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Specificity of DnaK–peptide Binding

Alexander Gragerov[1,3], Li Zeng[1], Xun Zhao[2], William Burkholder[1,2] and Max E. Gottesman[1,2]

[1]*Institute of Cancer Research,* [2]*Department of Biochemistry and Molecular Biophysics College of Physicians and Surgeons Columbia University, 701 W 168 Street, New York, NY 10032, U.S.A.*

[3]*Institute of Molecular Genetics Russian Academy of Sciences, 123182 Moscow, Russia*

The sequence specificity of DnaK substrate binding has been studied using a peptide display library. Based on the amino acid patterns that appeared in this selection, short peptides were synthesized for direct measurements of DnaK affinity. The results show that peptides enriched in internal hydrophobic residues are preferential DnaK substrates, and negatively charged peptides have poor affinity.

The isolated C-terminal domain of DnaK binds peptides. Peptide dissociation studies indicate that bound peptides are released from the C-terminal fragment and from DnaK at identical rates. ATP stimulates peptide dissociation from DnaK but not from the C-terminal fragment.

Keywords: molecular chaperone; heat shock protein; hsp70; peptide display library; protein folding

1. Introduction

Members of the hsp70 family of heat shock proteins (hsps†) serve in many cellular processes involving unfolded polypeptides (for reviews, see Pelham, 1990; Gething & Sambrook, 1992). These functions entail the capacity to differentiate unfolded from native proteins (for a review, see Rothman, 1989). The mechanism of hsp70 binding to substrate is known mostly through studies with synthetic peptides. Peptides bind to hsp70 in fully extended conformation, and most interactions occur between the protein and peptide backbone (Landry *et al.*, 1991). Peptides differ in their affinity for hsp70s (Flynn *et al.*, 1989), indicating that the absence of a stable secondary structure, common for all peptides, is not the sole determinant of hsp70 recognition. BiP, an hsp70 homologue found in the endoplasmic reticulum, binds peptides with a minimal length of 7 amino acids, and prefers peptides enriched in hydrophobic amino acids (Flynn *et al.*, 1991). Binding of hsp70 to exposed hydrophobic residues is suggested to prevent unfolded (e.g. nascent) polypeptides from aggregation.

In this work, we study the peptide specificity of DnaK, the only *Escherichia coli* hsp70. We first selected peptides with affinity to DnaK in a random peptide-display phage library, and then designed heptapeptides according to the sequences of these phage. Our results indicate that peptides containing internal hydrophobic residues and terminal polar residues are preferential substrates for DnaK. Peptides with a net negative charge bind poorly. We also show that the DnaK C-terminal domain binds peptides. Bound peptides rapidly dissociate when ATP binds to the N-terminal domain.

2. Materials and Methods

(a) *Isolation of DnaK and its C-terminal domain*

DnaK protein was purified from *E. coli* carrying the plasmid pJM2 (McCarty & Walker, 1991) with the *dnaK* operon under the control of the *lac* promoter. Briefly, cells were lysed with lysozyme and sodium deoxycholate; cleared lysate was precipitated with Polymin P (0·8% (v/v) final concentration); proteins were eluted from the Polymin pellet with 1 M NaCl and precipated with $(NH_4)_2SO_4$ (60% saturation). Redissolved proteins were applied to an ATP-agarose column (Zylicz *et al.*, 1987), the column was washed with a buffer plus 0·5 M NaCl, then with the same buffer plus 1 mM GTP, and DnaK was eluted with the same buffer plus 1 mM ATP. Peak fractions were applied to DEAE-Sephacel column, which was

† Abbreviations used: hsp, heat shock protein; PCR polymerase chain reaction; MHC, major *histocompatibility complex*.

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washed and eluted with a linear 50 mM to 250 mM NaCl gradient. DnaK-containing fractions were concentrated and stored frozen at −20°C.

A fragment of *dnaK* encoding the C-terminal domain of the protein (amino acids 383 to 637) was amplified by PCR using the forward primer 5′GCGGATCCATCGAGGGTAGAGGTGACGTAAAAGACGTACTGCTG-3′ containing a *Bam*HI site and coding for Factor Xa cleavage site, and the reverse primer 5′GCCAAGCTTCCCTAGATGAATGCACGG-3′ containing a *Hin*dIII cleavage site. The PCR fragment was inserted into the hexahistidine fusion expression vector, pQE30 (Qiagen). The DnaK C-terminal fragment was purified using Ni-NTA affinity resin essentially as recommended by Qiagen. The imidazole-eluted C-terminal fragment was concentrated and stored at −20°C.

(b) *Selection of DnaK-binding phage from epitope library*

The f1 phage epitope library (Scott & Smith, 1990) was kindly provided by G. P. Smith. The library displays 6 residue random peptides located at positions 5 to 10 of the phage pIII surface protein. The phage library (10^9 plaque-forming units) was mixed with 1 to 5 μg of biotinylated DnaK (see below) for 15 min at room temperature in 300 μl of 10 mM Tris (pH 7·9), 150 mM NaCl. Then 200 μl of streptavidin-coated magnetic beads suspension (Promega) were added for an additional 10 min, and DnaK-bound phage were separated from free phage in a magnetic field. The beads were washed extensively with 10 mM Tris (pH 7·9), 150 mM NaCl, 0·1% (v/v) Tween 20. The presence of non-ionic detergent was required to prevent non-specific (biotinylated protein-independent) binding of phage to magnetic beads. Bound phage were eluted with 1 mM ATP, 5 mM $MgCl_2$, 2 mM $CaCl_2$ in the same buffer. DnaK was biotinylated by treating DnaK at 2 mg/ml in 0·1 M $NaHCO_3$ with 0·4 mM Bio-LC-NHS (Pierce) for 1 h at room temperature. Biotinylated DnaK retained ATPase activity.

(c) *ATPase activity measurements*

DnaK ATPase was measured in 24 μl of 50 mM Hepes (pH 7·9), 40 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 10% (v/v) glycerol, 25 μg ovalbumin/ml, 1 μM ATP, containing 0·2 μCi of [γ^{32}P]ATP (Amersham), and 12 ng of DnaK. Incubation, in the presence or absence of peptides, was at 32°C for varying lengths of time. ATP and ADP were separated by chromatography on PEI plates (Merck) in 0·8 M LiCl, 0·8 M acetic acid. Radioactive spots were visualized and quantified using the Betascope 603 Blot Analyser (Betagen).

(d) *Peptide binding experiments*

Peptides were labelled for binding experiments by reductive methylation with [^{3}H]$NaBH_4$ (Amersham) and formaldehyde (Tack *et al.*, 1980). Radioactive peptides were diluted with the respective unlabelled peptides to the final concentration of 0·1 mM. Labelled peptide was incubated with 10 μg of DnaK in ATPase buffer (without ATP and carrier ovalbumin) for ≥10 min at 37°C, and complexes were separated from unbound peptide on Bio-Gel 30 spincolumns (BioRad). The amount of peptide in the flow-through was determined by scintillation counting and normalized to the amount of DnaK as determined by the Bradford (1976) protein assay (BioRad). In peptide competition experiments, labelled peptide was premixed with varying amounts of unlabelled peptide before DnaK addition. To measure kinetics of peptide–DnaK complex dissociation, preformed complexes were challenged with 50-fold excess of unlabelled peptide at zero time, and portions were withdrawn at various times and immediately spun through the columns.

(e) *Model of DnaK structure*

The model of the DnaK C terminus was constructed using the HOMOLOGY module of the INSIGHT II molecular modelling package (Biosym Technologies) based on the alignment of hsp70 and HLA class I sequences described by Rippmann *et al.* (1991) (model 1). Structural co-ordinates for human MHC class I antigen A2 were used (Bjorkman *et al.*, 1987; Saper *et al.*, 1991; Brookhaven Protein Databank file 3HLA). Surface contours and electrostatic potentials were calculated and displayed using the GRASP program (Anthony Nichols and Barry Honig, Columbia University).

3. Results

To determine if DnaK binds preferentially to peptides of different sequences, we used an f1 phage peptide-display library. The phage carry random six amino acid insertions near the N terminus of the pIII surface protein (Scott & Smith, 1990). The library was mixed with biotinylated DnaK, and the DnaK-bound phage were isolated using streptavidin-coated magnetic beads and magnetic separation (see Materials and Methods). The phage–DnaK complex was dissociated with ATP, and the released phage expanded and the sequence of the insert determined. Control isolations, using biotinylated GroEL or biotinylated total *E. coli* proteins, or unbiotinylated DnaK, yielded 5 to 15-fold less phage in the ATP eluate.

The purified phage were subjected to the same isolation protocol and shown, in fact, to have significantly higher affinity for DnaK than the original library, or wild-type phage (Table 1). The relatively low selectivity of the protocol might reflect the instability of complexes between DnaK and its substrates. Complexes of DnaK with model peptides have short half-lives in the absence of ATP, and are further destabilized by ATP (see below).

We considered the possibility that the six amino acid insert in the selected phage might not represent the true DnaK ligand. It might, for example, distort

Table 1

Binding of f1, f1 epitope library, and three selected phage to biotinylated DnaK

			Selected phage		
	f1	Library	1	2	3
Initial titre ($\times 10^{-8}$)	60	3	60	15	60
ATP eluate ($\times 10^{-2}$)	80	5	600	150	400
Relative affinity	1	1·3	7·7	7·7	5·2

Assays were performed as described in Materials and Methods. Relative affinity compares the ratio of the titre of the ATP eluate to the initial titre with respect to wild-type f1.

Table 2
DnaK-selected peptides

A	D	G	A	**G**	**K**	**W**	**V**	**Y**	**I**	G	A	A	G
A	D	G	A	**A**	**K**	**R**	**E**	**T**	**K**	G	A	A	G
A	D	G	A	**K**	**W**	**V**	**H**	**L**	**F**	G	A	A	G
A	D	G	A	**R**	**L**	**V**	**L**	**V**	**L**	G	A	A	G
A	D	G	A	**W**	**K**	**W**	**G**	**I**	**Y**	G	A	A	G
A	D	G	A	**S**	**S**	**H**	**A**	**S**	**A**	G	A	A	G
A	D	G	A	**W**	**G**	**P**	**W**	**S**	**F**	G	A	A	C
A	D	G	A	**A**	**I**	**P**	**G**	**K**	**V**	G	A	A	G
A	D	G	A	**R**	**V**	**H**	**D**	**P**	**A**	G	A	A	G
A	D	G	A	**R**	**S**	**V**	**S**	**S**	**F**	G	A	A	G
A	D	G	A	**L**	**G**	**T**	**R**	**K**	**G**	G	A	A	G
A	D	G	A	**K**	**D**	**P**	**L**	**F**	**N**	G	A	A	G
A	D	G	A	**L**	**S**	**Q**	**H**	**T**	**N**	G	A	A	G
A	D	G	A	**N**	**R**	**L**	**L**	**L**	**T**	G	A	A	G
A	D	G	A	**Y**	**P**	**L**	**W**	**V**	**I**	G	A	A	G
A	D	G	A	**L**	**L**	**I**	**I**	**D**	**R**	G	A	A	G
A	D	G	A	**R**	**V**	**I**	**S**	**L**	**Q**	G	A	A	G
A	D	G	A	**E**	**V**	**S**	**R**	**E**	**D**	G	A	A	G
A	D	G	A	**S**	**I**	**L**	**R**	**S**	**T**	G	A	A	G
A	D	G	A	**P**	**G**	**L**	**V**	**W**	**L**	G	A	A	G
A	D	G	A	**V**	**K**	**K**	**L**	**Y**	**I**	G	A	A	G
A	D	G	A	**N**	**N**	**R**	**L**	**L**	**D**	G	A	A	G
A	D	G	A	**S**	**K**	**G**	**R**	**W**	**G**	G	A	A	G
A	D	G	A	**I**	**R**	**P**	**S**	**G**	**I**	G	A	A	G
A	D	G	A	**A**	**S**	**L**	**C**	**P**	**T**	G	A	A	G
A	D	G	A	**D**	**V**	**P**	**G**	**L**	**R**	G	A	A	G
A	D	G	A	**R**	**H**	**R**	**E**	**V**	**Q**	G	A	A	G
A	D	G	A	**L**	**A**	**R**	**K**	**R**	**S**	G	A	A	G
A	D	G	A	**S**	**V**	**L**	**D**	**H**	**V**	G	A	A	G
A	D	G	A	**N**	**L**	**L**	**R**	**R**	**A**	G	A	A	G
A	D	G	A	**S**	**G**	**I**	**S**	**A**	**W**	G	A	A	G
A	D	G	A	**F**	**Y**	**P**	**W**	**V**	**R**	G	A	A	G
A	D	G	A	**K**	**L**	**F**	**L**	**P**	**L**	G	A	A	G
A	D	G	A	**T**	**P**	**T**	**L**	**S**	**D**	G	A	A	G
A	D	G	A	**T**	**H**	**S**	**L**	**I**	**L**	G	A	A	G
A	D	G	A	**L**	**L**	**L**	**L**	**S**	**R**	G	A	A	G
A	D	G	A	**L**	**L**	**R**	**V**	**R**	**S**	G	A	A	G
A	D	G	A	**E**	**R**	**R**	**S**	**R**	**G**	G	A	A	G
A	D	G	A	**R**	**M**	**L**	**Q**	**L**	**A**	G	A	A	G
A	D	G	A	**R**	**G**	**W**	**A**	**N**	**S**	G	A	A	G
A	D	G	A	**R**	**P**	**F**	**Y**	**S**	**Y**	G	A	A	G
A	D	G	A	**S**	**S**	**S**	**W**	**N**	**A**	G	A	A	G
A	D	G	A	**L**	**G**	**H**	**L**	**E**	**E**	G	A	A	G
A	D	G	A	**S**	**A**	**V**	**T**	**N**	**T**	G	A	A	G

N termini of sequenced f1 phage pIII proteins are shown. Variable epitope amino acids are in bold.

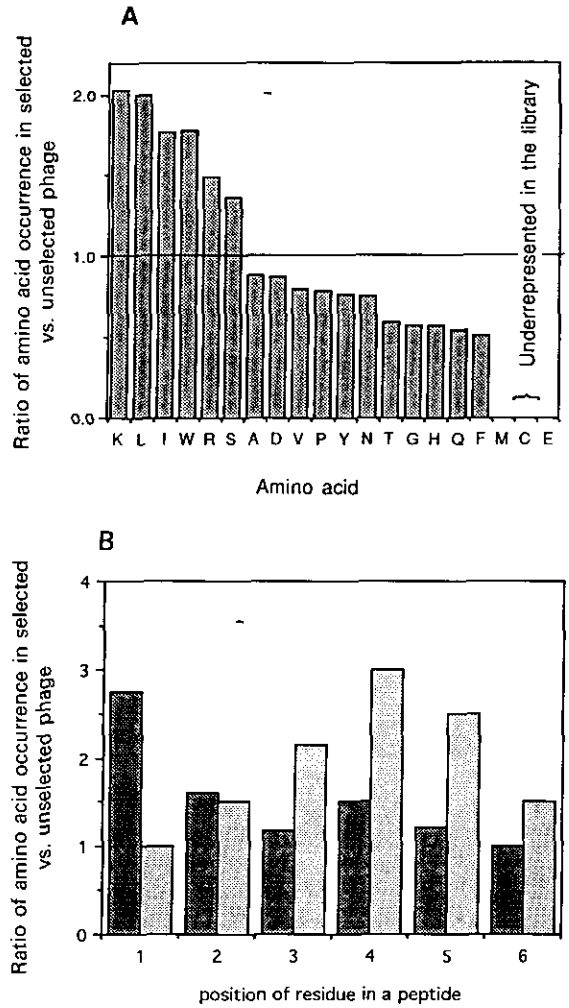

Figure 1. A, Ratio of frequencies of occurrence of amino acid residues in peptides exposed on the surface of DnaK-selected phage and unselected phage. The 3 last amino acids are significantly underrepresented in the unselected pool. B, Distribution of frequently occurring amino acids in DnaK-selected peptides. Positively charged and hydrophobic residues were grouped to reduce random variations due to taking ratios of small numbers. K + R, heavy shading; L + I + W, light shading.

pIII, revealing a DnaK ligand located elsewhere in the pIII protein. To test this notion, we compared the thermal stabilities of the selected and unselected phage. The thermal stability of f1 phage is determined by the resistance of pIII protein to heating (Rossomando & Zinder, 1968). Although some selected phage were labile, the majority of the isolated clones were identical in thermal stability to wild-type f1 (data not shown). This result supports the idea that the inserted peptide did, in fact, represent a preferential DnaK substrate.

Forty-four selected phage (Table 2) and 48 unselected phage from the random pool were sequenced. Insert amino acid frequencies in the random phage pool corresponded roughly to the values expected from codon frequencies, with the exception of Met, Cys and Glu, which were strongly underrepresented.

The data of Table 2 are analysed in Figure 1. While there is no apparent consensus sequence, clones with affinity for DnaK were enriched in hydrophobic and positively charged amino acids. The hydrophobic amino acids often appeared in tandem, and tended to be located near the centre of the peptide epitope. In contrast, positively charged residues were found principally at N-terminal positions.

Given the large size of phage as compared with the target peptide, and a potential for DnaK to interact weakly with the vast phage particle surface, we did not feel that the epitope library approach was completely free of artefacts. Therefore, we used the epitope library data as a guide to synthesize defined peptides. We chose initially three peptides: dodecamer F6 (MQER-ITLKDYAM) known to have affinity for DnaK (Sherman & Goldberg, 1991), and two heptapep-

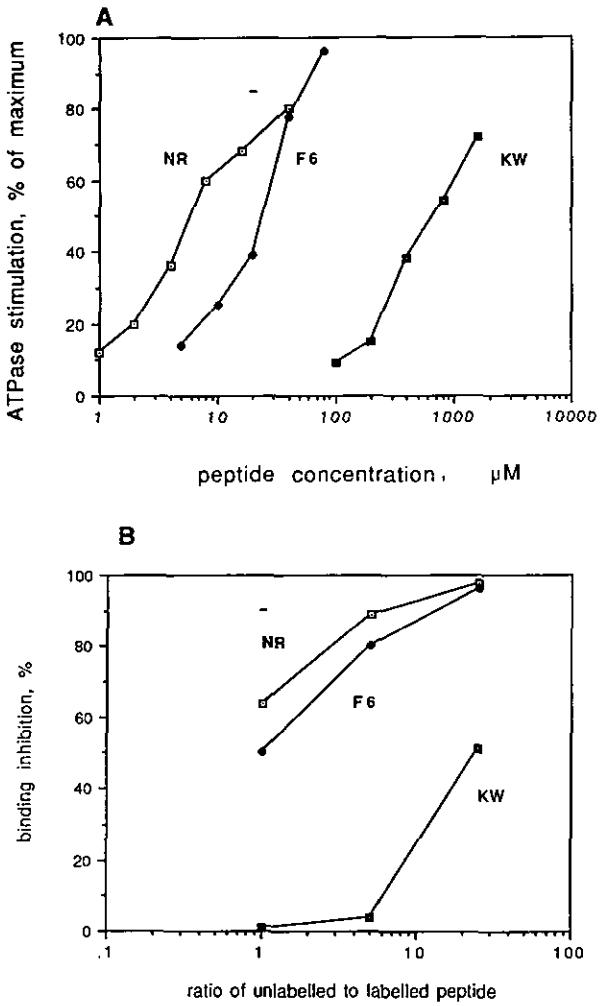

Figure 2. Two methods of estimating the relative affinity of a peptide to DnaK. The same 3 peptides were tested in A, stimulation of ATPase activity, showing affinity of a peptide to DnaK in the presence of ATP; and in B, binding competition experiments, in which ^{3}H-labelled F6 peptide was premixed with varying amounts of unlabelled peptides prior to DnaK addition. These experiments show the relative affinity of peptides to DnaK in the absence of ATP.

tides, KW (KWVHLFG) and NR (NRLLLTG), corresponding in sequence to the peptide epitopes of individual selected phage (see Table 2). The heptapeptides carry a Gly residue added at the C terminus as a seventh residue: heptapeptides are the minimal length peptides that significantly stimulate BiP ATPase activity (Flynn *et al.*, 1991), and Gly is the C-terminal neigbour of the variable peptide epitope in the phage library. Our decision to concentrate on short peptides was based on two considerations. First, short peptides allow better definition of the real binding site in a peptide. Second, they limit the number of sequences that must be tested in order to obtain meaningful results.

The affinity of a given peptide for DnaK was measured by two assays. First, the stimulation of DnaK ATPase as a function of peptide concentration was determined. Second, we measured the binding of ^{3}H-labelled peptide to DnaK, using spin-columns to separate free from bound peptide (Flynn *et al.*, 1989). Note that in the last assay only one peptide was labelled. The affinity of the remaining peptides was evaluated in competition experiments with the labelled peptide. Both methods gave qualitatively comparable values for the relative affinities of the different peptides for DnaK (Fig. 2), although they measure binding under different conditions. The ATPase assay determines affinity in the presence of ATP, whereas the direct binding assay measures affinity in the absence of nucleotide.

Table 3

Specificity of DnaK–peptide interaction

Peptide	Sequence	Affinity	K_a of ATPase stimulation
Kemptide	LRRASLG	−	>200
Kemptide*	LRRWSLG	+	>200
KW	KWVHLFG	+	1000
NR	NRLLLTG	++++	10
1	ARLLLTG	++++	nd
2	NALLLTG	++	20
3	NRLALTG	++	30
4	NDLLLTG	−	nd
5	NLLRLTG	++++	nd
6	LLTNRGL	−	nd
7	NRLWLTG	+++	nd
8	NRLLLAG	++++	nd

The Table shows results from the study of DnaK substrate specificity with heptapeptides. Kemptide and kemptide* are peptide substrates for protein kinase A included as arbitrary heptapeptides. KW and NR peptides correspond to 2 phage-displayed epitopes selected for DnaK binding. The remaining peptides are versions of the peptide NR. The column Affinity summarizes the results of competition experiments with labelled peptide F6 and unlabelled peptides. The symbols indicate the effectiveness of each peptide in the competition relative to peptide NR: ++++, the peptide inhibits binding at the same concentration as does NR; +++, at a concentration 1·5 times higher than required for NR; ++, at a concentration 2 to 3 times higher than required for NR; +, at a concentration approximately 10 times higher than required for NR; −, no competition at concentrations tested. The column K_a shows the results of ATPase stimulation experiments for selected peptides. Stimulatory concentrations of the peptides in μM are shown; the error of these experiments constitutes approximately 30% of the value; nd, not determined. Both types of experiments were performed as shown in Fig. 2.

The DnaK binding affinities of 12 heptapeptides and the activation constants for DnaK ATPase for some of the peptides are summarized in Table 3. The peptides vary widely in affinity. The differences in DnaK affinity of peptides KW and NR is around 100-fold, both in the presence or absence of ATP.

Peptide NR binds best to DnaK of the four peptides shown in the upper part of the Table. To probe the structural basis for the strong binding, we tested different substituted versions of peptide NR (designated by numbers in Table 3). Comparison of peptides NR, 3, and 7 demonstrates the importance of the tandem hydrophobic residues. Interruption of the stretch of three consecutive Leu residues in NR with Ala (peptide 3) significantly reduced binding. The more hydrophobic Trp located in the same position (peptide 7) was less inhibitory. Peptide 6 is

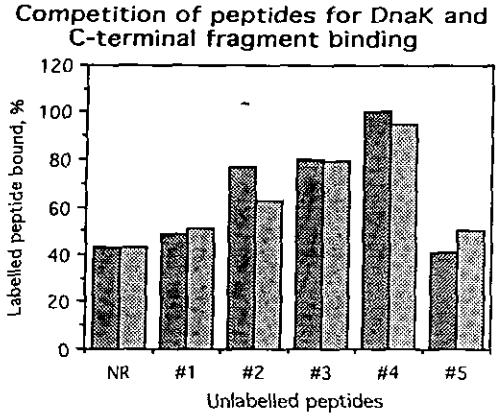

Figure 3. Relative affinities of different peptides to DnaK (heavy shading) and its C-terminal (light shading) fragment. The bars represent inhibition of [^{3}H]F6 by equimolar amounts of different peptides.

a permutation of NR; the hydrophobic residues have been displaced to the termini, and the polar residues internally. This peptide bound with low affinity, implying that the hydrophobic residues must be centrally located. Substitution of the positively charged Arg residue for a neutral Ala (peptide 2), reduced but did not eliminate binding. However, a negatively charged Asp (peptide 4) drastically reduced affinity for DnaK. The position of the Arg may not be relevant (peptide 5). The apparent preference of a positively charged residue at the N terminus of peptides selected from the epitope library might reflect the influence of neighbouring residues of the recombinant pIII protein, e.g. Asp at position two (see Table 2).

DnaK and other hsp70s consist of at least two domains, an N-terminal domain with ATPase activity (Chappell *et al.*, 1987; Cegielska & Georgopoulos, 1989; Flaherty *et al.*, 1990) and a C-terminal, presumably responsible for peptide binding. We cloned and expressed the C-terminal

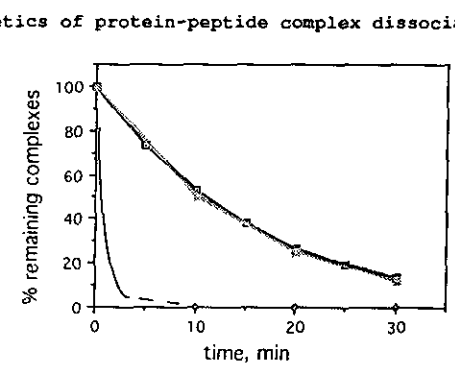

Figure 4. Dissociation kinetics of F6-DnaK (■) and F6-C-terminal fragment (⊡) complexes. The complexes of the proteins and [^{3}H]F6 were formed at 37 °C for 10 min, and challenged with 50-fold excess of unlabelled F6. Where indicated, ATP (1 mM) was added together with unlabelled peptide: (◇) DnaK + ATP; (◆) C-terminal fragment + ATP.

domain in *E. coli*, and purified the polypeptide to near homogeneity (see Materials and Methods). The relative affinities of the heptapeptides for the C-terminal domain were identical to intact DnaK (Fig. 3). The rates of dissociation of peptides bound to DnaK or to the C-terminal fragment were likewise identical. However, the DnaK complexes, unlike the C-terminal fragment complexes, were significantly destabilized by ATP (Fig. 4). We conclude that, in the absence of ATP, the peptide binding properties of DnaK are determined solely by the C-terminal domain. ATP bound to the N-terminal domain evidently alters the C-terminal domain in a way that compromises the stability of the DnaK–peptide complex.

4. Discussion

We have explored the substrate specificity of DnaK and demonstrated that its C-terminal domain is responsible for peptide binding. The N and C-terminal domains interact: peptide binding stimulates the ATPase activity of the N-terminal domain, whereas ATP stimulates the dissociation of the bound peptide (Flynn *et al.*, 1989). We note that the half-life of the peptide–DnaK complex in the presence of ATP is considerably shorter than the rate of ATP hydrolysis. This indicates that peptide dissociation is stimulated by ATP binding, and that hydrolysis may occur after several cycles of peptide binding and release. Other kinetic measurements of DnaK support this idea (A. Fink & R. McMacken, personal communications).

Secondary structure predictions suggest that the C-terminal domain of DnaK resembles the major histocompatibility (MHC) antigens which, like the hsp70s, can bind peptides (Rippmann *et al.*, 1991). The most prominent structural feature of MHC antigens is a deep peptide-binding cleft. We have constructed a model based on an alignment of MHC and hsp70s structures (Rippmann *et al.*, 1991, model 1), substituting residues of DnaK for those of MHC class I antigen A2 with known structure (Bjorkman *et al.*, 1987; Saper *et al.*, 1991). The model displays a putative peptide binding cleft, whose characteristics may explain the peptide binding specificities of DnaK. Consistent with the requirement for internal hydrophobic amino acids in substrate peptides, the middle of the peptide-binding cleft reveals a hydrophobic cluster. In addition, both the surface of the C-terminal domain and the peptide binding cleft are predominantly negatively charged. The bottom of the cleft is largely negatively charged, but contains a positively charged region at one extremity. These features agree well with the charge dependence for peptide binding; positively charged peptides are preferred to peptides bearing negatively charged residues.

How does the specificity of peptide recognition relate to the interaction of DnaK with its *in vivo* substrates? DnaK plays a role in many cellular processes that have a common theme, the recognition of unstructured polypeptide chains. These

include: the productive folding of newly synthesized proteins (Gragerov *et al.*, 1991, 1992), renaturation of denatured proteins *in vivo* (Gaitanaris *et al.*, 1990) and *in vitro* (Langer *et al.*, 1992), the secretion of periplasmic proteins (Phillips & Silhavy, 1990; Wild *et al.*, 1992), and the degradation of unstable proteins (Straus *et al.*, 1988). DnaK has been found associated with ribosome-bound nascent polypeptides (G. Gaitanaris *et al.*, unpublished results), and with polypeptides destined for proteolysis (Sherman & Goldberg, 1992), including σ^{32} (Liberek *et al.*, 1992; Gamer *et al.*, 1992).

Although DnaK binds to peptides in the fully extended conformation (Landry *et al.*, 1991), absence of stable structure is not the sole determinant of polypeptide recognition. Different structureless peptides vary significantly in their affinity for DnaK (this work) or BiP (Flynn *et al.*, 1991). The sequence specificities of DnaK and BiP are similar; both hsp70s prefer peptides with internal hydrophobic residues. Such residues may be buried within a native protein and are displayed only during synthesis or after denaturation. A limited search of the protein database of proteins with known structures indicates that runs of hydrophobic residues occur only in the non-polar cores of the proteins or at intersubunit boundaries (data not shown). Alternatively, preference for apolar residues may be important not only for the recognition, but also for protection of these residues from intermolecular interactions which may lead to aggregation. Finally, there is a possibility that an interaction of DnaK with a loosely folded protein that displays hydrophobic core amino acids may lead to DnaK-driven protein unfolding.

The significance of the preference of DnaK for positively charged peptides is less obvious. We note that the signal peptides of secretory proteins carry an N-terminal positive charge, followed by a run of hydrophobic amino acids. Signal peptides should, therefore, have affinity for DnaK. Although a global role for DnaK in secretion has not been demonstrated, genetic evidence for a redundancy between DnaK and SecB has, in fact, been reported (Wild *et al.*, 1992). Peptide binding properties of DnaK and SecB (Randall, 1992) also bear general similarity.

Finally, the sequence preference shown by DnaK, while similar to that of BiP, differs in certain details (A. Gragerov *et al.*, unpublished results). This may not be surprising in view of the differences in the polypeptides that react with these proteins *in vivo*.

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