What is the molten globule?

Sir–"The chaperonin GroEL does not recognize apo-α-lactalbumin in the molten globule state" is the title of an article published lately in this journal by Kuwajima and co-workers[1]. By contrast, our contemporary paper on the same subject, using the same two proteins, reaches the seemingly opposite conclusion "that the chaperonin interacts with the hydrophobic surfaces exposed by proteins in a flexible compact intermediate or molten globule state"[2]. How can such a discrepancy be explained? Thankfully, it does not arise from any substantial difference in the experimental observations: the two studies concur in their finding that GroEL does not bind apo-α-lactalbumin (apo–αLA) with the four native disulphide bonds, but will bind it when the disulphide bonds have been reduced. They appear to differ in their conclusions because different molten globule states are being described.

The term 'molten globule' has been applied to all compact denatured states that have substantial secondary structure but little or no fixed tertiary structure, which covers a wide range of more or less disordered, partially folded proteins, with and without disulphide bonds[3–5]. In common with its fully unfolded state, and in contrast to its comparatively rigid and closely constrained native state, the molten globule is not a single conformation but is very flexible and comprises a large number of mobile, rapidly interconverting conformations, which are also in relatively rapid equilibrium with more unfolded states.If such a conformationally mobile protein binds to a molecule like GroEL, it could be any or all of the molten globule, and more unfolded, conformations that are binding; this is a fundamental difficulty with such studies.

In its native state with four disulphide bonds, αLA binds a single calcium ion. In the absence of Ca^{2+}, apo-αLA exists in an equilibrium between native and molten globule conformations that depends critically on the salts present. Both studies[1,2] found that this species does not bind tightly to GroEL, which indicates that this molten globule state is not recognized. But, this molten globule state is tightly cross-linked by the four native disulphide bonds, and we attribute the poor binding to GroEL to these constraints[2]. The native disulphide bonds are not intrinsic to the molten globule state of αLA, as shown by the many more, stable disulphide isomers that arise spontaneously when disulphide interchange is permitted and that retain the molten globule conformation[6]. The constraints of the native disulphide bonds tend to stabilize preferentially molten globule conformations that are native-like[6].

The fully reduced form of αLA can also exist in the molten globule state, as was recognized previously by Kuwajima and co-workers[7,8]: "under an appropriate condition [at acid pH], it can assume secondary structure similar to that found in the molten globule state of the disulfide intact protein"[7]. Under the more physiological conditions used in the binding studies, reduced αLA exists in an equilibrium between molten globule and unfolded conformations[2,6], and the data presented by Okazaki *et al.*[1] confirm this. Consequently, the observation that GroEL binds reduced αLA tightly would be consistent with binding involving either the unfolded or the molten globule conformations.

The problem of conformational flexibility on binding was one reason why we used other forms of αLA, particularly the two- and three-disulphide forms in which the disulphides were permitted to rearrange under physiological or unfolding conditions. The sub-populations of disulphide isomers that bound to GroEL could be characterized[2]. Those isomers that bound tightest were found to be compact and to have exposed hydrophobic surfaces, and were deduced to be in a molten globule state. Significantly, protection from hydrogen exchange has been demonstrated in the bound species, suggesting the persistence of secondary structure elements[9].

All the available data, including that of Okazaki *et al.*[1], indicate that GroEL binds preferentially the molten globule state of αLA so long as it is not tightly cross-linked by the four native disulphide bonds.

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In reply —

Sir–In their correspondence to *Nature Structural Biology*, Creighton *et al.* discuss a discrepancy between two articles, one by them[1] and the other by us[2], on recognition of the molten globule by the chaperonin GroEL. As they mention, however, both experimental observations described in the two articles are not inconsistent with each other. Both studies concur in their finding that GroEL does not bind apo-α-lactalbumin (apo-αLA) in the molten globule state when the four native disulfide bonds are intact but does bind α-lactalbumin in the presence of KCl when the disulphide bonds have been reduced. The discrepancy between the conclusions is ascribable to different meanings of the term 'molten globule' used in the two articles.