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Creaming in Black Tea

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Tea cream is the precipitate formed as tea cools. Its formation has been studied by X-ray scattering, and it is shown that a higher tea concentration leads to earlier onset of creaming and larger particles and that addition of theaflavin and calcium promotes creaming. Association constants between the major components of black tea have been obtained using NMR and show that calcium and glucose enhance the self-association of caffeine, polyphenols, and theaflavin but have little effect on hetero-association. Glycosylation of a polyphenol reduced self-association and reduced binding to caffeine. We conclude that theaflavin is important for the initiation of creaming, forming nanoclusters of typically 3 nm diameter, whereas caffeine acts more to fill in the gaps within the clusters and thus adds to the bulk of tea cream without being necessary for its initiation. Tea creaming may be reduced by increasing the solubility of the polyphenols (i.e., by glycosylation) or by removing calcium.

Tea cream; theaflavin; caffeine; small-angle X-ray scattering; NMR; colloid

INTRODUCTION

Hot black tea infusions produce cloudy coacervates on cooling, which are known as tea cream, and contain up to 30% of the total solids. Tea cream contains many of the compounds that provide taste and color in black tea, and its formation therefore gives rise to a loss of both taste and color, as well as a cloudy appearance to the tea. It is thus deleterious both for the consumer and for the producer, especially for producers of ready-to-drink teas, who need to prepare tea infusions at high solid concentrations and for whom cloudy solutions are undesirable. By removing polyphenols, creaming is also likely to reduce many of the health-giving properties of tea, which are generally held to lie in the polyphenols present (*1*).

The major components of tea are theaflavin **1** (TF, which here is taken to include mono- and digallates of TF), caffeine (**2**), and polyphenols (**Table 1**and **Figure 1**) (*2*−*5*). These latter range in size from gallic acid (**3**) and simple gallate esters (**4**: epigallocatechin gallate, a major constituent of black tea) to much larger products of the oxidative fermentation process that makes black tea, which in particular includes a poorly characterized set of molecules known collectively as thearubigins, which are thought to be catechins that have been polymerized, oxidized, and cross-linked to other cellular components such as protein and saccharides (*6*). Black tea also contains polysaccharides, which are of interest because they have been shown to inhibit the precipitation of polyphenol−protein mixtures (*7*), and calcium. Ca^{2+} is of particular interest since the concentration of this mineral is relatively high in a tea infusion (0.045% w/w or approximately 11 mM) and it partitions strongly into the tea cream (D. J. Scollard and D. P. Jones, personal communication). Processed teas typically contain even higher calcium concentrations than the tea leaf because calcium is added during the processing to remove oxalic acid as its insoluble calcium salt. Adding a chelating agent, such as ethylendiaminetetraacetic acid (EDTA), has been shown to decrease the extent of cream formation by up to 50% (*8*). Calcium is thus likely to play a role in tea cream formation. Finally, approximately 10% of the tea solids is nitrogenous, of which approximately half is free amino acids and the other half belongs to an ill-defined group that has been proposed to consist of proteins and peptides conjugated to thearubigins. Tea cream is generally of a similar

Table 1. Composition of Black Tea (% w/w)

constituent	Balentine (*41*)	Sanderson et al. (*42*)	Balentine et al. (*43*)
Catechins			
simple catechins	4	11	3–10
thearubigins	17	36	23
Theaflavins	2	3	ND
Caffeine	7	ND	3–6
Protein	11	6	6
Free amino acids	5	7	6
Carbohydrate	14	4	11
Organic acids	11	2	2
Ash	ND	10	10–13

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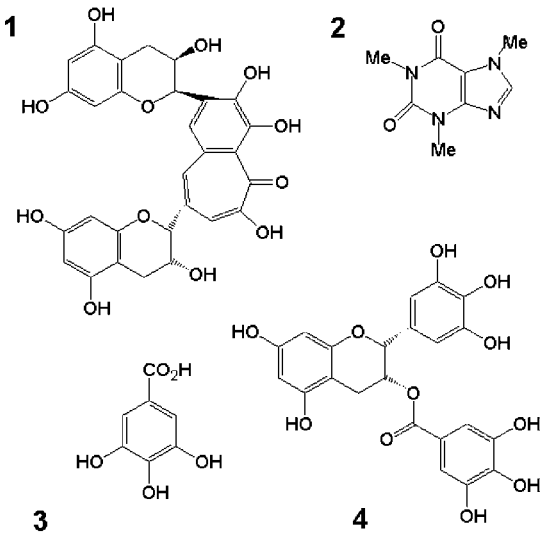

Figure 1. Structures of components of tea. **1**: Theaflavin. **2**: Caffeine. **3**: Gallic acid. **4**: (–)-Epigallocatechin gallate.

composition to tea, containing mainly thearubigins, theaflavins, and caffeine (*4*, *9*−*15*) but contains higher Ca^{2+} and TF than tea and lower concentrations of simple catechins (*4*, *16*).

The mechanism of formation of tea cream varies, depending particularly strongly on the tea solid concentration (*17*) but has for a long time been held to be led by polyphenols (*18*), which are of low solubility and therefore come out of solution on cooling, in complex with TF and other tea constituents (*12*, *19*), essentially accomplishing phase separation into two immiscible phases (*20*). Caffeine is involved but is not critical since, for example, decaffeinated tea is still capable of forming creams (*17*). Here, we have measured a range of binding affinities and cream properties, which we have used to investigate the formation of tea cream in molecular detail.

MATERIALS AND METHODS

TF was purified from tea by Unilever Tea Science, Colworth, UK and had a purity of >90% as determined by NMR. All other chemicals (except PG Tips granules) were purchased from Sigma.

Small-angle X-ray scattering was performed using commercially available freeze-dried tea granules (PG Tips) dissolved at 70 °C. The experiments were performed at the SRS Daresbury, Warrington on beamlines 2.1 and 16.1 at camera lengths between 1.5 and 3.25 m, and the measurements were static. The temperature was lowered in 5 °C steps, and the solution was equilibrated 5 min at each temperature prior to measurement, each measurement taking 5 min (5 × 60 s frames). Sector integration between 60 and 120° of the scattering pattern was performed using the program *bsl*, the program *otoko* was used for the correction of experimental artifacts, and the processed scattering data were fitted using the program *GNOM4.5a* (*21*−*23*).

Self-association was measured using NMR in H_2O/^{d}DMSO 9:1, pH 4.0 on a Bruker DRX-500 spectrometer at 300 K. Starting with a 100 mM stock solution, defined volumes of the solution were replaced by the solvent until an end concentration of 0.1 mM was reached. Quercitin and quercitrin had low solubilities, and stock solutions of 0.5 and 10 mM were used, respectively. Chemical shift changes were fitted to the isodesmic model by a least-squares method (*24*−*26*). All available ^{1}H signals were used, fitted independently, and averaged to obtain the best estimate. The protons used were therefore the following: caffeine, methyls 1, 3, 7, and H8 (in the presence of glucose, H8 only as the methyl proton signals are obscured by glucose signals); gallic acid, aromatic signal; methyl gallate, methyl and aromatic (in glucose, aromatic only); theaflavin, protons H3, H2′, H6′, H6, H8′, H8, Hc, He, and Hg using the nomenclature of ref *27* (in glucose, Hc, He, and Hg only); and quercitin and quercitrin, aromatic protons. Hetero-association experiments were conducted as titrations, whereby solutions of phenolic compounds in 1 mM caffeine were titrated into a 1 mM solution of caffeine, and the chemical shifts of caffeine protons were followed. At this concentration, caffeine is >99% monomeric, which allows simplified equations to be used (*26*). For the caffeine−TF titration, caffeine was titrated into a 1 mM TF solution, and fitting was to a different equation because TF binds in a two-step process (*27*). Chemical shifts of theaflavin protons were used. Because glucose signals overlap with TF signals, the association could not be measured in the presence of glucose.

When investigating the self-association of caffeine in the presence of calcium, various equilibria may occur simultaneously, which in theory all need to be considered to investigate the self-association process. If the association constant of caffeine binding to calcium is much weaker than the self-association of caffeine, then the presence of calcium will not affect measured association constants. On the other hand, if the association constant of caffeine binding to calcium is much greater than the self-association of caffeine, it can be assumed that caffeine binds to calcium before binding to a like molecule. In this case, the measured apparent self-association is not a true self-association but an association of caffeine with itself, bound to one or more calcium ions. This poses the question as to whether the model for self-association can be applied in this case, where self-association and association with calcium take place simultaneously. However, the complexity of the system is too great to allow a complete analysis of the equilibria occurring, particularly since there is no simple way of differentiating between calcium-bound and -free species because the chemical shifts of the relevant molecules do not change on addition of calcium. Similar arguments apply to binding in the presence of glucose. It is therefore assumed here, provided that the chemical shift titrations can be fitted to the simple association equations, that it is not unreasonable to treat the system as a simple association.

RESULTS

Small-Angle X-ray Scattering. A commercial freeze-dried tea powder was dissolved in water at 70 °C, and small-angle X-ray scattering (SAXS) measurements were made as the temperature was reduced stepwise from 70 to 25 °C. Solutions prepared using greater than 2% tea solids were very turbid, which gave problems in fitting (*28*). Consequently, the concentrations 1 and 2% were identified as the most suitable tea concentrations for SAXS measurements. Typical drinking tea infusions are in the range 0.5−1%. The SAXS curves were normalized and analyzed to generate pair distance distribution functions, which were used to obtain estimates of the maximum particle dimension D_{max} and the radius of gyration R_g for the tea cream particles (*21*, *22*). Values for R_g were also calculated using the Guinier approximation (*29*) and gave slightly higher values but the same trends. **Figure 2** shows pair distance distribution functions for a 1% tea solution at three different temperatures, showing the increase in both D_{max} and R_g as the temperature is lowered. Both D_{max} and R_g are larger at higher tea solid concentrations (data not shown). The pair distance distribution functions also show that the typical cream particle initially formed is a few nanometers in diameter. When D_{max} and R_g are plotted against temperature (**Figure 3**), they demonstrate a marked step change during the cooling process, reflecting a phase change in the tea infusate (*20*), the temperature of which is identified as the creaming temperature. Measurements were repeated in the presence of caffeine, TF, and calcium, and the creaming temperature was determined for each solution. The results are shown in **Table 2** and indicate that the creaming temperature is higher at higher tea solid concentrations, that addition of caffeine reduces the creaming temperature, but that addition of calcium or TF results in an increase in the creaming temperature.

To ensure that the rate of cooling of the tea infusate was not influencing the measurements, the R_g and D_{max} values of tea

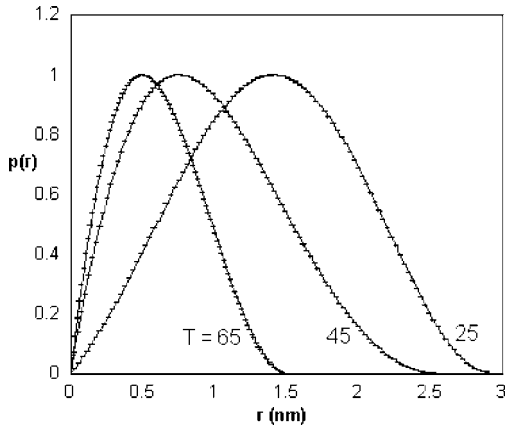

Figure 2. Normalized pair distance distribution functions $p(r)$ as a function of distance r for a tea solution 1 wt % at three different temperatures (25, 45, and 65 °C). The maximum particle dimension D_{max} can be determined where the curve returns to the x-axis. An increase in D_{max} of the tea cream particles with decreasing temperature can be seen.

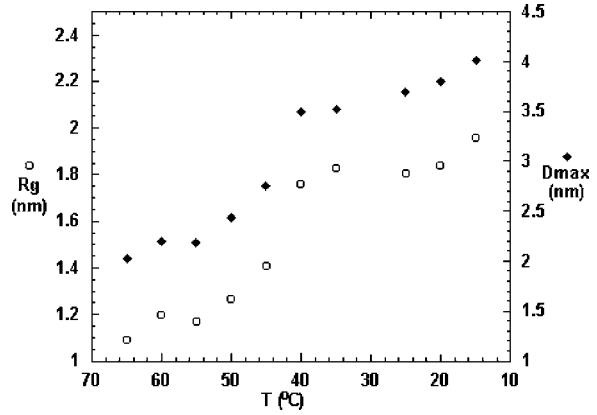

Figure 3. Radius of gyration R_g and maximum particle dimension D_{max} for a 2% tea infusion as a function of temperature. The increase of the particle size with decreasing temperature is illustrated, and the creaming temperature can be identified by the discontinuity in the curves, which in this figure occurs between 45 and 40 °C. The error in the fitted values is of the order of 1–3% for both R_g and D_{max}, although these are probably an underestimate of the true errors.

Table 2. Creaming Temperatures of Tea Solutions

sample	creaming temperature (°C)
1% solids	35–40
2% solids	40–45
2% solids + caffeine	35–40
2% solids + calcium	45–50
2% solids + TF	45–50

particles of a 2% tea solution were measured at a constant temperature (40 °C) for 1 h. The measurement shows that the particle size remained stable after an initial 5 min of temperature equilibrium, implying that the equilibration time was adequate to ensure a stable measurement.

Self-Association of Tea Cream Components. The association of tea components can be either self-association (one molecule to another molecule of the same type) or hetero-association. Both are important, not least because the extent of hetero-association can only be calculated once the extent of self-association has been determined. The self-association of different black tea components was studied using NMR, by measuring chemical shift changes on dilution, which were fitted to standard

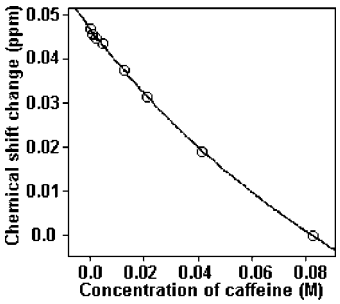

Figure 4. Chemical shift change of the methyl 7 signal of caffeine on dilution of caffeine from 80 to 0.1 mM. The curve is the best-fitted curve assuming formation of infinite stacks on the isodesmic model (see Materials and Methods).

Table 3. Self-Association Constants for Black Tea Components[a]

	K_M (M^{-1})	K_M (M^{-1}) + 11 mM Ca^{2+}	K_M (M^{-1}) + 1 mM glucose
caffeine	6 ± 1	4 ± 1	40 ± 1.5
gallic acid	2200 ± 400	7000 ± 2200	2000 ± 200
methyl gallate	2 ± 0.5	11 ± 2	113 ± 8
TF	304 ± 68	3100 ± 1100	2100 ± 300
quercitin	370 ± 140	ND[b]	ND
quercitrin	16 ± 3	ND	ND

[a] Self-association constants were measured in H_2O/[d]DMSO 9:1 v/v, pH 4.0 ± 0.5, 300 ± 1 K. [b] ND: not determined.

equations (*23*−*25*). All measurements were carried out in H_2O/[d]DMSO (dimethyl sulfoxide) 9:1 v/v because of the limited solubility of some of the components measured in pure water and to maintain comparability with earlier studies. On the basis of our earlier studies (*24*, *30*), we expect association in water to be stronger than in 10% DMSO. Measurements were made at pH 4.0 ± 0.5 (approximately the pH of black tea) and at 300 ± 1 K. A typical chemical shift dilution curve is shown in **Figure 4**, and the results are shown in **Table 3**, which also shows results obtained in the presence of 11 mM Ca^{2+} or 1 mM glucose. The self-association constants for caffeine and TF are in good agreement with previously obtained values (*26*, *27*). Gallic acid and TF self-associate much more strongly than does caffeine, and quercitin self-associates much more strongly than does its glycosylated equivalent, quercitrin. The addition of calcium or glucose generally strongly promotes self-association. Calcium presumably enhances self-association by bridging polar groups. However, the reason for the effect of glucose is less obvious.

Hetero-Association of Tea Cream Components. Hetero-association between caffeine and other components was measured by NMR. The association with methyl gallate and gallic acid is straightforward to model since these compounds (and caffeine) are planar and form stacks, and hetero-association occurs via addition of a caffeine molecule either to the end of a gallate stack or intercalated inside a stack. There are, therefore, two different association constants. Our previous results (*26*) have shown that binding in the middle of the stack is approximately twice as strong as binding at the end, presumably because similar binding interactions can occur on both sides of the planar caffeine when it inserts into the middle of a stack of gallate. The fitted association constants for gallic acid and methyl gallate are shown in **Table 4**, which also show this trend. However, we note that binding of caffeine to quercitin and quercitrin does not follow the normal trend. This is most likely because these compounds are not planar and therefore do not fit well to the isodesmic model. The fitted association constants

Table 4. Hetero-Association Constants for Caffeine Binding to Gallic Acid, Methyl Gallate, Quercitin, and Quercitrin[a]

	K_e (M^{-1})	K_s (M^{-1})
caffeine–gallic acid	13 ± 0.4	33 ± 6
caffeine–methyl gallate	14 ± 0.5	30 ± 0.7
caffeine–quercitin	775 ± 40	390 ± 20
caffeine–quercitrin	820 ± 40	590 ± 30
+Ca^{2+} (11 mM)		
caffeine–gallic acid	8 ± 0.4	23 ± 6
caffeine–methyl gallate +glucose (1 mM)	14 ± 1	53 ± 15
caffeine–gallic acid	9 ± 2	18 ± 6
caffeine–methyl gallate	16 ± 3	27 ± 8

[a] K_e is the association constant for binding at the end of a stack, and K_s is the association constant for binding in the middle of a stack.

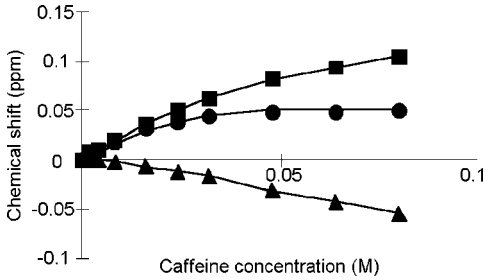

Figure 5. Least-squares fits of the chemical shift data of TF upon titration with caffeine. The association is assumed to occur in two steps; therefore, two association constants, K_A and K_B, are fitted to the experimental data. (●) Experimental data. (■) Contribution of first binding (K_A). (▲) Contribution of second binding (K_B).

are therefore less reliable than the other data presented in **Table 4**. Although the binding of caffeine to polyphenols is generally weak, the binding constants suggest that it will intercalate into polyphenolic stacks, particularly in the case of methyl gallate, forming intermolecular complexes. In solutions containing both caffeine and methyl gallate, both molecules will be more associated than in isolated solutions of either species. In agreement with our earlier results, binding of caffeine to complex polyphenols (e.g., quercitin) is much stronger than to simple ones (e.g., methyl gallate) (**Table 4**): in particular, binding to galloyl esters seems to be preferred (*24*, *30*). Calcium and glucose have little effect on hetero-association.

Binding of caffeine to TF is more complicated. TF is almost entirely dimeric in solution, but it dimerizes via the 7/6 fused ring system, leaving the two catechin moieties from each molecule free to bind independently. The heteromeric binding therefore behaves like monomeric TF binding to caffeine, with the two TF monomers binding independently of each other. Binding of caffeine to the two catechin moieties on each TF goes by a two-step mechanism, with a second caffeine molecule binding only when the first is bound (*27*). This can be seen from the unusual chemical shift titration data (**Figure 5**), which cannot be fitted to a standard saturation curve. Fitting to the appropriate equation produces two association constants, one for each step (**Table 5**). The binding is weak and is not influenced by calcium. Although the binding of caffeine to most tea components measured is weak, it should be remembered that the concentration of caffeine in tea is high (approximately 4 mM in our solutions, roughly 10 times greater than that of TF on a molar basis), and therefore, it is involved in widespread binding.

Table 5. Hetero-Association Constants for Caffeine Binding to TF[a]

	K_A (M^{-1})	K_B (M^{-1})
caffeine–TF	14 ± 1	20 ± 2
+Ca^{2+} (11 mM)		
caffeine–TF	11 ± 2	20 ± 7

[a] K_A and K_B are the association constants for the first and second binding steps, respectively.

DISCUSSION

Black tea contains a very large number of compounds, whose concentrations vary markedly from one tea to another, and many of which are of low solubility and tend to bind together. It is therefore highly likely that there is no single pathway for formation of tea cream (*12*). Our studies have concentrated on concentrations of 1−2% tea solids, which is approximately the concentration of a strong drinking tea brew.

The SAXS results show that that increasing the tea solid concentration leads to an increase in creaming temperature and particle size, as expected (*31*). Increases both in TF and in calcium lead to a higher creaming temperature (**Table 2**). The importance of TF has long been recognized (*32*). The effect of calcium can be attributed to charge compensation. TF has acidic properties and is likely to carry a negative charge at the pH of a standard tea solution (*33*, *34*). This leads to electrostatic repulsion between the charged surfaces of the cream particles, hence stabilizing the tea colloid (*19*, *35*). The positively charged divalent Ca^{2+} ion has the ability to compensate these negative charges, hence promoting aggregation and precipitation. Other multiply charged metal ions, in particular, magnesium and aluminum, which are also present at high concentrations in tea, are likely to have similar effects, although of these ions, calcium partitions most strongly into tea cream.

The particle sizes seen by SAXS are a few nanometers in radius. By contrast, the particles identified using light scattering techniques are typically 10−40 times larger, approaching 100 nm in radius (*19*). Light scattering and X-ray scattering are optimal for different size ranges, and it is therefore likely that both size ranges are present in tea infusions and that the smaller particles seen by SAXS aggregate to form the larger particles seen by light scattering. The aggregation is likely to involve the thearubigin component, which has been identified as a major constituent in mature tea cream particles (*36*). This conclusion again serves to emphasize that the results reported here pertain to the initial formation of tea cream rather than maturation of the cream particle, which may involve different processes.

Caffeine produces a reduction in the creaming temperature and binds only weakly to many cream components. This supports the suggestions made by others that caffeine does not play an essential part in, and is not required for the initiation of, the tea cream process. It does, however, clearly bind to the forming cream particle, and in particular to galloyl groups (*37*), does contribute strongly to the mass and radius of the resultant particle (*19*, *38*, *39*), and does form a major fraction of tea cream (*12*, *37*). Our results therefore support earlier conclusions that caffeine stimulates tea cream formation only in the presence of sufficient substances with galloyl groups (*32*, *37*), presumably by increasing the bulk and decreasing the solubility of cream (*17*), in effect filling in vacant binding sites. This implies that degallation of tea polyphenols is likely to lead to reduced cream formation, although it is also likely to lead to a reduction in favorable characteristics of the tea such as color and astringency.

A comparison of quercitin and quercitrin is interesting. The main effects of glycosylation are to increase the solubility of

the polyphenol (by approximately a factor of 20) and also to weaken its self-association by about the same amount. This latter is presumably a consequence of the bulk of the sugar, which prevents proper stacking of one molecule against another. Both of these are expected to result in reduced cream formation. This result would imply that one possible way of reducing tea cream formation during tea processing is to partially glycosylate the polyphenols prior to concentration, for example, by adding saccharides to the tea leaf before fermentation, as reported in ref *40*.

In conclusion, we have shown that the main driver of tea cream formation is the insolubility of theaflavin and polyphenols, which associate together via their galloyl groups (*15*). Caffeine binds to tea cream particles and increases their mass and density. Calcium increases cream formation by neutralizing the charges that stabilize small colloidal particles. Tea creaming may therefore be reduced by increasing the solubility of the polyphenols (i.e., by glycosylation or possibly by degallation) or by removing calcium. An understanding of the mechanism, especially the initiation and driving forces of tea creaming, may lead to the development of a production procedure, where creaming can be controlled.

ABBREVIATIONS USED

DMSO, dimethyl sulfoxide; SAXS, small-angle X-ray scattering; TF, theaflavin.

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