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Minireview

Equine lysozyme: The molecular basis of folding, self-assembly and innate amyloid toxicity

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Abstract **Calcium-binding equine lysozyme (EL) combines the structural and folding properties of c-type lysozymes and α-lactalbumins, connecting these two most studied subfamilies. The structural insight into its native and partially folded states is particularly illuminating in revealing the general principles of protein folding, amyloid formation and its inhibition. Among lysozymes EL forms one of the most stable molten globules and shows the most uncooperative refolding kinetics. Its partially-folded states serve as precursors for calcium-dependent self-assembly into ring-shaped and linear amyloids. The innate amyloid cytotoxicity of the ubiquitous lysozyme highlights the universality of this phenomenon and necessitates stringent measures for its prevention.**

Keywords: Lysozyme; Molten globule; Calcium-binding; Amyloid; Oligomers; Cytotoxicity

1. Introduction

EL occupies a special position within the extended family of structurally homologous proteins – lysozymes and α-lactalbumins. These are among the most studied objects providing a wealth of information on the mechanisms of structural organization, folding and amyloidogenicity of polypeptides. EL is viewed as an evolutionary link between them, as it possesses the structural and folding features of both subfamilies [1,2]. Specifically, it contains the active-site residues Glu 35 and Asp 52, involved in lysozyme enzymatic (EL) activity [3,4], and the conserved, high-affinity calcium-binding site of α-lactalbumins [5] (Fig. 1). Consequently, EL acts as a bacteriolytic enzyme similar to lysozymes, ubiquitous components in the many body fluids and tissues of all mammalians, while α-lactalbumins take part in lactose biosynthesis in the mammary glands [5]. Due to EL's extremely low stability and cooperativity compared to non-calcium-binding c-type lysozymes, it forms equilibrium partially folded states similar to α-lactalbumins [6–8]. However, like c-type lysozymes it populates well-defined transient kinetic intermediates [9], which enables us to compare the equilibrium and kinetic species of the same protein. EL can also self-assemble into amyloids with a very distinctive ring-shaped and linear morphology [10,11]. They differ from the fibrils of both lysozymes involved in systemic amyloidoses [12,13] and α-lactalbumins, forming amyloids in vitro [14]. Here we review the EL folding, misfolding and amyloid properties in view of their general implications for understanding the underlying mechanisms and causes of these processes.

2. Calcium-binding property

Lysozymes/α-lactalbumins consist of two sub-domains (Fig. 1): the α-domain is rich in α-helical structure, while the β-domain contains a triple-stranded β-sheet and several loops. They possess also a conserved pattern of four disulfides. The EL calcium-binding property was initially predicted [15], since in the domain interface it has the same calcium-binding ligands as α-lactalbumins (Fig. 1). Then the binding constant of $2 \times 10^6\ M^{-1}$ was determined experimentally [1] and the structure of the binding-site confirmed by crystallographic and NMR structural analysis [2–4]. The presence of the calcium-binding site most likely contributes to EL's significantly lower stability and cooperativity compared to c-type lysozymes [6–9]. If hen and human lysozymes undergo highly cooperative two-state thermal denaturation above 70 °C, and even the amyloidogenic variants of human lysozyme are destabilized by only ca. 12 °C [16,17], then, by contrast, apo-EL displays a three-state transition in a wide range between room temperature and 80 °C (Fig. 2a) [18,19]. The first denaturation transition is calcium-dependent and leads to molten globule formation; upon calcium-binding it is shifted to significantly higher temperatures. EL's unfolding behaviour, however, cannot be described by two-state model even at very high calcium content. Similarly, the three-state unfolding of EL was observed upon chemical denaturation [7]. The thermodynamic behaviour of EL within the first thermal unfolding transition was described by a four-state scheme [18], where N and I correspond to apo-native and apo-intermediate states, and NM and IM – to holo-forms, respectively.

$$\begin{array}{ccc} & \alpha(T) & \\ N & \leftrightarrow & I \\ + & & + \\ M & & M \\ K_n(T) \updownarrow & & \updownarrow K_u(T) \\ NM & \leftrightarrow & IM \\ & \beta(T) & \end{array} \qquad (1)$$

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Abbreviations: EL, equine lysozyme

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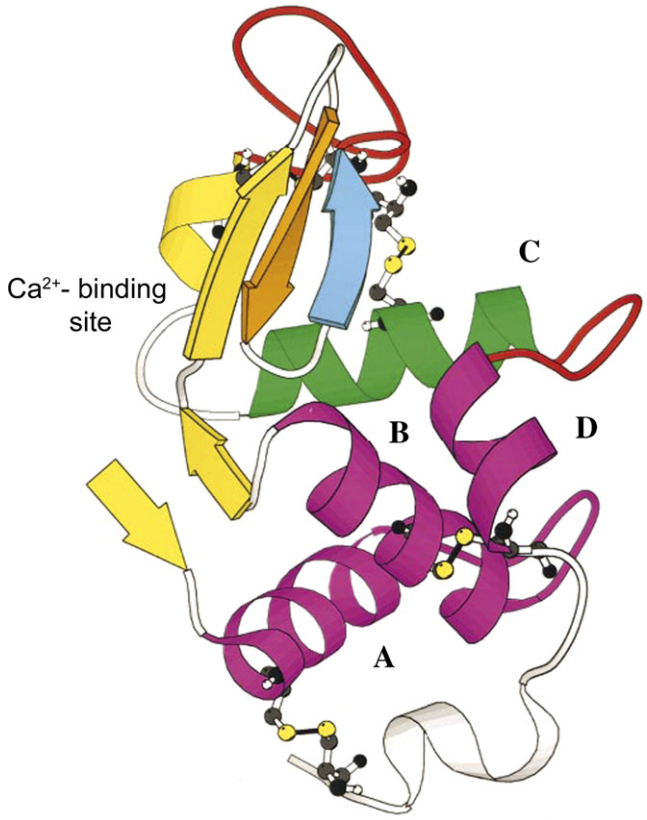

Fig. 1. Schematic diagram of EL demonstrating the folding areas with local cooperatively [9]. They are colour-coded as follows: violet, corresponding to the rapidly folded A, B and D α-helical core, blue – the 59–61 residue β-strand stabilized with 50–100 ms time constants, green-the C-helix, 120–150 ms time constants, yellow – the elements of secondary structures attaining persistent structure with 200–250 ms time constants, orange – the middle 51–55 residue β-strand, ca. 400 ms time constants, and red, denoting the most slowly stabilized loops. The disulphide bridges are shown in ball-and-stick.

Determining all thermodynamic parameters of the scheme, the "phase-diagram" was build, visualizing the population of EL conformations in the coordinates of free-calcium concentration and temperature. The "phase diagrams" of EL and α-lactalbumin both share a similarity (Fig. 2b) [18,20], indicating that stabilization of the structure around the calcium-binding site and domain interface is critical for both proteins.

3. EL structural characterization

The EL crystal structure has been determined at a 2.5 Å resolution, demonstrating a structural homology with lysozymes and a calcium-binding loop similarity to α-lactalbumins [4]. The ^{1}H NMR spectrum of holo-EL shows the high degree of resonance dispersion characteristic of lysozymes [3,21]. Backbone chemical shifts as well as sequential and long-range NOEs closely resemble those of human and hen lysozymes, providing evidence of the similarity between their solution structures. As in crystallographic studies the major chemical shift deviations were observed for the residues located in the calcium-binding loop [3,21]. The calcium-binding site was analyzed by ^{113}Cd and ^{43}Ca NMR, confirming its match with those of bovine and human α-lactalbumins [22,23].

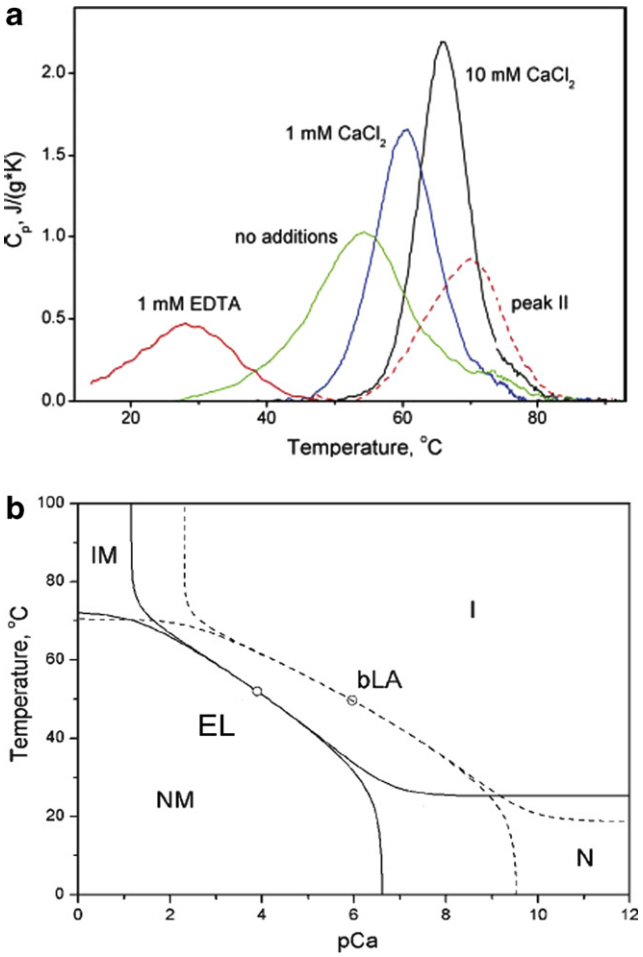

Fig. 2. (a) Specific heat capacity of EL at various calcium concentrations [18]. Concentration of calcium was controlled by adding 1 mM EDTA (red), 1 mM $CaCl_2$ (blue), 10 mM $CaCl_2$ (black) or no additives in control measurements (green). (b) Phase-diagrams of EL (solid lines) and bovine α-lactalbumin (dashed curve); EL states are denoted as in Eq. (1).

The amide hydrogen exchange protection measurements of holo-EL, probing both the structure and dynamic of molecule, show a pattern similar to those of human and hen lysozymes, i.e. the protection of amides in all major secondary structure elements [21]. However, the protection factors are on average 600-fold lower than in human and hen lysozymes. This correlates well with the EL's lower stability [6–8,18] and with the thermodynamic calculations of the protein equilibrium folding pathways [24].

4. The origin of molten globule stability

The molten globules of α-lactalbumins were the earliest discovered and most studied partially folded states [25,26]. Classic molten globules are compact states with native-like secondary structures, but with a few tertiary contacts compared to native proteins. Understanding the differences between the molten globule and native states is critical for clarifying the mechanisms of protein folding and stability. Most lysozymes do not readily form equilibrium molten globules, but EL is a notable exception, as it enters into various molten globule

states during thermal, chemical or pH denaturation [6–8,18,21]. They display some features of α-lactalbumin molten globules, being compact and possessing conformational mobility in the millisecond time scale. However, they are significantly more stable, exhibiting cooperative unfolding transitions and the pronounced circular dichroism signals both in the near and far-UV regions, indicative of the presence of persistent tertiary interactions [6–8,18,21].

Amide proton exchange analysis provided residue-specific information on the nature of EL molten globule stability [8,21]. The acidic A-state of EL showed high protection of the A, B and D α-helices and particularly the amides of hydrophobic residues located at the helical interface, making contacts with other residues of the core (Fig. 1). A model of the molten globule was proposed, which possesses an extended hydrophobic core based on the native-like tertiary interactions of the three out of four major α-helices. This was the most stable molten globule within the lysozyme-α-lactalbumin family [8,21]. Recently, a molten globule with a similar core, but more stabilized α-helices, was observed in canine calcium-binding lysozyme [27,28]. Thus, if the first thermodynamic transition of EL corresponds to the conversion of the native state to the molten globule, then the unfolding of the core, rather than the whole α-domain [19], gives an enthalpic contribution to the second transition to the thermally-unfolded state [7,8,27,28]. The particular role of the D helix in maintaining the native-like core was demonstrated by designing the chimeras of human and bovine α-lactalbumins, carrying the D helix of EL and displaying the stability of EL molten globule [29,30]. Using polypeptide constructs of different length, Chowdhury et al. [31] showed that more extensive interactions than those in α-domain are required to generate a stable molten globule in two calcium-binding equine and canine lysozymes. The structural characterization of the EL molten globule clearly demonstrates that the native-like packing can significantly contribute to non-specific rudimentary hydrophobic interactions initially persistent in the intermediate states.

5. Ensemble of kinetic intermediates

The kinetics of EL native state formation is described as an extremely uncooperative process, occurring via the heterogeneous assembly of the numerous regions characterized by independent nucleation and local cooperativity (Fig. 1) [9]. The kinetic events were monitored by using several techniques such as amide hydrogen exchange pulse-labelling coupled with NMR and stopped-flow circular dichroism, intrinsic and dye fluorescence spectroscopy, providing an insight into the stabilization of the secondary and tertiary structures and the burial of hydrophobic surfaces [9,32]. The folding reactions attributed to different molecular regions were characterized by time constants from a few to hundreds milliseconds. A burst-phase folding intermediate was formed in the whole population of molecules within the dead time of experiments [9]. It possesses the persistent A, B and D α-helical core, closely resembling the core observed in the EL equilibrium molten globule (Fig. 1) [8,21]. The most protected B helix is central to both equilibrium and kinetic intermediates. Mizuguchi et al. [32] also demonstrated the similarity between the EL burst-phase intermediate and equilibrium molten globule. Both results emphasize that the former is an obligatory intermediate on the folding pathway and the latter is an important structural model of short-lived kinetic species [8,9,32,33].

The overall assembly into the native state proceeded in a different order within the given population of EL molecules, implying the involvement of multiple folding pathways. In some molecules the further step, occurring on a timescale of hundred milliseconds, involved the stabilization of the remaining C helix in the α-domain. In others the persistent structure began to develop in the β-domain, involving three kinetically distinguishable steps, with the middle β-strand stabilized last of all (Fig. 1). Residues in the loop regions throughout the protein attained persistent structures most slowly. Based on these observations, the model of the most uncooperative EL folding compared to other c-type lysozymes was proposed [9,34,35], which provided an experimental basis for the theoretical simulation of multiple folding pathways in a free energy folding landscape [36]. It is interesting to note, that in α-lactalbumins the transient species with some persistent structure in the α-domain were also detected. However, the weak protection against solvent exchange, measured for bovine α-lactalbumin, suggests that the ensemble of molecules formed early in the folding reaction contains not much common and persistent structure, which is preserved through the folding process [37]. There is no evidence of the existence of well-defined discrete folding intermediates of the type populated during refolding of EL, human and hen lysozymes [9,34,35]. The folding of members of lysozyme-α-lactalbumins super-family emphasizes that the native state in structurally homologous proteins can be attained via very diverse folding pathways. The common feature for all members of this super-family is that the nucleation site is located in the α-domain, but the structural elements involved in the initial core as well as the order and rate of assembly of subsequent structural regions are highly sequence specific.

6. Partially folded states as amyloid precursors

The interest in both equilibrium and kinetic intermediates was stimulated recently due to their implication as precursor-states in aggregation and amyloid formation [38,39]. It was suggested that the amyloidogenicity of human lysozyme variants involved in systemic amyloidosis originates from their lower global stability and cooperativity compared to the wild-type protein and the decrease in the stability of the native-fold relative to partially-folded intermediates [40–42]. Indeed, both wild-type lysozyme and Asp57Ile/His67Thr variants form amyloid fibrils in vitro, if their partially folded states are populated [12]. Therefore, the stabilization of lysozyme was suggested as a valuable pharmaceutical approach [43]. EL as a naturally destabilized lysozyme also assembles into amyloid protofilaments under the conditions of its molten globule formation at acidic pH [10]. However, compared to human lysozyme fibrils, which undergo maturation, lengthening and thickening [12], the EL protofilaments remain single-stranded and never exceed a few hundred nanometers (Fig. 3b and c) [10]. This fact may be attributed to the presence of the very stable core in its structure, persistent even under amyloid forming conditions [10]. Although the polypeptide sequences most likely forming the fibrillar cross-β-sheet are encompassed in the β-domain [10,44], the stable α-helical core provides a non-adhesive fibrillar interface, preventing significant fibrillar growth. These results suggest that it is not only

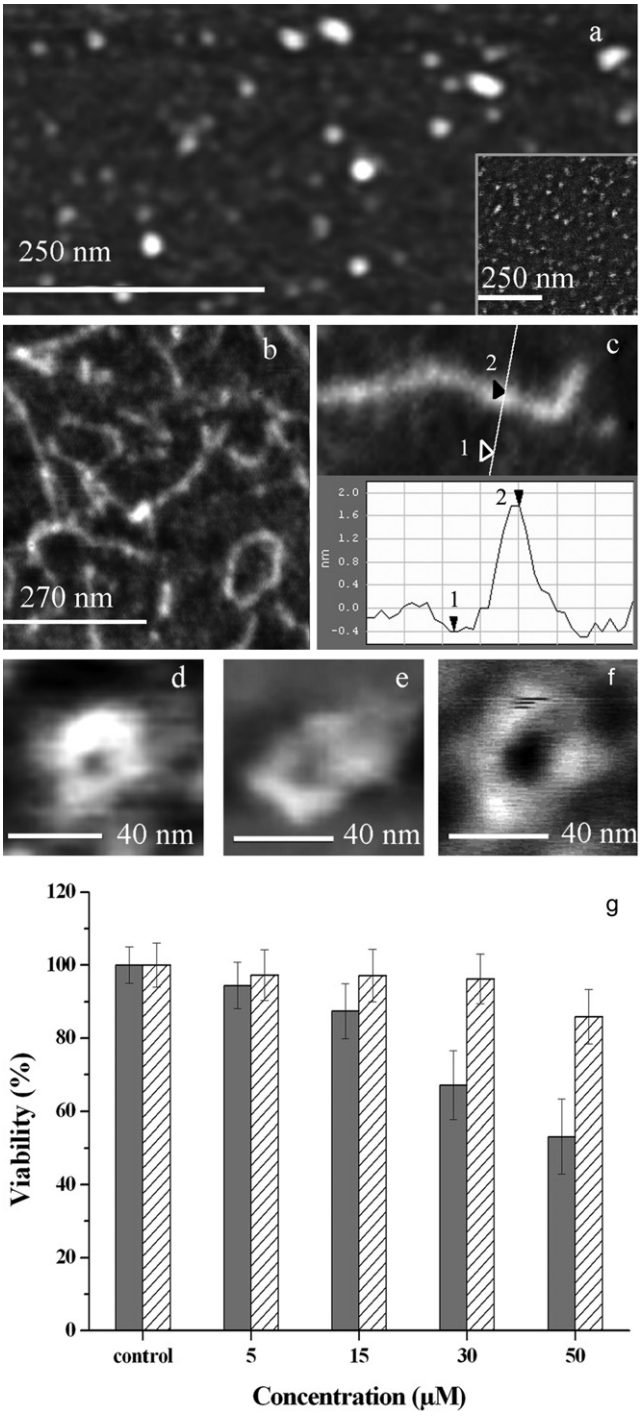

Fig. 3. Relating EL amyloid morphology and cytotoxicity [10,11]. (a) Atomic force microscopy images of cytotoxic amyloid oligomers produced at pH 4.5, calcium content was not controlled; (b) non-toxic EL protofilaments; (c) an individual protofilament and its cross-section; (d–f) EL amyloid-rings constituted of oligomers; (g) the decrease of viability of primary neuronal cell culture in the presence of EL samples, containing the amyloid tetramers, octamers and eikosimers (20) (black bars) and the tetramers and octamers (striped bars), respectively.

the propensity to form β-sheet structure which governs the amyloid formation, but also the conformational plasticity of the polypeptide chains involved in the fibrillar interface. Therefore, the design of the regions with a high local stability and cooperativity, in addition to the overall stabilization of protein molecules, can be broadly explored as an amyloid-preventing strategy in the applications involving proteinaceous compounds [45].

It is important to note that the amyloid precursor states of both EL and human lysozyme share one common feature – the formation of polyproline-type II helix [46,47]. In both proteins the C-helix, located at the domain interface, is a potential candidate for this conformation. This helix is characterized by a significant conformational plasticity and a propensity to convert into β-sheet. Polyproline-type II helix was observed in the amyloid precursor states of other proteins and can be considered as a signature of this state or a "killer conformation" [46,47].

7. Calcium-regulated amyloid formation

The morphology of EL amyloids depends strongly on solution conditions, including calcium concentration, pH and temperature [10]. The holo-EL assembles into linear protofilaments, while the apo-protein forms ring-shaped amyloids (Fig. 3b–f). All amyloid samples are polymorphic as shown in Fig. 3a and b, consisting of a mixture of various size oligomers and protofilaments. The amyloid-rings are varied in diameters, which equal to 45–50 nm at pH 4.5 and 70–80 nm at pH 2.0, respectively [10]. They bind amyloid dyes Congo red and thioflavin T similar to the disease related amyloid-rings of Aβ peptide and α-synuclein. Amyloid-ring formation was suggested as a major pathogenic cause in Alzheimer's and Parkinson's diseases [48]. The observation of EL amyloid-rings highlights that they are not limited to disease-related polypeptides, but represent a second generic type of amyloids in addition to more common linear filaments [10].

8. Innate amyloid cytotoxicity

Cytotoxicity is one of the major properties gained by proteins as a result of their self-assembly into amyloids. Already EL amyloid tetramers and even more markedly octamers and eikosimers (20), determined by atomic force microscopic analysis, exhibit this innate cytotoxic activity on various cell types (Fig. 3a and g) [11]. This property has a transient nature and diminishes as soon as protofilaments are formed. It has been suggested that the amyloid cross-β-sheet core development is critical for the oligomeric toxicity, stabilizing them from dissociation upon penetration through cellular membranes and within the cellular environment [49]. Soluble amyloid oligomers may assemble into rings. However, there was no correlation between the population of the amyloid-rings and the cytotoxicity of the samples with an equal protein concentration, demonstrating that the size and the structural properties of the constituting oligomers rather than the ring-formation determine the amyloid toxicity [11].

It is interesting to note that the amyloids of hen lysozyme, formed under similar conditions of acidic pH as EL's ones, also exhibit cytotoxicity [50]. The amyloid oligomers initiate apoptosis-like pathways, while the fibrils induce necrosis-like cell death. This suggests that the type of cytotoxicity may depend on amyloid morphology or/and the primary structure

of precursor-protein, but that is not limited to a particular amyloid species, being an inherent property of a wide range of amyloids.

9. Concluding remarks

EL proved to be a rich and invaluable resource for protein researchers. In-depth studies of EL have enriched our understanding of the general folding mechanisms, revealing the link between equilibrium and kinetic intermediates, the origin of their stability and their pivotal role in amyloid assembly. The observation of an ensemble of kinetic intermediates and multiple folding pathways provided an experimental demonstration of the complexity of the theoretically simulated energy folding landscape. The calcium-regulated amyloid self-assembly and innate amyloid toxicity of such an abundant protein as lysozyme emphasizes the universality of this phenomenon. Undesired protein aggregation and amyloid assembly, arising from protein instability, is a common event, occurring spontaneously in vivo and in vitro and being potentially highly hazardous. Therefore, they demand careful assessments, particularly in the growing areas of industrial applications of polypeptide-based products, such as biopharmaceuticals [45]. Analyzing the folding and amyloid properties of EL we suggest that amyloid propagation can be effectively limited or prevented by a targeted design within the protein molecule of the relevant local areas with a high stability and cooperativity. This strategy can be explored in biopharmaceutical applications, in order to ensure more highly functional and safe products [45].

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References

[1] Nitta, K., Tsuge, H., Sugai, S. and Shimazaki, K. (1987) The calcium-binding property of equine lysozyme. FEBS Lett. 223, 405–408.
[2] Acharya, K.R., Stuart, D.I., Phillips, D.C., McKenzie, H.A. and Teahan, C.G. (1994) Models of the three-dimensional structures of echidna, horse and pigeon lysozymes: calcium-binding lysozymes and their relationship with α-lactalbumins. J. Prot. Chem. 6, 569–584.
[3] Tsuge, H., Koseki, K., Miyano, M., Shimazaki, K., Chuman, T., Matsumoto, T., Noma, M., Nitta, K. and Sugai, S. (1991) A structural study of calcium-binding equine lysozyme by two-dimensional ^{1}H NMR. Biochim. Biophys. Acta 1078, 77–84.
[4] Tsuge, H., Ago, H., Noma, M., Nitta, K., Sugai, S. and Miyano, M. (1992) Crystallographic studies of a calcium binding lysozyme from equine milk at 2.5 Å resolution. J. Biochem. 111, 141–143.
[5] Permyakov, E.A. and Berliner, L.J. (2000) α-Lactalbumin: structure and function. FEBS Lett. 473, 269–274.
[6] Morozova, L., Haezebrouck, P. and Van Cauwelaert, F. (1991) Stability of equine lysozyme. Thermal unfolding behaviour. Biophys. Chem. 41, 185–191.
[7] Van Dael, H., Haezebrouck, P., Morozova, L., Arico-Muendel, C. and Dobson, C.M. (1993) Partially folded states of equine lysozyme. Structural characterization and significance for protein folding. Biochemistry 32, 11886–11894.
[8] Morozova, L.A., Haynie, D.T., Arico-Muendel, C., Van Dael, H. and Dobson, C.M. (1995) Structural basis of the stability of a lysozyme molten globule. Nat. Struct. Biol. 10, 171–175.
[9] Morozova-Roche, L.A., Jones, J.A., Noppe, W. and Dobson, C.M. (1999) Independent nucleation and heterogeneous assembly of structure during folding of equine lysozyme. J. Mol. Biol. 289, 1055–1073.
[10] Mališauskas, M., Zamotin, V., Jass, J., Noppe, W., Dobson, C.M. and Morozova-Roche, L.A. (2003) Amyloid protofilaments from the calcium-binding equine lysozyme: formation of rings and linear structures depends on pH and metal ion concentration. J. Mol. Biol. 330, 879–890.
[11] Malisauskas, M., Ostman, J., Darinskas, A., Zamotin, V., Liutkevicius, E., Lundgren, E. and Morozova-Roche, L.A. (2005) Does the cytotoxic effect of transient amyloid oligomers from common equine lysozyme in vitro imply innate amyloid toxicity? J. Biol. Chem. 280, 6269–6275.
[12] Morozova-Roche, L.A., Zurdo, J., Spencer, A., Noppe, W., Receveur, V., Archer, D., Joniau, M. and Dobson, C.M. (2000) Amyloid fibril formation and seeding by wild type human lysozyme and its disease related mutational variants. J. Struct. Biol. 130, 339–351.
[13] Pepys, M.B., Hawkins, P.N., Booth, D.R., Vigushin, D.M., Tennent, G.A., Soutar, A.K., Totty, N., Nguyen, O., Blake, C.C., Terry, C.J., Feest, T.G., Zalin, A.M. and Hsuan, J.J. (1993) Human lysozyme gene mutations cause hereditary systemic amyloidosis. Nature 362, 553–557.
[14] Goers, J., Permyakov, S.E., Permyakov, E.A., Uversky, V.N. and Fink, A.L. (2002) Conformational prerequisites for α-lactalbumin fibrillation. Biochemistry 41, 12546–12551.
[15] Stuart, D.I., Acharya, K.R., Walker, N.P., Smith, S.G., Lewis, M. and Phillips, D.C. (1986) α-Lactalbumin possesses a novel calcium binding loop. Nature 324, 84–87.
[16] Privalov, P.L. (1979) Stability of proteins: small globular proteins. Adv. Protein Chem. 33, 167–241.
[17] Booth, D.R., Sunde, M., Bellotti, V., Robinson, C.V., Hutchinson, W.L., Fraser, P.E., Hawkins, P.N., Dobson, C.M., Radford, S.E., Blake, C.C. and Pepys, MB. (1997) Instability, unfolding and aggregation of human lysozyme variants underlying amyloid fibrillogenesis. Nature 385, 787–793.
[18] Permyakov, S.E., Khokhlova, T.I., Nazipova, A.A., Zhadan, A.P., Morozova-Roche, L.A. and Permyakov, E.A. (2006) Calcium-binding and temperature induced transitions in equine lysozyme: new insights from the pCa-temperature ''phase diagrams''. Proteins 65, 984–998.
[19] Griko, Y.V., Freire, E., Privalov, G., van Dael, H. and Privalov, P.L. (1995) The unfolding thermodynamics of c-type lysozymes: a calorimetric study of the heat denaturation of equine lysozyme. J. Mol. Biol. 252, 447–459.
[20] Permyakov, E.A., Morozova, L.A. and Burstein, E.A. (1985) Cation binding effects on the pH, thermal and urea denaturation transitions in α-lactalbumin. Biophys. Chem. 21, 21–31.
[21] Morozova-Roche, L.A., Arico-Muendel, C., Haynie, D.T., Emelyanenko, V.I., Van Dael, H. and Dobson, C.M. (1997) Structural characterisation and comparison of the native and A-states of equine lysozyme. J. Mol. Biol. 268, 903–921.
[22] Aramini, J.M., Hiraoki, T., Ke, Y., Nitta, K. and Vogel, H.J. (1995) Cadmium-113 NMR studies of bovine and human α-lactalbumin and equine lysozyme. J. Biochem. 117, 623–628.
[23] Aramini, J.M., Drakenberg, T., Hiraoki, T., Ke, Y., Nitta, K. and Vogel, H.J. (1992) Calcium-43 NMR studies of calcium-binding lysozymes and α-lactalbumins. Biochemistry 31, 6761–6768.
[24] Hilser, V.J. and Freire, E. (1996) Structure-based calculation of the equilibrium folding pathway of proteins. Correlation with hydrogen exchange protection factors. J. Mol. Biol. 262, 756–772.
[25] Ptitsyn, O.B., Pain, R.H., Semisotnov, G.V., Zerovnik, E. and Razgulyaev, O.I. (1990) Evidence for a molten globule state as a general intermediate in protein folding. FEBS Lett. 262, 20–24.
[26] Kuwajima, K. (1989) The molten globule as a clue for understanding the folding and cooperativity of globular protein structures. Proteins 6, 87–103.
[27] Kobashigawa, Y., Demura, M., Koshiba, T., Kumaki, Y., Kuwajima, K. and Nitta, K. (2000) Hydrogen exchange study of canine milk lysozyme: stabilization mechanism of the molten globule. Proteins 40, 579–589.
[28] Koshiba, T., Yao, M., Kobashigawa, Y., Demura, M., Nakagawa, A., Tanaka, I., Kuwajima, K. and Nitta, K. (2000) Structure and thermodynamics of the extraordinarily stable molten globule state of canine milk lysozyme. Biochemistry 39, 3248–3257.

[29] Mizuguchi, M., Masaki, K. and Nitta, K. (1999) The molten globule state of a chimera of human α-lactalbumin and equine lysozyme. J. Mol. Biol. 292, 1137–1148.
[30] Tada, M., Kobashigawa, Y., Mizuguchi, M., Miura, K., Kouno, T., Kumaki, Y., Demura, M., Nitta, K. and Kawano, K. (2002) Stabilization of protein by replacement of a fluctuating loop: structural analysis of a chimera of bovine α-lactalbumin and equine lysozyme. Biochemistry 41, 13807–13813.
[31] Chowdhury, F.A., Fairman, R., Bi, Yu., Rigotti, D.J. and Raleigh, D.P. (2004) Protein dissection experiments reveal key differences in the equilibrium folding of α-lactalbumin and the calcium binding lysozymes. Biochemistry 43, 9961–9967.
[32] Mizuguchi, M., Arai, M., Ke, Y., Nitta, K. and Kuwajima, K. (1998) Equilibrium and kinetics of the folding of equine lysozyme studied by circular dichroism spectroscopy. J. Mol. Biol. 283, 265–277.
[33] Ikeguchi, M., Kuwajima, K., Mitani, M. and Sugai, S. (1986) Evidence for identity between the equilibrium unfolding intermediate and a transient folding intermediate: a comparative study of the folding reactions of α-lactalbumin and lysozyme. Biochemistry 25, 6965–6972.
[34] Hooke, S.D., Radford, S.E. and Dobson, C.M. (1994) The refolding of human lysozyme: a comparison with the structurally homologous hen lysozyme. Biochemistry 33, 5867–5876.
[35] Matagne, A., Chung, E.W., Ball, L.J., Radford, S.E., Robinson, C.V. and Dobson, C.M. (1998) The origin of the α-domain intermediate in the folding of hen lysozyme. J. Mol. Biol. 277, 997–1005.
[36] Dobson, C.M., Šali, A. and Karplus, M. (1998) Protein folding: a perspective from theory and experiment. Angew. Chem. Int., Ed. Eng. 37, 868–893.
[37] Forge, V., Wijesinha, R.T., Balbach, J., Brew, K., Robinson, C.V., Redfield, C. and Dobson, C.M. (1999) Rapid collapse and slow structural reorganisation during the refolding of bovine α-lactalbumin. J. Mol. Biol. 288, 673–688.
[38] Dobson, C.M. (2001) The structural basis of protein folding and its links with human disease. Phil. Trans. R. Soc. Lond. B 356, 133–145.
[39] Buswell, A.M. and Middelberg, A.P.J. (2003) A new kinetic scheme for lysozyme refolding and aggregation. Biotechnol. Bioeng. 83, 567–577.
[40] De Felice, F.G., Nascimento, M.N., Meirelles, M.N.L., Morozova-Roche, L.A., Dobson, C.M. and Ferreira, S.T. (2004) Formation of amyloid fibrils from human lysozyme and its disease related variants by using hydrostatic pressure. FASEB J. 18, 1099–1101.
[41] Canet, D., Sunde, M., Last, A.M., Miranker, A., Spencer, A., Robinson, C.V. and Dobson, C.M. (1999) Mechanistic studies of the folding of human lysozyme and the origin of amyloidogenic behavior in its disease-related variants. Biochemistry 38, 6419–6427.
[42] Dumoulin, M., Canet, D., Last, A.M., Pardon, E., Archer, D.B., Muyldermans, S., Wyns, L., Matagne, A., Robinson, C.V., Redfield, C. and Dobson, C.M. (2005) Reduced global cooperativity is a common feature underlying the amyloidogenicity of pathogenic lysozyme mutations. J. Mol. Biol. 346, 773–788.
[43] Merlini, G. and Bellotti, V. (2005) Lysozyme: a paradigmatic molecule for the investigation of protein structure, function and misfolding. Clin. Chim. Acta 357, 168–172.
[44] Krebs, M.R., Wilkins, D.K., Chung, E.W., Pitkeathly, M.C., Chamberlain, A.K., Zurdo, J., Robinson, C.V. and Dobson, C.M. (2000) Formation and seeding of amyloid fibrils from wild-type hen lysozyme and a peptide fragment from the β-domain. J. Mol. Biol. 300, 541–549.
[45] Morozova-Roche, L.A. and Malisauskas, M. (2007) A false paradise – mixed blessings in the protein universe: the amyloid as a new challenge in drug development. Curr. Med. Chem. 14, 1221–1230.
[46] Blanch, E.W., Morozova-Roche, L.A., Hecht, L. and Barron, L.D. (2000) Raman optical activity characterisation of native and molten globule states of equine lysozyme and comparison with hen lysozyme and bovine α-lactalbumin. Biopolymers 57, 235–248.
[47] Blanch, E.W., Morozova-Roche, L.A., Cochran, D.A.F., Doig, A.J., Hecht, L. and Barron, L.D. (2000) Is polyproline II helix the killer conformation? A Raman optical activity study of the amyloidogenic prefibrillar intermediate of human lysozyme. J. Mol. Biol. 301, 553–563.
[48] Lashuel, H.A., Hartley, D., Petre, B.M., Walz, T. and Lansbury, P.T. (2002) Neurodegenerative disease: amyloid pores from pathogenic mutations. Nature 418, 291.
[49] Zamotin, V., Gharibyan, A., Gibanova, N.V., Lavrikova, M.A., Dolgikh, D.A., Kirpichnikov, M.P., Kostanyan, I.A. and Morozova-Roche, L.A. (2006) Cytotoxicity of albebetin oligomers depends on cross-β-sheet formation. FEBS Lett. 580, 2451–2457.
[50] Gharibyan, A.L., Zamotin, V., Yanamandra, K., Moskaleva, O.S., Margulis, B.A., Kostanyan, I.A. and Morozova-Roche, L.A. (2007) Lysozyme amyloid oligomers and fibrils induce cellular death via different apoptotic/necrotic pathways. J. Mol. Biol. 365, 1337–1349.