organic small molecules is useful for drug screening and development.

In this study, we have first examined the effect of lauroyl-L-glutamate on the solubility of alkyl gallates as model drug substances. In addition, we investigated the solubilization effect of lauroyl-L-glutamate on alkyl gallates in the presence of arginine or other additives.

Materials and Methods

Chemicals

All alkyl gallates and sodium dodecyl sulphate (SDS) were obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Arginine hydrochloride (Arg) and lauroyl-L-glutamate were provided by Ajinomoto Co., Inc. (Tokyo, Japan). Lysine hydrochloride (Lys), guanidine hydrochloride (Gdn), glycine (Gly) and sodium chloride (NaCl) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All compounds used were of the highest commercially available grade.

Solubility measurement

The solubility of all alkyl gallates at pH 7.0 in the absence and presence of lauroyl-L-glutamate and solvent additives were measured as follows. Aqueous stock solutions of the additives were prepared by mixing water and additives. Weight concentrations were converted to molar concentrations from the densities of the solutions prepared. Appropriate amounts of alkyl gallate powders were transferred into test tubes, to which 0.5 ml of water or the solution containing the additive or lauroyl-L-glutamate or both were added. The suspension was heated at 40°C for 1 h with frequent vortexing to achieve complete dissolution of alkyl gallate powders. The solution was then incubated at 25°C for 3 days with frequent vortexing. Subsequently, the suspension was centrifuged at 25°C and 16,000 *g* for 20 min to obtain a supernatant saturated with alkyl gallates. After appropriate dilution of the supernatant with water, its absorbance was measured spectrophotometrically at 271 nm using a UV-VIS spectrophotometer (ND-1000; NanoDrop Technologies Inc., Wilmington, DE, USA). The absorbance value was converted to concentration using a standard curve determined for each alkyl gallates. Solubility was determined in triplicate, from which the averages and standard errors were obtained.

Calculation of transfer free energy and synergistic effect

The transfer free energy (ΔG_{tr}) of the alkyl gallates from water to the additive solutions was calculated according to the following equations:

$$\Delta G_{\mathrm{tr}} = \mu_a^0 - \mu_w^0 = -RT\ln(x_a/x_w) \tag{1}$$

$$\begin{cases} \mu_w = \mu_w^0 + RT\ln x_w \\ \mu_a = \mu_a^0 + RT\ln x_a \end{cases} \tag{2}$$

$$\begin{cases} x_w = n_{g,w}/(n_{g,w} + n_{\mathrm{H_2O},w}) \\ x_a = n_{g,a}/(n_{g,a} + n_{\mathrm{H_2O},a} + 2n_{a,a}) \end{cases}. \tag{3}$$

In these equations, μ_a and μ_w are the chemical potentials of the alkyl gallates in the presence and absence of the solvent additive or lauroyl-L-glutamate or both, respectively, and μ_a^0 and μ_w^0 are the corresponding standard chemical potentials. The transfer free energy of the alkyl gallates from water to the additive solution can be calculated from the solubility of the alkyl gallates in the respective solutions x_a and x_w and are expressed as the mole fraction solubility of the alkyl gallates in the presence and absence of the additive or lauroyl-L-glutamate or both. The mole fraction concentration is calculated using $n_{i,a}$ and $n_{i,w}$, which correspond to the molarity of the component i at saturation in the presence and absence of the additive or the detergent or both. Subscript g, H_2O and a are used to express the molarities of the alkyl gallates, water and the additive at saturation of the alkyl gallates, respectively. For example, μ_a indicates the chemical potential of an alkyl gallates in an aqueous solution containing an additive or lauroyl-L-glutamate or both, and $n_{g,a}$ is the molarity of the alkyl gallates in the same solution. The activity coefficient was considered to be close to unity because of the poor solubility of the alkyl gallates. R and T correspond to the gas constant and absolute temperature, respectively.

The excess transfer free energy (ε) of alkyl gallates from water to the detergent solution containing the additives was calculated according to the following equation:

$$\varepsilon = \Delta G_{\mathrm{tr,mixture}} - (\Delta G_{\mathrm{tr,detergent}} + \Delta G_{\mathrm{tr,co-solvent}}). \quad (4)$$

Thus, ε showed the difference in the free energy between the overall interaction in the presence of both the additive and detergent, and their individual interaction. In Eq. 4, $\Delta G_{\mathrm{tr,mixture}}$, $\Delta G_{\mathrm{tr,detergent}}$ and $\Delta G_{\mathrm{tr,co\text{-}solvent}}$ are the transfer free energies of the alkyl gallates from the water to the detergent solution containing the additive and the detergent solution plus the additive solution.

Results

Solubility of alkyl gallates in detergent solution

The potential of lauroyl-L-glutamate as a drug solubilizing agent was examined as a function of concentration for several alkyl gallates with different alkyl chain lengths, such as methyl ($C=1$), ethyl ($C=2$), propyl ($C=3$), butyl ($C=4$), octyl ($C=8$) and dodecyl ($C=12$) gallates. Fig. 1 shows the solubility of alkyl gallates as a function of lauroyl-L-glutamate concentration. In the absence of detergent, the solubility of alkyl gallates in water decreased in the order of ethyl, methyl, propyl, butyl, octyl and dodecyl indicating that increasing alkyl chain length resulted in greater hydrophobicity resulting in lower aqueous solubility. However, the solubility of ethyl gallate was greater than that of methyl gallate. We have attributed this result to interference of molecular interaction by ethyl group (*32*). For all the alkyl gallates examined, solubility increased with increasing lauroyl-L-glutamate concentration; however, such concentration dependence for dodecyl gallate was not clearly observed in this study.

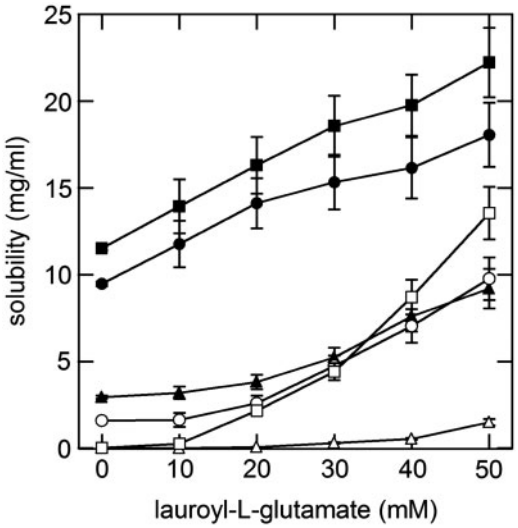

Fig. 1 Solubility of alkyl gallates in the presence of 0–50 mM lauroyl-L-glutamate as a function of concentration. Closed circles, methyl gallate; closed squares, ethyl gallate; closed triangles, propyl gallate; open circles, butyl gallate; open squares, octyl gallate; open triangles, dodecyl gallate.

At certain lauroyl-L-glutamate concentration, the concentration dependence curves cross each other, leading to alterations in the solubility order. For example, the solubility increased in the order of propyl > butyl > octyl below 20 mM, but in the reverse order above 40 mM. This result may be related to micelle formation, which occurs at ~10.6 mM for this detergent (*35*). The linear increase observed for short-chain alkyl gallates suggested that both monomeric and micellar forms of lauroyl-L-glutamate can interact with these short-chain alkyl gallates and exert a similar solubilization effect. On the other hand, the non-linear increase observed for long-chain alkyl gallates suggested that micellar forms of lauroyl-L-glutamate interact more strongly with long-chain alkyl gallates than lauroyl-L-glutamate monomers.

Figure 2A plots the solubility ratio in 10 and 50 mM lauroyl-L-glutamate. The increase in solubility was modest at 10 mM for short alkyl chains (methy, ethyl, propyl and butyl gallates) only by ~1.0- to 1.3-fold. At 50 mM (i.e. above critical micelle concentration (CMC)), lauroyl-L-glutamate was more effective for long alkyl chains in the order of methyl < ethyl < propyl < butyl, perhaps due to stronger interactions between alkyl chain and micellar forms of lauroyl-L-glutamate. Although solubilization effects were much stronger for octyl and dodecyl gallates both at 10 and 50 mM (see Fig. 2A, inset), the trend was similar i.e. lauroyl-L-glutamate was less effective for dodecyl gallate than for octyl gallate at 10 mM and more effective in this order at 50 mM. It should be noted that there was a large difference in solubilization effect of lauroyl-L-glutamate between butyl and octyl gallates at both 10 and 50 mM. The effects were only ~1.0-fold increase at 10 mM and ~6.5-fold at 50 mM in the solubility of butyl gallate, but there was ~15-fold at 10 mM and ~750-fold at 50 mM in the solubility of octyl gallate. A critical alkyl chain length may be present (i.e. between carbon number 4 for butyl and carbon number 8 for octyl), above which aliphatic interactions between the alkyl chain and lauroyl-L-glutamate become qualitatively different. The solubility of dodecyl gallate increased by 2,500-fold on the addition of 50 mM lauroyl-L-glutamate, demonstrating the strong potential of micellar forms of lauroyl-L-glutamate in solubilizing poorly water-soluble organic compunds.

The transfer free energy reflects the interactions of alkyl gallates with the monomeric lauroyl-L-glutamate at 10 mM and with the micellar forms above this concentration. As shown in Fig. 2B, the values of the transfer free energy were all negative, indicating that

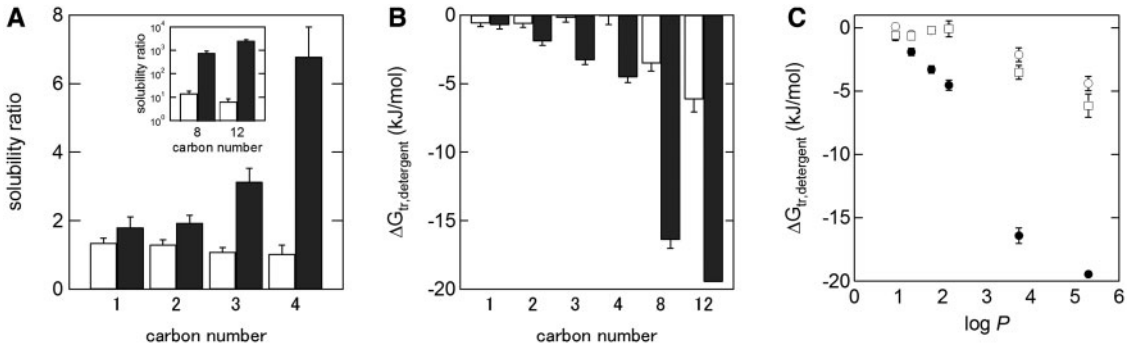

Fig. 2 Solubility of the alkyl gallates in the presence of lauroyl-L-glutamate. (A) The solubility ratio of the alkyl gallates in the presence of 10 mM (white bar) and 50 mM (black bar) lauroyl-L-glutamate to that in its absence as a function of alkyl chain length (expressed as carbon number). (B) Transfer free energy of the alkyl gallates from water to 10 mM (white bar) and 50 mM (black bar) lauroyl-L-glutamate as a function of carbon number in the alkyl chain. (C) Transfer free energy of the alkyl gallates from water to 5, 10 and 50 mM lauroyl-L-glutamate as a function of log *P* of the alkyl gallates. Open circles, 5 mM lauroyl-L-glutamate; open squares, 10 mM lauroyl-L-glutamate; closed circles, 50 mM lauroyl-L-glutamate. The values of *P* are taken from the Ref. (*36*).

alkyl gallates were stabilized by the addition of 10 and 50 mM lauroyl-L-glutamate. Each transfer free energy for lauroyl-L-glutamate was greater at 50 mM than at 10 mM. The values of the transfer free energy for octyl and dodecyl gallates were much greater than that of short-chain alkyl gallates. Figure 2C plots the transfer free energy against the hydrophobic parameter log *P* for each alkyl gallates. The values of transfer free energy from water at 5 and 10 mM lauroyl-L-glutamate were independent of the hydrophobic parameters of methyl to butyl gallates. Note that these concentrations of lauroyl-L-glutamate are less than CMC. Thus, the result shown in Fig. 2C indicates that the hydrophobic interaction between a monomeric lauroyl-L-glutamate and an alkyl group of gallates is not adequate for the stabilization of short-chain alkyl gallates. On the other hand, the transfer free energy for octyl and dodecyl gallates decreased with the hydrophobic parameter, indicating that these alkyl gallates favourably interact with lauroyl-L-glutamate propably. In contrast to the results obtained with 10 mM lauroyl-L-glutamate, the transfer free energy from water to 50 mM lauroyl-L-glutamate showed a linear relationship with the hydrophobic parameter.

As shown in Fig. 3, arginine increased the solubility of all alkyl gallates in a concentration-dependent manner, except for dodecyl gallate, in the presence of 50 mM lauroyl-L-glutamate. While arginine almost linearly increased the solubility of methyl, ethyl and propyl gallates with increasing concentration, the effect of arginine appeared to level off for higher concentrations of both butyl and dodecyl gallates. In particular, the increasing effect for dodecyl gallate leveled off at 0.4 M. These results suggest that arginine interacts directly even in the presence of lauroyl-L-glutamate with short-chain alkyl gallates, whereas arginine interacts with long-chain alkyl gallates by co-operating with lauroyl-L-glutamate. Direct arginine interaction should lead to a monotonic increase in solubility (*32*, *33*). On the other hand, the cooperative effect of arginine with lauroyl-L-glutamate may become saturated at a certain arginine concentration. For example, it is possible that 0.4 M arginine is

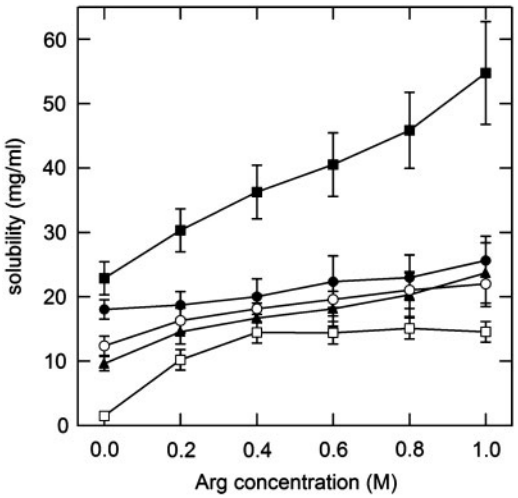

Fig. 3 The solubility of alkyl gallates in the presence of 50 mM lauroyl-L-glutamate containing 0-1 M arginine. Closed circles, methyl gallate; closed squares, ethyl gallate; closed triangles, propyl gallate; open circles, butyl gallate; open squares, dodecyl gallate.

sufficient to alter the micellar forms of lauroyl-L-glutamate, and thereby to increase the dodecyl gallate solubility to the maximum. We have previously shown that arginine is moderately effective in increasing the solubility of various aromatic compounds, such as alkyl gallates studied here (*32*, *33*). Thus, its effect on the alkyl gallates solubility was examined in the presence of 50 mM lauroyl-L-glutamate. It is noted that the measurement could not be made for the octyl gallate because the addition of 1 M arginine to the octyl gallate solution in the presence of 50 mM lauroyl-L-glutamate resulted in phase separation of the solution. Although we could not pinpoint the reason for this observation of phase separation, it is possible that the structure of the octyl gallate/lauroyl-L-glutamate complex is altered by arginine.

Figure 4A compares the solubilizing effects of various additives at 1 M in the presence of 50 mM lauroyl-L-glutamate. Among the additives tested, 1 M arginine most significantly increased the solubility of all alkyl

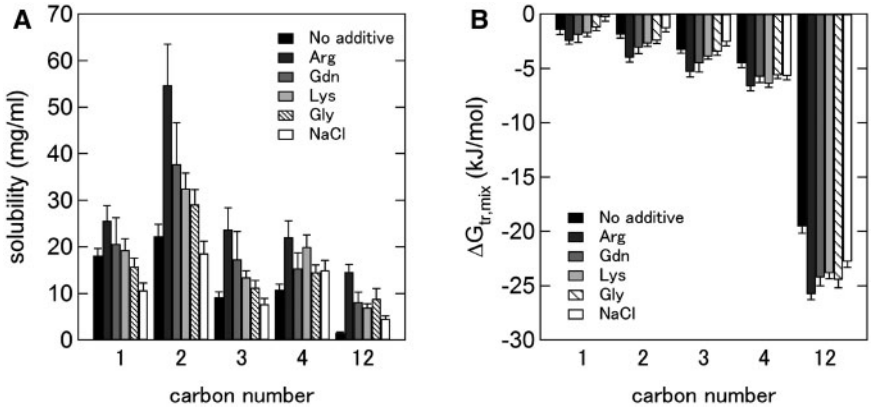

Fig. 4 Solubility of the alkyl gallates in the presence of lauroyl-L-glutamate and various additives. (A) The solubility of alkyl gallates in the presence of 50 mM lauroyl-L-glutamate containing various additives at 1 M as a function of carbon number in the alkyl chain. (B) Transfer free energy of alkyl gallates from water to 50 mM lauroyl-L-glutamate containing various additives at 1 M as a function of alkyl chain length (expressed as carbon number).

gallates. The effect of 1 M arginine on the solubility of dodecyl gallate was particularly strong with an increase from ~1 mg/ml in 50 mM detergent alone to over ~14 mg/ml in the presence of both the detergent and arginine. The pattern of effects of 1 M additives was similar for methyl, ethyl and propyl gallates with the order of arginine > Gdn > lysine > no additive > NaCl. This order was identical to their effects on gallate solubility in the absence of lauroyl-L-glutamate (*33*). Thus, it is possible that these additives and lauroyl-L-glutamate are independent of each other in affecting the solubility of these three alkyl gallates.

Pattern of the additive effects shown in Fig. 4 was significantly different for butyl and dodecyl gallates. All additives, including 1 M NaCl, increased the solubility of these two alkyl gallates in the presence of 50 mM lauroyl-L-glutamate. Nevertheless, arginine was still the most effective, increasing the dodecyl gallate solubility by ~10-fold, which was above its solubility in 50 mM lauroyl-L-glutamate alone. Other four additives were less effective in solubilizing both butyl and dodecyl gallates. The transfer free energy of the alkyl gallates from water to the additive solution containing 50 mM lauroyl-L-glutamate was calculated from the solubility data (Fig. 4B). In all cases, the values were negative, indicating that none of the additives co-operating with lauroyl-L-glutamate destabilized the alkyl gallates. One molar arginine had the strongest stabilizing effects for any alkyl gallates. As expected from the effects of additives on the solubility of methyl, ethyl and propyl gallates in 50 mM lauroyl-L-glutamate, the values of the transfer free energy were reduced in the order of arginine > Gdn > lysine > Gly > no additive > NaCl. The destabilizing effect of NaCl on the three alkyl gallates in 50 mM lauroyl-L-glutamate suggests that NaCl interferes with the favourable interaction between these alkyl gallates and lauroyl-L-glutamate. It is interesting that the profile for butyl and dodecyl gallates was qualitatively different from that for short-chain alkyl gallates. Especially, butyl and dodecyl gallates were stabilized even by NaCl.

From these data, it is unclear whether such enhanced stability of alkyl gallates with additives in 50 mM lauroyl-L-glutamate is due to their direct interaction with alkyl gallates. The indirect effect can be calculated as the difference between the value of the transfer free energy for the mixed solution, containing both additive and lauroyl-L-glutamate, and the total value of the energy for each additive solution as shown in Eq. 4. Such excess transfer free energy (ε) is plotted for the above additives in Fig. 5. This parameter (ε) is an indication of excess transfer free energy and can be positive or negative. When ε is negative, the stabilization of the alkyl gallates by the mixture is greater than the sum of each stabilization by the additive and lauroyl-L-glutamate. When ε is positive, the mixture reduced the stabilizing effect of the alkyl gallate and lauroyl-L-glutamate. The excess transfer free energy is slightly positive or negative for these additives against methyl, ethyl and propyl gallates within experimental errors. Figure 5 clearly shows that there is no synergistic effect of the additive and lauroyl-L-glutamate on the solubilization of these short-chain alkyl gallates. Against butyl gallate, the excess transfer free energy was significantly negative for NaCl. Interestingly, all these additives acted synergistically with lauroyl-L-glutamate in solubilizing dodecyl gallate. Among them, 1 M arginine showed the greatest synergistic effect with lauroyl-L-glutamate in solubilizing this alkyl gallate. An intriguing question raised is why arginine and lauroyl-L-glutamate showed no synergy against short-chain alkyl gallates, whereas the strongest synergistic effect against dodecyl gallate.

Next, we compared the solubilization effect of lauroyl-L-glutamate with that of a typical detergent, SDS (Fig. 6). The effect of lauroyl-L-glutamate was greater than or equal to that of SDS for methyl, ethyl, propyl and butyl gallates. However, it was significantly less effective for the dodecyl gallate. Structural differences between SDS and lauroyl-L-glutamate are attributed by the charged head group that most likely contributes to their micellar property. This suggests that micellar forms of lauroyl-L-glutamate interact more favourably with short-chain alkyl gallates than SDS, while micellar forms SDS interact more favourably with dodecyl gallate than lauroyl-L-glutamate.

When 1 M arginine was added to these detergent systems, the solubility of all the alkyl gallates was more effectively increased in the presence of lauroyl-L-glutamate than in the presence of SDS. Since 1 M arginine is a common component in these two systems, the observed difference between lauroyl-L-glutamate and SDS in the solubilization effect is due to a different synergy between these two detergents. Such synergy can be expressed as the excess transfer free energy (ε) as described above. The excess transfer free energy was positive for SDS (Fig. 6B), indicating that the presence of arginine together with SDS resulted in the reduction of their respective solubilization effects. The differential effect between SDS and lauroyl-L-glutamate was most evident for the solubilization of dodecyl gallate. Thus, SDS had no synergistic effect on the solubilization in comparison to lauroyl-L-glutamate.

Discussion

Lauroyl-L-glutamate, which is one of *N*-acyl amino acid detergents, may be biocompatible solubilizing agent for drug substances due to its biocompatibility (*28*, *29*). However, the solubilizing effect of lauroyl-L-glutamate on small molecules has never been reported. Thus, this is the first report of the effect of lauroyl-L-glutamate on poorly water-soluble molecules, alkyl gallates, as model drug substances. For all the alkyl gallates examined, the solubilities increased with increasing lauroyl-L-glutamate concentration (Fig. 1). Comparison of the transfer free energy from water to 10 or 50 mM lauroyl-L-glutamate showed that the alkyl gallates with a longer alkyl chain were more effectively stabilized by the micellar forms of the lauroyl-L-glutamate (Fig. 2C).

It has previously been argued that arginine effectively increased the solubility of aromatic compounds and the mechanism has been interpreted in terms of specific interactions between arginine and aromatic groups (*30–34*). Despite such studies, synergistic effect of arginine and detergents on solubilizing drugs has not been discussed. Actually, the addition of arginine concentration dependently increased the solubility of short-chain alkyl gallates in the presence of lauroyl-L-glutamate though the effect of arginine appeared to level off for long-chain alkyl gallates (Fig. 3), which result from the nature of the interaction between arginine and alkyl gallates as described in previous articles (*32*). The effects of arginine and the other additives on short-chain alkyl gallates (methyl, ethyl and propyl gallates) were obtained in the order of arginine > Gdn > lysine > Gly > no additive > NaCl (Fig. 4), which is identical to that obtained in the absence of lauroyl-L-glutamate (*33*). Namely, these additives affected the solubility of short-chain alkyl gallates independently of lauroyl-L-glutamate. On the other hand, all of these additives increased the solubility of long-chain alkyl gallates (butyl and dodecyl gallates), which was different from their effects in the absence of the detergent. This result suggests a synergy between the additive and lauroyl-L-glutamate.

Here, we defined this synergistic effect as the excess transfer free energy (ε). This parameter clearly demonstrates an insignificant synergy between additives and 50 mM lauroyl-L-glutamate on short-chain alkyl

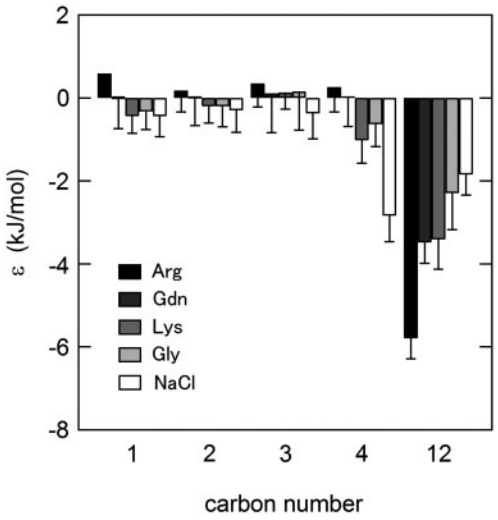

Fig. 5 Excess transfer free energy of alkyl gallate from water to 50 mM lauroyl-L-glutamate containing various additives at 1 M as a function of carbon number in the alkyl chain.

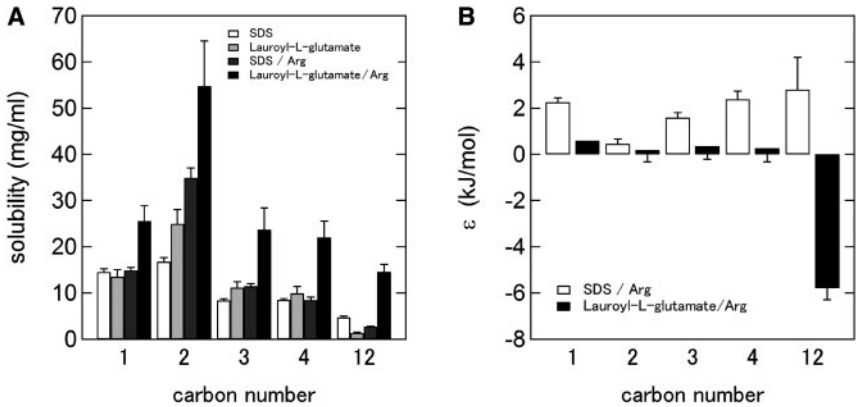

Fig. 6 Solubility of the alkyl gallates in the presence of detergents and Arg. (A) The solubility of the alkyl gallates in 50 mM detergent in the absence and presence of 1 M Arg as a function of alkyl chain length (expressed as carbon number). White bar, 50 mM SDS; light grey bar, 50 mM lauroyl-L-glutamate; dark grey bar, 50 mM SDS/1 M Arg; black bar, 50 mM lauroyl-L-glutamate/1 M Arg. (B) Excess transfer free energy of the alkyl gallates from water to 50 mM detergent containing 1 M Arg as a function of carbon number in the alkyl chain. White bar, 50 mM SDS/1 M Arg; black bar, 50 mM lauroyl-L-glutamate/1 M Arg.

gallates, and a significant synergy on long-chain alkyl gallates (Fig. 5). Among the additives tested, 1 M arginine showed the greatest synergy (∼−5 kJ/mol) on dodecyl gallates. It remains unclear how synergy occurred only for long-chain alkyl gallates. Nevertheless, the comparison with SDS may shed light on the mechanism of the synergistic effect between arginine and lauroyl-L-glutamate (Fig. 6). Fifty millimolar lauroyl-L-glutamate was more effective than 50 mM SDS against short-chain alkyl gallates, which may reflect a different micellar form between these two detergents. Importantly, 1 M arginine was more effective in augmenting the solubilization effect of lauroyl-L-glutamate than that of SDS. Lauroyl-L-glutamate and SDS has the same alkyl group as hydrophobic tail, but has a different head group. These data imply that the head groups interact differently with arginine, which in turn may alter the cooperative effect between the detergent and arginine.

Arginine, which has no chaotropic properties, preferentially interacts with proteins without any adverse effects on them (*37*, *38*). Furthermore, an *N*-acyl amino acid detergent such as lauroyl-L-glutamate is also known to have high biocompatibility and low toxicity (*24*). Therefore, the strong synergistic effect of arginine with lauroyl-L-glutamate would be advantageous for solubilization of substances having poor aqueous solubility.

Conflict of Interest
None declared.

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