Supplementary material

N- and C-terminal regions of the small heat shock protein IbpA from Acholeplasma laidlawii competitively govern its oligomerization pattern and chaperone-like activity

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Table S1.	Plasmids	used in	this	study
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Plasmid	Description	Primers used for	Source
		AlibpA amplification	
pET15b	Expression vector	-	Novagene
pET15b lbpA	Overexpression of His-tagged IbpA in	-	Vishnyakov et
	E.coli		al., 2012
pET15b lbpA∆N12	Overexpression of His-tagged	lbpA_N12 up-	This study
	AllbpA∆N12 in <i>E.coli</i>	lbpA_C lw	
pET15b lbpA∆N25	Overexpression of His-tagged	lbpA_N25 up-	This study
	AllbpA∆N25 in <i>E.coli</i>	lbpA_C lw	
pET15b lbpAΔN12C14	Overexpression of His-tagged	lbpA_N12 up-	This study
	<i>Al</i> IbpAΔN12C14 in <i>E.coli</i>	lbpA_C14 lw	
pET15b lbpAΔN25C14	Overexpression of His-tagged	lbpA_N25 up-	This study
	AllbpA∆N25C14 in <i>E.coli</i>	lbpA_C14 lw	
pET15b lbpA∆C14	Overexpression of His-tagged	IbpA_N up-	This study
	AllbpA∆C14 in <i>E.coli</i>	lbpA_C14 lw	
pET15b lbpAN11N12	Overexpression of His-tagged	lbpA_N11N12 up-	This study
	AllbpAN11N12 in E.coli	lbpA_N11N12 lw	
pET15b lbpASEP	Overexpression of His-tagged	IbpA_N up- IbpA_SEP	This study
	<i>Al</i> lbpASEP in <i>E.coli</i>	lw	
pET15b lbpASEPΔN12	Overexpression of His-tagged	lbpA_N12 up-	This study
	AllbpASEPΔN12 in <i>E.coli</i>	IbpA_SEP lw	
pET15b lbpASEPΔN25	Overexpression of His-tagged	lbpA_N25 up-	This study
	AllbpASEPΔN25 in <i>E.coli</i>	lbpA_SEP lw	

Table S2. Primers used in this study

Primer	Sequence
IbpA_N up	5' CTG GTG CCG CGC GGC AGC CAT ATG CTC GAG GAT CCG ATG
	TTG AGT TTA TTG AAC AAG AAT AGA AG 3'
IbpA_N12 up	5' CTG GTG CCG CGC GGC AGC CAT ATG CTC GAG GAT CCG GAT
	GAT TTC TTC GAA GAC TTC AAT GTG C 3'
IbpA_N25 up	5' CTG GTG CCG CGC GGC AGC CAT ATG CTC GAG GAT CCG ACT
	ACT TCT AAC TTA ATG AGA ACA G 3'
IbpA_C lw	5' CCA ACT CAG CTT CCT TTC GGG CTT TGT TAG CAG CCG GAT
	CCT TAT TTA AGT TCT AAA TAA CGT TTT TCA GGC 3'
IbpA_C14 lw	5' CCA ACT CAG CTT CCT TTC GGG CTT TGT TAG CAG CCG GAT
	CCT TAT TTT GGA AGT TCG ATA TGT AAC ATA CCG 3'
IbpA_N11N12	5' CAA GAA TAG AAG TAA CAA TGA TGA TTT CTT CGA AGA C 3'
up	
IbpA_N11N12	5' GTC TTC GAA GAA ATC ATC ATT GTT ACT TCT ATT CTT G 3'
lw	
IbpA_SEP lw	5'CCA ACT CAG CTT CCT TTC GGG CTT TGT TAG CAG CCG GAT CCT
	TAT TTG GGT TCG GAA TAA CGT TTT TCA GGC 3'

Table S3. The identity of full-length proteins and their features for AllbpA compared to EclbpA or EclbpB

	Full-length	N-termini	ACD	C-termini
<i>Ec</i> IbpA	18.0%	18.0%	22.0%	29.4%
<i>Ec</i> IbpB	20.3%	20.3%	28.1%	12.5%

Table S4. The temperature stability (Tm₅₀) of various proteins

Protein	Can No	Tm ₅₀ , °C
	(Sigmaaldrich)	
Alcohol dehydrogenase	55689	53±1.1
Bovine insulin	16634	48±5.1
Human transferrin	T3705	58±2.3
Trypsin inhibitor	10109886001	73±1.2
Catalase	C9322	53±2.5
A/IbpA	-	53±4.5

Table S5. The chaperone-like activities (Tm₅₀) of full-length and truncated IbpA proteins

AllbpA variant	Protein structure	Tm ₅₀ , °C	AllbpA variant	Tm ₅₀ , °C
			Insulin	48±5.1
A/IbpA	N- ACD LEL -C 1 12 25 123 137	53±4.5	Insulin +AllbpA	53±5.7
A/lbpA∆N12	N- ACD LEL -C 12 25 123 137	56±5.2	Insulin +A/IbpA∆N12	58±6.7
A/lbpA∆N25	N- ACD LEL -C 25 123 137	56±5.4	Insulin <i>+Al</i> IbpA∆N25	58±6.9
A/IbpAN11N12	N- ACD SEP -C 1 10 25 123 137	58±6.1	Insulin +A/IbpAN11N12	59±4.5
A/lbpA∆C14	N- ACD -C 1 12 25 123	46±5.2	Insulin <i>+Al</i> IbpA∆C14	45±5.7
A/lbpA∆N12C14	N- ACD -C 12 25 123	48±4.1	Insulin +A/IbpA∆N12C14	42±5.6
AllbpA∆N25C14	N- ACD -C 25 123	40±4.8	Insulin +A/IbpA∆N25C14	39±3.5
A/IbpASEP	N- ACD SEP -C 1 12 25 123 137	51±4.6	Insulin +A/IbpASEP	50±4.7
A/IbpASEPΔN12	N- ACD SEP -C 12 25 123 137	52±6.6	Insulin +A/IbpASEPΔN12	51±5.1
A/IbpASEPΔN25	N- ACD SEP -C 25 123 137	54±5.9	Insulin <i>+Al</i> IbpASEP∆N25	53±7.3

¹ Determined by measuring the SYPRO Orange fluorescence

Table S6. Distribution of various oligomeric fractions (%) in solutions of full-length *Al*IbpA and various truncated versions of the protein

		Distribution of various oligomeric fractions, %			Distribution of various oligomeric fractions, %	
		Determined from gel-filtration data			Determined by quantification of TEM-images	
<i>Al</i> lbpA variant	Protein structure	I peak	ll peak	1×-4×-mers	l peak	ll peak
A/IbpA	N- ACD LEL -C 1 12 25 123 137	23±10.3	74±17.8	3±0.2	26±4.1	74±15.3
A/IbpA∆N12	N- ACD LEL -C 12 25 123 137	88±17.1	10±1.1	3±0.1	83±23.1	17±3.5
A/IbpA∆N25	N- ACD LEL -C 25 123 137	77±21.3	12±2.4	11±2.1	85±17.9	15±3.1
A/IbpAN11N12	N- ACD SEP -C 1 10 25 123 137	8±1.7	88±12.2	4±0.7	36±8.5	64±15.7
AllbpA∆C14	N- ACD-C 1 12 25 123	7±4.1	86±23.4	7±2.3	21±4.6	79±15.3
A/IbpA∆N12C14	N- ACD C 12 25 123	1±0.1	86±22.8	14±10.3	7±2.5	93±21.6
A/IbpA∆N25C14	N- ACD C 25 123	1±0.2	50±13.5	49±10.3	1±0.3	99±24.8
A/IbpASEP	N- ACD SEP -C 1 12 25 123 137	18±4.4	56±7.8	26±5.9	33±7.9	67±20.3
A/IbpASEP∆N12	N- ACD SEP -C 12 25 123 137	2±0.8	59±10.1	39±9.9	11±2.2	89±18.9
A/IbpASEP∆N25	N- ACD SEP -C 25 123 137	69±15.5	23±3.2	8±2.0	96±23.6	4±0.8



(b)

Figure S1. The models of tertiary structures of sHSPs from *A. laidlawii* (red) and *E.coli* (yellow) were obtained by using Phyre2 server and their superposition was obtained with SuperPose online software. (a) – superposition of *A*/lbpA and *Ec*lbpA, (b) – superposition of *A*/lbpA and *Ec*lbpB.

	가는 11 가는 12 가는 15 가는 15	α-crystallin	
A. laidlawii A. granularum A. brassicae A. modicum A. axanthum Acholeplasma sp.	M-LSLLNKNRSFFDDFFEI M-FNLSRQNRGFFDDFFEI M-TNLLKRQRDIFDGFFD M-LEVSKRGKNFFSDFFDI M-FDLLRKDKTFFDDFFG M-MMIPRRKDFDILEDIFF	DFNVLNPVTTSNLMRTDIKETQNGYSLSVELPGFA DFKLLNPGLSSNLMKTDIKETDKSYELSVELPGFA SFRLSPFFSNESIMRTDIKETDTHFLLDIELPGFA DLITTDNNLMKTDIKEVDGRYEFLIDLPGYA SIAGSKNALMKTDIKENDTSYTFEIDLPGFA	KKEDVKVS OKNDVKVS OKKDVKVT KKEDIKIS OKKDIKVG NKEDIKVS
A. laidlawii A. granularum A. brassicae A. modicum A. axanthum Acholeplasma sp.	LEDGYLTIEAHTSKNSET LDNGYLIIEATTDKTTED IDDGYLTVEANKKEEKDE IKNNYLTVEAVKEEILDE IENGYLTVSASKNDEVEE VEDGYLTIHATMNSENEE	KDQATKYIRKERYEGTMKRSYYVG-NLHLDEING NAKDGRFIKRERHYGSMQRSYYVG-NLTLDDIKG KSKDGKIIRQERYYGNLKRSYYVG-NVSINDVKG EKKDFIKKERHYGLISRSFYVG-DVTMKDLKA KKDNYIRRERHFGSFTRSFYVG-NVKLDTLAAN KEKG-KFVRRERYFGECSRSFYVGDDITTEDIKA	FFENGMLH FFKNGILH KFDQGILR SYKDGTLK NYKDGILS SYKNGSLK
A. laidlawii A. granularum A. brassicae A. modicum A. axanthum Acholeplasma sp.	IELPKETKKEPEKRYLI LDIPKENKKEPEKRYLI LEIPKEIKRIEQKKYLI VDCKKVTEEKTKYLI IDVPKEIKEETKYLI LEVPKKEVRKEIPEKTYV	ELK- ELK- ELE- DIEW EIK- ELED	

Figure S2. Multiple alignment of IbpA proteins from various *Acholeplasma spp.* Residues highlighted in gray show homologous substitutions. Amino acids in black represent similar residues. Putative (W/F)(D/F) PF and V/IXI/V motifs are underlined.



Figure S3. The SDS-PAGE analysis of purified recombinant truncated/mutated *Al*IbpA-His₆ proteins produced in *E.coli* BL21.



Figure S4. The SPR-analysis of various proteins interaction with *Al*IbpA. *Al*IbpA-His₆ was immobilized on NTA chip until ~2000 resonance units (RU). All proteins (1 mg/ml solution in running buffer) were injected in a volume of 15 μ l with a flow rate of 15 μ l/min.