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D-Peptide Ligands for the Co-chaperone DnaJ*

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The molecular chaperone DnaK, the Hsp70 homolog of *Escherichia coli*, binds hydrophobic polypeptide segments in extended conformation. The co-chaperone DnaJ (Hsp40) has been reported to bind native and denatured proteins as well as peptides. We tested pseudopeptides of D-amino acids as ligands for both chaperones. In comparison to the parent *all*-L peptide, these mimetics had either enantiomorphic side chain positions combined with retained main chain direction (*normal all*-D peptide) or unchanged side chain topology together with reverse direction of the peptide backbone (*retro all*-D peptide). The peptides were labeled with acrylodan (*a*), and their binding to DnaK and DnaJ was monitored by the accompanying increase in fluorescence intensity. The parent *all*-L peptide *a*-CALLLSAARR bound to both DnaK (K_d = 0.1 μM) and DnaJ (K_d = 9.2 μM). In contrast, the *normal all*-D and *retro all*-D peptides did not bind to DnaK; they bound, however, to DnaJ with K_d values of 6.8 μM and 0.9 μM, respectively. The emission spectra of the DnaJ-bound peptides suggests that DnaJ bound both D-peptides with the same main chain direction as L-peptides. Binding of the *normal all*-D and *all*-L peptides inhibited the DnaJ-induced stimulation of DnaK ATPase. However, binding of the *retro all*-D analog to DnaJ did not impair the stimulation, indicating the existence of separate binding sites for peptides and DnaK.

Chaperones of the Hsp70[1] family fulfill essential functions in protein folding by preventing and reversing off-pathway interactions that lead to aggregation (1, 2). Hsp70s are also required for membrane translocation of precursor polypeptides (3, 4) and participate in the degradation of misfolded proteins (2).

The Hsp70 chaperone system of *Escherichia coli* comprises the Hsp70 homolog DnaK and the co-chaperones DnaJ and GrpE. DnaK consists of a 44-kDa ATPase domain, the crystal structure of which has recently been determined in a complex with a dimer of truncated GrpE (5), and a 27-kDa peptide-binding domain, the crystal structure of which has been solved in a complex with a synthetic peptide ligand (6). The chaperone effects of the DnaK/DnaJ/GrpE system are based on the ability of DnaK to bind extended hydrophobic segments of proteins in a reversible manner and possibly to exert conformational work on them (2, 7). Binding and release of polypeptides are modulated by ATP binding and hydrolysis (8), which on their part are controlled by the co-chaperones GrpE and DnaJ, respectively (7, 9). DnaJ interacts with DnaK through its highly conserved N-terminal J-domain (10, 11). However, DnaJ appears to exert also a chaperone function of its own (12). The co-chaperone associates with denatured polypeptides independently of DnaK, *e.g.* luciferase and rhodanese (13, 14), preventing their aggregation and targeting them to DnaK.

In previous studies, we have found various peptides derived from the presequence of mitochondrial aspartate aminotransferase to be high affinity ligands of DnaK (7).[2] In a search for peptide mimetics that might be used as inhibitors of the chaperone system, we tested *normal all*-D and *retro all*-D *(retro-inverso)* analogs of a parent *all*-L peptide for binding to DnaK and DnaJ. In the *normal all*-D peptide mimetics, all L-amino acid residues have been replaced by the corresponding D-enantiomers; the main chains of these peptide analogs have the same direction as in the parent peptides, whereas the side chains occupy enantiomorphic positions. In contrast, *retro all*-D peptides, *i.e.* mimetic peptides of D-amino acids with, as compared with the reference L-peptides, reverse sequence, exhibit in extended conformation the same side chain topology as their native counterparts (15). In certain cases they may therefore retain the biological activity of the reference L-peptides while being much less susceptible to proteolytic degradation (16–18).

Here we show that the co-chaperone DnaJ binds the tested pseudo-peptides with similar affinities as the parent *all*-L peptide. In contrast, DnaK did not bind any of the D-peptides with measurable affinity.

EXPERIMENTAL PROCEDURES

Purification of Proteins—DnaK from an overproducing strain of *E. coli* bearing the plasmid pTTQ19dnak$^+$ was purified and prepared for experimentation as described previously (19). Purified DnaK contained less than 0.1 mol of ADP/mol of DnaK (20). Protein concentration was determined photometrically with ϵ_{280} = 14.6 mM^{-1} cm^{-1} (20). DnaJ and GrpE were prepared as described (21, 22); stock solutions were 120 μM and 240 μM, respectively, in 50 μM Tris/HCl, 100 mM NaCl, pH 7.7. Proteins were stored at −80 °C.

Preparation of Peptides—The *all*-L peptide p5′ (NH_2-CALLLSAARR-COOH), *normal all*-D peptide p5′ (NH_2-calllsaarr-$CONH_2$), two *retro all*-D analogs of p5′ (*retro all*-D p5′: NH_2-rraaslllac-$CONH_2$ and *retro all*-D p5′′: NH_2-crraasllla-$CONH_2$), and the N-terminal rhodanese peptide (NH_2-VHQVLYRALVSTKWLAE-COOH) were purchased with a purity of >90% from ANAWA (all sequences are indicated in the conventional CO-NH direction of peptide bonds). Peptides were labeled at their sulfhydryl groups with acrylodan (from Molecular Probes, Eugene, OR) and purified as published (19). The mass of monolabeled peptides was checked by mass spectrometry. Labeled and unlabeled peptides were stored at −20 °C as 1 mM stock solutions in 0.1% acetic acid containing 10 mM dithiothreitol. Samples of *all*-L peptide p6 (NH_2-CARSLLLSS-COOH), synthesized and purified as described (19), and *normal all*-D p5′ were labeled at the sulfhydryl group with *N*-[*ethyl*-2-

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[1] The abbreviations used are: Hsp70, 70-kDa heat shock protein; NEM, *N*-ethylmaleimide; *NEM, *N*-[*ethyl*-2-^{3}H]maleimide; *a*-p5′, acrylodan-labeled peptide p5′.

[2] S. M. Gisler, E. V. Pierpaoli, and P. Christen, submitted for publication.

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TABLE I
Peptides used in this study: fluorescence properties and K_d values of their complexes with DnaJ

Amino acid sequences are given in conventional peptide bond direction, L- and D-amino acids are denoted by capital and lowercase letters, respectively. The acrylodan group is attached at the single cysteine residue. K_d values were determined as described in the legend to Fig. 2 for *retro all-D* p5′-*a*, except that in the case of the other peptides the differences in fluorescence at 520 nm were entered into the equation.

Sequence	Denotation	λ_{max}^{em}	K_d
		nm	*μM*
H_2N-CLLLSAPRR-COOH	*all*-L *a*-p5	520	37[a]
H_2N-CALLLSAARR-COOH	*all*-L-*a*-p5′	522	9.2
H_2N-calllsaarr-$CONH_2$	*normal all*-D *a*-p5′	520	6.8
H_2N-rraaslllac-$CONH_2$	*retro all*-D p5′-*a*	490	0.9
H_2N-crraaslla-$CONH_2$	*retro all*-D *a*-p5″	520	1.9

[a] From Ref. 7.

^{3}H]maleimide (*NEM) as follows. 50 μl of *NEM (50 μCi, 60 Ci/mmol) in pentane was mixed with the same volume of 50 mM Hepes, pH 7.0. Pentane was removed by evaporation, and 5 μl of a 1 mM peptide solution were added. After 5 min, 50 μl of 5.3 mM nonradioactive NEM was added and the solution was kept for 1 h at 25 °C. *NEM-labeled peptides were purified to homogeneity by high performance liquid chromatography using an analytical reversed-phase C-8 column. The specific radioactivity of the peptides was about 7000 cpm/pmol.

Fluorescence Measurements—Fluorescence spectra of the acrylodan-labeled peptides and their reactions with the chaperones were recorded with a Spex Fluorolog spectrofluorimeter. The excitation wavelength was set at 370 nm (band pass 4.6 nm). For fluorescence titration, the reactions were followed at an emission wavelength of 500 nm (band pass 4.5 nm) until equilibrium was reached. All reactions were performed in assay buffer (25 mM Hepes-NaOH, 100 mM KCl, pH 7.0), if not indicated otherwise, at 25 °C.

Size Exclusion Chromatography—Gel filtration experiments were performed on a fast protein liquid chromatography system from Amersham Pharmacia Biotech using a Superose 12 column (10 × 300 mm) at a flow rate of 0.5 ml/min. Running buffer was 25 mM Tris/HCl, 100 mM KCl, pH 7.5. The peptides were detected by their absorption at 225 nm.

Materials—[2,5,8′-^{3}H]Adenosine 5′-triphosphate ammonium salt, 45 Ci/mmol, was purchased from Amersham Pharmacia Biotech and *N*-[*ethyl*-2-^{3}H]maleimide, 60 Ci/mmol, from NEN Life Science Products. *N*-Ethylmaleimide was purchased from Fluka. Poly(ethyleneimine)-cellulose F thin layer plates were from Merck.

RESULTS AND DISCUSSION

Binding of Acrylodan-labeled D-Peptides to DnaJ—The labeled peptide *a*-p5 (CLLLSAPRR), which represents part of the presequence of mitochondrial aspartate aminotransferase, has previously been shown to bind to both DnaK and DnaJ, with K_d values of 0.06 μM and 37 μM, respectively (7). The similar *all*-L peptide *a*-p5′ (CALLLSAARR), which binds to DnaK with a K_d of 0.1 μM (not shown), was used as reference peptide in this study. Addition of DnaJ to *normal all*-D *a*-p5′ and *retro all*-D *a*-p5′′ (for the structures of the peptides, see Table I) resulted in an increase in fluorescence and a shift in the emission maximum from 526 nm to 520 nm, indicating binding of these peptides (Fig. 1). Similar binding-induced changes in fluorescence were observed with *all*-L *a*-p5 and *all*-L *a*-p5′ (Table I), suggesting that the acrylodan-labels of DnaJ-bound *normal all*-D *a*-p5′ and *retro all*-D *a*-p5′′ are located in an environment similar to that of the DnaJ-bound *all*-L peptides. In contrast, addition of DnaJ to *retro all*-D p5′-*a*, which has the fluorophore attached to its C-term, shifted the fluorescence maximum to 490 nm. This more pronounced blue shift (Fig. 2) is an exception among all peptides tested and suggests that the acrylodan label of *retro all*-D p5′-*a* is situated at the opposite site of the peptide-binding region of DnaJ (Table I). This interpretation implies that *retro all*-D *p5′-a*, as the other tested D-peptides, binds with the same peptide backbone direction as the L-peptides. *All*-L and *normal all*-D peptides were bound in a biphasic process, while the binding of both *retro all*-D peptides could be fitted to single-exponential functions (Fig. 1). The two *retro all*-D peptides are the most strongly binding ligands; *normal all*-D *a*-p5′ binds with lower affinity, similar to that of *all*-L *a*-p5′ (Table I). For comparison, the 17-residue N-terminal peptide of rhodanese has been reported to bind with a $K_d < 20$ nM to DnaJ but not to DnaK (23). We used this high affinity peptide ligand to check the specificity of binding of the tested D-peptides. Addition of 4 μM unlabeled rhodanese peptide to preformed complexes of *normal all*-D *a*-p5′ and *retro all*-D p5′-*a* with DnaJ (conditions as described in the legend to Fig. 1, except that 1.5 μM DnaJ was used) decreased the fluorescence signal of the DnaJ-bound labeled peptides in a time-dependent

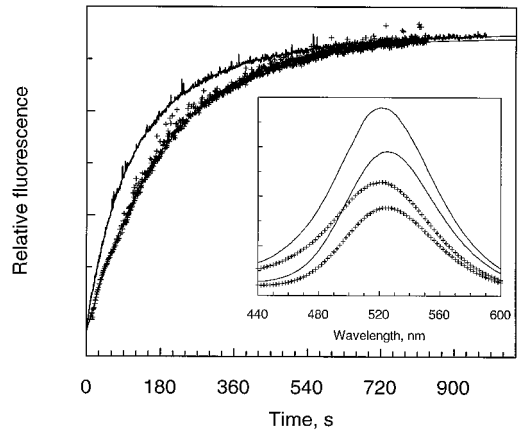

FIG. 1. **Binding of D-peptides to DnaJ.** Reactions were started by adding 1 μM DnaJ to 50 nM acrylodan-labeled *normal all*-D *a*-p5′(——) or *retro all*-D *a*-p5′′(+) in assay buffer. The reaction curves were fitted either to a double-exponential function with $k_{obs1} = 0.013\ s^{-1}$ and $k_{obs2} = 0.004\ s^{-1}$ in the case of *normal all*-D *a*-p5′, the first and second phase contributing 55% and 45% to the total amplitude, respectively, and to a single-exponential function with $k_{obs} = 0.0052\ s^{-1}$ in the case of *retro all*-D *a*-p5′′. The reaction traces of the *retro all*-D peptide and the *normal all*-D were normalized to the same total amplitude. The reaction of *retro all*-D p5′-*a* could also be fitted to a single-exponential function with $k_{obs} = 0.0058\ s^{-1}$ (not shown). *Inset*, corresponding spectra of *normal all*-D *a*-p5′ (——) and *retro all*-D *a*-p5′′(+) before and after addition of DnaJ. For details of the fluorescence measurements, see "Experimental Procedures."

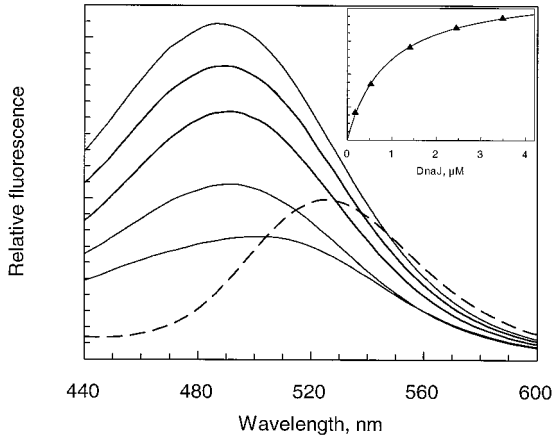

FIG. 2. **Fluorescence titration of the acrylodan-labeled *retro all-D* p5′ with DnaJ.** *Retro all*-D p5′-a (50 nm) in assay buffer (— — —) was titrated with increasing concentrations of DnaJ, and emission spectra were recorded after reaching equilibrium (——). *Inset,* the titration curve was obtained by plotting the differences in fluorescence at 490 nm against the total DnaJ concentration and fitting these data points to the following nonlinear regression equation: $\Delta F = (\Delta F_{max}/2 \times L_t) \times \{(L_t + P_t + K_d) - [(L_t + P_t + K_d)^2 - (4 \times L_t \times P_t)]^{0.5}\}$, where ΔF is the change in relative fluorescence, L_t is the total concentration of acrylodan-labeled peptide, and P_t is the total concentration of DnaJ. The calculated value of K_d was 0.9 μM.

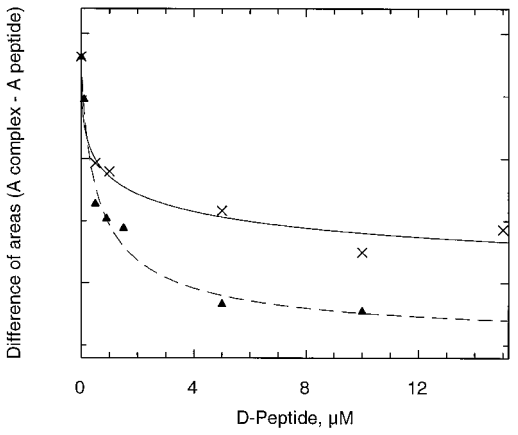

FIG. 3. **Competition of binding of *all-L a*-p5′ to DnaJ by D-peptides.** 0.9 μM DnaJ was added to 50 nM *all*-L *a*-p5′ in assay buffer containing *retro all*-D p5′ (▲) or *normal all*-D p5′ (X) at the indicated concentrations. Fluorescence spectra were recorded before and after the addition of DnaJ when equilibrium had been reached (after ~20 min). Each data point represents the difference in the integrated area from λ_{440} to λ_{600} between the spectrum of *all*-L *a*-p5′ prior to addition of DnaJ and the spectra after reaction with DnaJ in the presence of the respective D-peptide.

TABLE II
Stimulation of DnaK ATPase by DnaJ in the presence and absence of peptides

ATPase activity was determined in 50-μl reaction mixtures at 25 °C as described (20). In the absence of DnaJ, DnaK (10 μM) was preincubated for 30 min with saturating concentrations of peptides, before GrpE (5 μM) was added, and after another 5 min the reaction was initiated by the addition of 1.1 mM ATP, containing 2 μCi of [2,5,8′-^{3}H]ATP. In the experiments with DnaJ (1 μM), the co-chaperone was preincubated with peptides for 30 min before DnaK and GrpE were added. Samples of 5 μl were removed from the reaction mixtures at different times between 30 s and 8 min and quenched by formic acid. ATP and ADP were separated by thin layer chromatography and quantified by liquid scintillation counting. Rates were determined by a linear regression of all 9 data points of the amount of generated ADP *versus* times (not shown).

Peptide	Concentration	DnaJ	k_{cat}
	μM		min^{-1}
None		−	0.03
Retro all-D p5′	50	−	0.04
Normal all-D p5′	50	−	0.04
Normal all-L p5′	50	−	0.11
None		+	1.9
Retro all-D p5′	50	+	1.9
Retro all-D p5′	150	+	2.6
Normal all-D p5′	150	+	0.82
Normal all-L p5′	150	+	0.23

process (not shown); however, for yet unknown reasons, the fluorescence signal was not abolished completely. Apparently, the rhodanese peptide and the D-peptides bind at least in part to the same site. The hydrophobic acrylodan label itself seems to contribute only little to the binding; the K_d value of acrylodan-labeled 2-mercaptoethanol proved to be > 800 μM.[3]

Competition of Binding of all-L Peptide to DnaJ—To confirm that the D-analogs were bound to the same binding site as the L-peptides, we performed competition experiments in which unlabeled *normal all*-D p5′ and *retro all*-D p5′ competed with 50 nM labeled reference peptide *all*-L *a*-p5′ for binding to DnaJ (Fig. 3). The IC_{50} value, *i.e.* the concentration at which the D-peptides inhibited binding of *a*-p5′ to 50%, was 0.6 μM and 2.2 μM for the *retro all*-D and the *normal all*-D peptide, respectively. The K_i values were calculated, assuming competitive inhibition, with Equation 1 (24, 25)

$$K_i = \frac{IC_{50}}{1 + [a - p5']/K_d} \quad \text{(Eq. 1)}$$

where K_d (9.2 μM; Table I) is the equilibrium dissociation constant of DnaJ for *all*-L *a*-p5′ and $[a\text{-p5}'] \approx [a\text{-p5}']_{free}$, being > 90% of added *a*-p5′ in the absence of competitor.

The K_i for the *retro all*-D analog (0.6 μM) proved to be virtually the same as its IC_{50} value and close to the K_d of its acrylodan-labeled derivative determined by fluorescence titration (0.9 μM). In the case of the *normal all*-D peptide, the calculated K_i (2.2 μM) was identical with the IC_{50} value and of the same order of magnitude as the K_d value obtained by titration (6.8 μM). Apparently, the D-peptides bind to the same site in DnaJ as *all*-L *a*-p5′.

Testing of D-Peptides for Interaction with DnaK—On addition of DnaK to the acrylodan-labeled D-analogs we did not observe any fluorescence changes, probably reflecting a very low affinity of the chaperone for the pseudo-peptides (not shown). The stimulation of the ATPase activity by peptide ligands offers an additional possibility to examine the interaction of DnaK with potential ligands (20, 26, 27). The *normal* and the *retro all*-D peptide p5′ only weakly stimulated DnaK ATPase by a factor of 1.3, as compared with the 3.5-fold stimulation in the case of the reference L-peptide (Table II), again indicating only a weak interaction between D-peptides and DnaK. This notion was confirmed by a gel filtration experiment with radioactively labeled peptides. As a representative of the D-analogs we used *NEM-labeled *normal all*-D p5′ and as a positive control for DnaK binding *NEM-labeled peptide p6 which is known to be a high affinity ligand of DnaK (K_d in the micromolar range[4]). After preincubation of DnaK and peptides for 2 h, the DnaK-peptide complex was separated from unbound ligand by Superose 12 chromatography. In the D-peptide experiment, DnaK eluted without radioactivity; apparently, the interaction between DnaK and the D-analog is too weak to be detected by gel filtration. In the control experiment, DnaK-containing fractions showed radioactivity, indicating formation of a stable complex (not shown).

Inhibition of the Stimulatory Effect of DnaJ on DnaK ATPase Activity—The rate of steady-state ATP hydrolysis of DnaK is stimulated by DnaJ up to 200-fold (2, 7). Because the D-peptides bind to DnaJ but not to DnaK, we investigated their effect on the ability of DnaJ to stimulate the steady-state ATPase activity of DnaK (Table II). We preincubated DnaJ with saturating concentrations of peptides, then added DnaK plus GrpE and followed the generation of ADP. Peptide *retro all*-D p5′ slightly stimulated the ATPase activity in the presence of DnaJ. The binding of *retro all*-D p5′ without concomitant inhibition of DnaK ATPase indicates that the co-chaperone DnaJ possesses two separate binding sites for peptides and DnaK. *Normal all*-D p5′ and *all*-L p5′ markedly decreased the DnaJ-stimulated ATPase activity of DnaK to 44% and 12%, respectively, of the activity in the absence of peptide (Table II). It remains to be explored whether this inhibitory effect is due to direct binding of these peptides to the DnaK recognition site of DnaJ or to an allosteric effect caused by binding to the peptide-binding site that hinders or prevents interaction with DnaK.

Implications—1) In contrast to DnaK, DnaJ recognizes peptide-mimetics that are composed exclusively of D-amino acids. For DnaK, neither the identical positions of the side chains in the case of *retro all*-D analogs nor the same direction of the peptide backbone (*normal all*-D peptide) suffice to mimic the reference peptide p5′. Interactions with both the peptide back-

[3] E. V. Pierpaoli, personal communication.

[4] S. M. Gisler, E. V. Pierpaoli, H.-J. Schönfeld, and P. Christen, manuscript in preparation.

bone and the side chains seem to limit the binding capacity strictly to L-peptides. DnaJ, however, seems to be less exacting with respect to the spatial orientation of side chains of the target peptide. All tested L- and D-peptides were recognized as ligands, binding with different spectral and kinetic features. 2) Spectral differences between DnaJ complexes of peptide *retro all*-D p5′-a that has the fluorophore attached to the C-term and the other peptides (Table I) suggest that *retro all*-D p5′-a binds with unchanged backbone direction and thus, like *normal all*-D *a*-p5′, with enantiomorphic side chain positions. 3) The peptides *all*-L *a*-p5′, *normal all*-D *a*-p5′, and *retro all*-D p5′-a differentially inhibit DnaJ in stimulating the DnaK ATPase activity, *i.e.* the extent of DnaJ-induced ATPase activity of DnaK depends on the nature of the DnaJ-bound peptide ligand. 4) DnaJ apparently possesses two binding sites, one for polypeptides, binding *all*-L as well as *all*-D peptides, and one for interaction with DnaK. Upon binding of peptides, DnaJ may control the action of the DnaK system by means of an allosteric regulation of its DnaK-interaction site.

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REFERENCES

1. Hartl, F. U. (1996) *Nature* **381,** 571–580
2. Martin, J., and Hartl, F. U. (1997) *Curr. Biol.* **7,** 41–52
3. Schatz, G., and Dobberstein, B. (1996) *Science* **271,** 1519–1526
4. Horst, M., Opplinger, W., Feifel, B., Schatz, G., and Glick, B. S. (1996) *Protein Sci.* **5,** 759–767
5. Harrison, J. C., Hayer-Hartl, M., Di Liberto, M., Hartl, F. U., and Kuriyan, J. (1997) *Science* **276,** 431–435
6. Zhu, X., Zhao, X., Burkholder, W. F., Gragerov, A., Ogata, C. M., Gottesmann, M. E., and Hendrickson, W. A. (1996) *Science* **272,** 1606–1614
7. Pierpaoli, E. V., Sandmeier, E., Baici, A., Schönfeld, H.-J., Gisler, S., and Christen, P. (1997) *J. Mol. Biol.* **269,** 757–768
8. Theyssen, H., Schuster, H.-P., Packschies, L., Bukau, B., and Reinstein, J. (1996) *J. Mol. Biol.* **263,** 657–670
9. Georgopoulos, G. (1992) *Trends Biochem. Sci.* **17,** 295–299
10. Bork, P., Sander, C., Valencia, A., and Bukau, B. (1992) *Trends Biochem. Sci.* **17,** 129–132
11. Szyperski, T., Pellecchia, M., Wall, D., Georgopoulos, C., and Wüthrich, K. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 11343–11347
12. Georgopoulos, C., Liberek, K., Zylicz, M., and Ang, D. (1993) in *The Biology of Heat Shock Proteins and Molecular Chaperones* (Morimoto, R., Tissieres, A., and Georgopoulos, C., eds) pp. 209–245, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
13. Schröder, H., Langer, T, Hartl, F. U., and Bukau, B. (1993) *EMBO J.* **12,** 4137–4144
14. Langer, T., Lu, C., Echols, H., Flanagan, J., Hayer, M., and Hartl, F. U. (1992) *Nature* **356,** 683–689
15. Chorev, M., and Goodman, M. (1995) *Trends Biotechnol.* **13,** 438–445
16. Binkirane, N., Guichard, G., Van Regenmortel, M. H., Briand, J.-P., and Muller, S. (1995) *J. Biol. Chem.* **270,** 11921–11926
17. Ruovo, M., and Fassina, G. (1995) *Int. J. Peptide Protein Res.* **45,** 356–365
18. Bartnes, K. Hannestad, K., Guichard, G., and Briand, J. P. (1997) *Eur. J. Immunol.* **27,** 1387–1391
19. Schmid, D., Baici, A., Gehring, H., and Christen, P. (1994) *Science* **263,** 971–973
20. Feifel, B., Sandmeier, E., Schönfeld, H.-J., and Christen, P. (1996) *Eur. J. Biochem.* **237,** 318–321
21. Schönfeld, H.-J., Schmid, D., and Zulauf, M. (1995) *Progr. Colloid Polymer Sci.* **99,** 7–10
22. Schönfeld, H.-J., Schmid, D., Schröder, H., and Bukau, B. (1995) *J. Biol. Chem.* **270,** 2183–2189
23. Kudlicki, W., Odom, O. W., Kramer, G., and Hardesty, B. (1996) *J. Biol. Chem.* **271,** 31160–31165
24. Cheng, Y.-C., and Prusoff, W. H. (1973) *Biochem. Pharm.* **22,** 3099–3108
25. Takeda, S., and McKay, B. (1996) *Biochemistry* **35,** 4636–4644
26. Jordan, R., and McMacken, R. (1995) *J. Biol. Chem.* **270,** 4563–4569
27. McCarty, J. S., Buchberger, A., Reinstein, J., and Bukau, B. (1995) *J. Mol. Biol.* **249,** 126–137