Table 1. Experimental techniques used to characterize the partially folded (molten globule) states of α-lactalbumin

Technique[1]	Source of protein[2]	MG-generating conditions[3]	Refs.
Fluorescence			
Stopped-flow fluorescence	B	apo, A	1, 2
ANS-bound fluorescence	H	A, P, apo, T	3-6
Fluorescence quenching / anisotropy	rH	A	7
Circular Dichroism			
Steady-state CD	B, H	A, P, apo, T	5, 8, 9
Stopped-flow CD	B, G	A, apo	1, 2, 10-14
Viscometry	B, H	A, P, apo, T	8, 9, 15
Oxidative-reductive disulfide bridge exchange	H, B / B	apo / T, P	16-19 / 20-22
Differential scanning calorimetry	H, B	A, apo	8, 9, 15, 23 / 24-26 / 27 / 28 / 29
Sequence deletion / substitution analysis			
Domain-level studies	rH	(n.a.)	30-32
Amino acid point mutations (incl. Φ-value analysis)	rH / rG	(n.a.)	33-38 / 39
Chimeric protein generation	H, B	(n.a.)	40-42
NMR Spectroscopy			
^{1}H NMR	B, GP	A, T	9, 43-45
2D ^{1}H NOE	B, H, GP	A	43, 44, 46
Hydrogen Exchange	GP, B, rH / B	A / apo	2, 47, 48 / 49
Steady-state CIDNP, EPR	B	A, T / apo	50 / 51
Real-time ^{1}H NMR / CIDNP NMR	B	apo / A	2, 52-55
^{15}N-^{1}H HSQC NMR with progressive denaturation	rH, B, All-Ala, [28-111], others	A	56-61
NOE magnetization transfer	B	A	62
^{15}N transverse relaxation rate measurements	GP	A	63
Water ^{17}O magnetic relaxation dispersion	B	A	64
^{19}F NMR	rH	A	65
Pulse-labelled CIDNP	B, H	A, P, apo	66 & this study
Limited proteolysis	B	A, apo	67-69
IR and stopped-flow Fourier transform IR	B	A, apo	9 / 70
Mass spectrometry (Hydrogen exchange)	rH, [28-111], others	A	71
Synthetic peptide constructs	H	A	72-76
Vibrational Raman optical activity	B	A, apo	77-79
Small angle X-ray scattering	B	apo, A	80, 81
Quasielastic neutron scattering	B	A	82
Chemical labeling	B	apo, A	83, 84
Hydrostatic pressure	B / All-Ala	apo / A	85 / 59
Ultrasonic techniques	B, H	A	86, 87
Interaction with molecular chaperones	B, H	apo	88-91

[1] ANS, 8-anilino-1-naphthalene sulfonate; CD, circular dichroism; NOE, nuclear Overhauser effect spectroscopy; CIDNP, chemically-induced dynamic nuclear polarization; EPR, electron paramagnetic resonance; HSQC, heteronuclear single quantum correlation spectroscopy; IR, infrared

[2] B, bovine α-lactalbumin; H, human α-lactalbumin; rH, recombinant human α-lactalbumin (containing N-terminal Met); G, goat α-lactalbumin; rG, recombinant goat α-lactalbumin; GP, guinea pig α-lactalbumin; [28-111], (Cys6Ala/Cys120Ala) (Cys61Ala/Cys77Ala) (Cys73Ala/Cys91Ala)-recombinant human α-lactalbumin (containing a single disulfide bond between Cys28 and Cys111).

[3] MG, partially folded "molten globule" form; A, Acidic-state molten globule at pH 2; P, molten globule generated in moderate concentrations of denaturant ("partly-denatured") at pH 7; apo, calcium ion-depleted molten globule at pH 7; T, high temperature molten globule at pH 7.

References

1. Balbach, J., Forge, V., van Nuland, N. A. J., Winder, S. L., Hore, P. J. & Dobson, C. M. (1995) *Nat. Struct. Biol.* **2,** 865-870.
2. Forge, V., Wijesinha, R. T., Balbach, J., Brew, K., Robinson, C. V., Redfield, C. & Dobson, C. M. (1999) *J. Mol. Biol.* **288,** 673-688.
3. Ptitsyn, O. B., Pain, R. H., Semisotnov, G. V., Zerovnik, E. & Razgulyaev, O. I. (1990) *FEBS Lett.* **262,** 20-24.
4. Semisotnov, G. V., Rodionova, N. A., Razgulyaev, O. I., Uversky, V. N., Gripas, A. F. & Gilmanshin, R. I. (1991) *Biopolymers* **31,** 119-128.
5. Vanderheeren, G. & Hanssens, I. (1994) *J. Biol. Chem.* **269,** 7090-7094.
6. Uversky, V. N., Winter, S. & Löber, G. (1996) *Biophys. Chem.* **60,** 79-88.
7. Chakraborty, S., Ittah, V., Bai, P., Luo, L., Haas, E. & Peng, Z.-y. (2001) *Biochemistry* **40,** 7228-7238.
8. Dolgikh, D. A., Gilmanshin, R. I., Brazhnikov, E. V., Bychkova, V. E., Semisotnov, G. V., Venyaminov, S. Y. & Ptitsyn, O. B. (1981) *FEBS Lett.* **136,** 311-315.
9. Dolgikh, D. A., Abaturov, L. V., Bolotina, I. A., Brazhnikov, E. V., Bychkova, V. E., Gilmanshin, R. I., Lebedev, Y. O., Semisotnov, G. V., Tiktopulo, E. I. & Ptitsyn, O. B. (1985) *Eur. Biophys. J.* **13,** 109-121.
10. Kuwajima, K., Hiraoka, Y., Ikeguchi, M. & Sugai, S. (1985) *Biochemistry* **24,** 874-881.
11. Ikeguchi, M., Kuwajima, K., Mitani, M. & Sugai, S. (1986) *Biochemistry* **25,** 6965-6972.
12. Ikeguchi, M., Fujino, M., Kato, M., Kuwajima, K. & Sugai, S. (1998) *Protein Sci.* **7,** 1564-1574.
13. Chaudhuri, T. K., Arai, M., Terada, T. P., Ikura, T. & Kuwajima, K. (2000) *Biochemistry* **39,** 15643-15651.
14. Mizuguchi, M., Hashimoto, D., Sakurai, M. & Nitta, K. (2000) *Proteins: Struct., Funct., Genet.* **38,** 407-413.
15. Pfeil, W. (1998) *Proteins: Struct., Funct., Genet.* **30,** 43-48.
16. Ewbank, J. J. & Creighton, T. E. (1991) *Nature* **350,** 518-520.
17. Ewbank, J. J. & Creighton, T. E. (1993) *Biochemistry* **32,** 3677-3693.
18. Ewbank, J. J. & Creighton, T. E. (1993) *Biochemistry* **32,** 3694-3707.
19. Creighton, T. E. & Ewbank, J. J. (1994) *Biochemistry* **33,** 1534-1538.
20. Chang, J.-Y., Bulychev, A. & Li, L. (2000) *FEBS Lett.* **487,** 298-300.
21. Chang, J.-Y. & Li, L. (2001) *J. Biol. Chem.* **276,** 9705-9712.
22. Chang, J.-Y. (2002) *J. Biol. Chem.* **277,** 120-126.
23. Pfeil, W., Bychkova, V. E. & Ptitsyn, O. B. (1986) *FEBS Lett.* **198,** 287-291.
24. Xie, D., Bhakuni, V. & Freire, E. (1991) *Biochemistry* **30,** 10673-10678.
25. Xie, D., Bhakuni, V. & Freire, E. (1993) *J. Mol. Biol.* **232,** 5-8.
26. Griko, Y. V., Freire, E. & Privalov, P. L. (1994) *Biochemistry* **33,** 1889-1899.
27. Yutani, K., Ogasahara, K. & Kuwajima, K. (1992) *J. Mol. Biol.* **228,** 347-350.
28. Veprintsev, D. B., Permyakov, S. E., Permyakov, E. A., Rogov, V. V., Cawthern, K. M. & Berliner, L. J. (1997) *FEBS Lett.* **412,** 625-628.
29. Griko, Y. V. & Remeta, D. P. (1999) *Protein Sci.* **8,** 554-561.
30. Peng, Z.-y. & Kim, P. S. (1994) *Biochemistry* **33,** 2136-2141.
31. Wu, L. C., Peng, Z.-y. & Kim, P. S. (1995) *Nat. Struct. Biol.* **2,** 281-286.

32. Wu, L. C. & Kim, P. S. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 14314-14319.
33. Peng, Z.-y., Wu, L. C. & Kim, P. S. (1995) *Biochemistry* **34,** 3248-3252.
34. Schulman, B. A. & Kim, P. S. (1996) *Nat. Struct. Biol.* **3,** 682-687.
35. Wu, L. C. & Kim, P. S. (1998) *J. Mol. Biol.* **280,** 175-182.
36. Song, J., Bai, P., Luo, L. & Peng, Z.-y. (1998) *J. Mol. Biol.* **280,** 167-174.
37. Chakraborty, S. & Peng, Z.-y. (2000) *J. Mol. Biol.* **298,** 1-6.
38. Bai, P., Song, J., Luo, L. & Peng, Z.-y. (2001) *Protein Sci.* **10,** 55-62.
39. Saeki, K., Arai, M., Yoda, T., Nakao, M. & Kuwajima, K. (2004) *J. Mol. Biol.* **341,** 589-604.
40. Mizuguchi, M., Masaki, K. & Nitta, K. (1999) *J. Mol. Biol.* **292,** 1137-1148.
41. Mizuguchi, M., Masaki, K., Demura, M. & Nitta, K. (2000) *J. Mol. Biol.* **298,** 985-995.
42. Mizuguchi, M., Kobashigawa, Y., Kumaki, Y., Demura, M., Kawano, K. & Nitta, K. (2002) *Proteins: Struct., Funct., Genet.* **49,** 95-103.
43. Baum, J., Dobson, C. M., Evans, P. A. & Hanley, C. (1989) *Biochemistry* **28,** 7-13.
44. Alexandrescu, A. T., Evans, P. A., Pitkeathly, M., Baum, J. & Dobson, C. M. (1993) *Biochemistry* **32,** 1707-1718.
45. Shimizu, A., Ikeguchi, M. & Sugai, S. (1993) *Biochemistry* **32,** 13198-13203.
46. Alexandrescu, A. T., Broadhurst, R. W., Wormald, C., Chyan, C.-L., Baum, J. & Dobson, C. M. (1992) *Eur. J. Biochem.* **210,** 699-709.
47. Chyan, C.-L., Wormald, C., Dobson, C. M., Evans, P. A. & Baum, J. (1993) *Biochemistry* **32,** 5681-5691.
48. Schulman, B. A., Redfield, C., Peng, Z.-y., Dobson, C. M. & Kim, P. S. (1995) *J. Mol. Biol.* **253,** 651-657.
49. Wijesinha-Bettoni, R., Dobson, C. M. & Redfield, C. (2001) *J. Mol. Biol.* **307,** 885-898.
50. Improta, S., Molinari, H., Pastore, A., Consonni, R. & Zetta, L. (1995) *Eur. J. Biochem.* **227,** 87-96.
51. Berliner, L. J., Koga, K., Nishikawa, H. & Scheffler, J. E. (1987) *Biochemistry* **26,** 5769-5774.
52. Maeda, K., Lyon, C. E., Lopez, J. J., Cemazar, M., Dobson, C. M. & Hore, P. J. (2000) *J. Biomol. NMR* **16,** 235-244.
53. Hamang, M., Sanson, A., Liagre, L., Forge, V. & Berthault, P. (2000) *Rev. Sci. Instrum.* **71,** 2180-2183.
54. Kühn, T. & Schwalbe, H. (2000) *J. Am. Chem. Soc.* **122,** 6169-6174.
55. Wirmer, J., Kühn, T. & Schwalbe, H. (2001) *Angew. Chem. Int. Ed.* **40,** 4248-4251.
56. Schulman, B. A., Kim, P. S., Dobson, C. M. & Redfield, C. (1997) *Nat. Struct. Biol.* **4,** 630-634.
57. Redfield, C., Schulman, B. A., Milhollen, M. A., Kim, P. S. & Dobson, C. M. (1999) *Nat. Struct. Biol.* **6,** 948-952.
58. Wijesinha-Bettoni, R., Dobson, C. M. & Redfield, C. (2001) *J. Mol. Biol.* **312,** 261-273.
59. Lassalle, M. W., Li, H., Yamada, H., Akasaka, K. & Redfield, C. (2003) *Protein Sci.* **12,** 66-72.
60. Ramboarina, S. & Redfield, C. (2003) *J. Mol. Biol.* **330,** 1177-1188.
61. Quezada, C. M., Schulman, B. A., Froggatt, J. J., Dobson, C. M. & Redfield, C. (2004) *J. Mol. Biol.* **338,** 149-158.
62. Balbach, J., Forge, V., Lau, W. S., Jones, J. A., van Nuland, N. A. J. & Dobson, C. M. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 7182-7185.
63. Kim, S., Bracken, C. & Baum, J. (1999) *J. Mol. Biol.* **294,** 551-560.
64. Denisov, V. P., Jonsson, B.-H. & Halle, B. (1999) *Nat. Struct. Biol.* **6,** 253-260.
65. Bai, P., Luo, L. & Peng, Z.-y. (2000) *Biochemistry* **39,** 372-380.
66. Lyon, C. E., Suh, E.-S., Dobson, C. M. & Hore, P. J. (2002) *J. Am. Chem. Soc.* **124,** 13018-13024.
67. Polverino de Laureto, P., De Filippis, V., Di Bello, M., Zambonin, M. & Fontana, A. (1995) *Biochemistry* **34,** 12596-12604.
68. Polverino de Laureto, P., Frare, E., Gottardo, R. & Fontana, A. (2002) *Proteins: Struct., Funct., Genet.* **49,** 385-397.
69. Polverino de Laureto, P., Frare, E., Gottardo, R., Van Dael, H. & Fontana, A. (2002) *Protein Sci.* **11,** 2932-2946.
70. Troullier, A., Reinstädler, D., Dupont, Y., Naumann, D. & Forge, V. (2000) *Nat. Struct. Biol.* **7,** 78-86.
71. Last, A. M., Schulman, B. A., Robinson, C. V. & Redfield, C. (2001) *J. Mol. Biol.* **311,** 909-919.
72. Demarest, S. J., Fairman, R. & Raleigh, D. P. (1998) *J. Mol. Biol.* **283,** 279-291.
73. Demarest, S. J., Hua, Y. & Raleigh, D. P. (1999) *Biochemistry* **38,** 7380-7387.
74. Demarest, S. J., Horng, J.-C. & Raleigh, D. P. (2001) *Proteins: Struct., Funct., Genet.* **42,** 237-242.
75. Demarest, S. J., Zhou, S.-Q., Robblee, J., Fairman, R., Chu, B. & Raleigh, D. P. (2001) *Biochemistry* **40,** 2138-2147.
76. Chowdhury, F. A. & Raleigh, D. P. (2005) *Protein Sci.* **14,** 89-96.
77. Wilson, G., Ford, S. J., Cooper, A., Hecht, L., Wen, Z. Q. & Barron, L. D. (1995) *J. Mol. Biol.* **254,** 747-760.
78. Wilson, G., Hecht, L. & Barron, L. D. (1996) *J. Mol. Biol.* **261,** 341-347.
79. Barron, L. D., Hecht, L., Blanch, E. W. & Bell, A. F. (2000) *Prog. Biophys. Mol. Biol.* **73,** 1-49.
80. Kataoka, M., Kuwajima, K., Tokunaga, F. & Goto, Y. (1997) *Protein Sci.* **6,** 422-430.

81. Arai, M., Ito, K., Inobe, T., Nakao, M., Maki, K., Kamagata, K., Kihara, H., Amemiya, Y. & Kuwajima, K. (2002) *J. Mol. Biol.* **321,** 121-132.
82. Bu, Z., Cook, J. & Callaway, D. J. E. (2001) *J. Mol. Biol.* **312,** 865-873.
83. Lala, A. K. & Kaul, P. (1992) *J. Biol. Chem.* **267,** 19914-19918.
84. D'Silva, P. R. & Lala, A. K. (1999) *Protein Sci.* **8,** 1099-1103.
85. Kobashigawa, Y., Sakurai, M. & Nitta, K. (1999) *Protein Sci.* **8,** 2765-2772.
86. Nölting, B., Jiang, M. & Sligar, S. G. (1993) *J. Am. Chem. Soc.* **115,** 9879-9882.
87. Kharakoz, D. P. & Bychkova, V. E. (1997) *Biochemistry* **36,** 1882-1890.
88. Okazaki, A., Ikura, T., Nikaido, K. & Kuwajima, K. (1994) *Nat. Struct. Biol.* **1,** 439-446.
89. Hayer-Hartl, M. K., Ewbank, J. J., Creighton, T. E. & Hartl, F.-U. (1994) *EMBO J.* **13,** 3192-3202.
90. Ewbank, J. J., Creighton, T. E., Hayer-Hartl, M. K. & Hartl, F.-U. (1995) *Nat. Struct. Biol.* **2,** 10.
91. Okazaki, A., Ikura, T. & Kuwajima, K. (1995) *Nat. Struct. Biol.* **2,** 10-11.