# The insect antimicrobial peptide, L-pyrrhocoricin, binds to and stimulates the ATPase activity of both wild-type and lidless DnaK

Liudmila S. Chesnokova, Sergey V. Slepenkov, Stephan N. Witt*

*Department of Biochemistry and Molecular Biology, Louisiana State University Health Sciences Center, 1501 Kings Highway, Shreveport, LA 71130-3932, USA*

Received 25 February 2004; revised 20 March 2004; accepted 22 March 2004

First published online 12 April 2004

Edited by Jesus Avila

**Abstract Recent reports have indicated that insect antimicrobial peptides kill bacteria by inhibiting the molecular chaperone DnaK. It was proposed that the antimicrobial peptide, *all*-L-pyrrhocoricin (L-PYR), binds to two sites on DnaK, the conventional substrate-binding site and the multi-helical C-terminal lid, and that inhibition of DnaK comes about from the lid mode of binding. In this report, we show using two different assays that L-PYR binds to and stimulates the ATPase activity of both wild-type and a lidless variant of DnaK. Our study shows that L-PYR interacts with DnaK much like the *all*-L NR (NRLLLTG) peptide, which is known to bind in the conventional substrate-binding site of DnaK. L-PYR antimicrobial activity is thus a consequence of the competitive inhibition of bacterial DnaK.**



*Keywords:* Antimicrobial peptide; DnaK; Hsp70 chaperone; Pyrrhocoricin

## 1. Introduction

The *E. coli* 70-kDa molecular chaperone DnaK functions co-translationally and post-translationally to promote protein folding and to inhibit the formation of toxic protein aggregates [1–3]. The DnaK reaction cycle is regulated by the two co-chaperones, DnaJ and GrpE. Homologs of the DnaK/DnaJ/GrpE chaperone machine are found in nearly all organisms, which indicates the important role this chaperone machine plays in maintaining proper protein cellular homoeostasis.

In an ATP-dependent reaction cycle, DnaK grabs a stretch of exposed hydrophobic residues on partially denatured protein and, by an unknown mechanism, refolds the protein molecule in concert with DnaK-mediated ATP hydrolysis. Because the DnaK chaperone machine is absolutely essential to repair partially denatured proteins that occur as a consequence of stress, inhibiting DnaK would devastate a bacterial cell. There have been intriguing reports of novel classes of DnaK (Hsp70) [4] and DnaJ (Hsp40) [5] inhibitors. Such inhibitors, if they selectively bind to the prokaryotic but not to the eukaryotic chaperone, could be an important new class of antimicrobial agents. Having new antimicrobial agents is important given the frequency of multi-drug resistant bacterial infections in hospital patients [6,7].

Multicellular organisms such as plants, animals and insects synthesize protective peptides that kill bacteria. Antimicrobial peptides are thought to kill by binding to the negatively charged outer plasma membrane of bacteria, altering membrane structure, and, in some cases, entering the cell to attack cytosolic targets [8]. The class of antimicrobial peptides secreted by insects, such as pyrrhocoricin, apidaecin, and drosocin, are thought to kill bacteria by entering cells and inhibiting the molecular chaperone DnaK [9]. Otvos and colleagues proposed that L-pyrrhocoricin (L-PYR, VDKGSYLPRPTPPRPIYNRN) binds predominately to a 'non-conventional binding site' on DnaK, that is, the αE and αD helices of the multi-helical lid subdomain (Fig. 1), and that L-PYR prevents DnaK's lid from opening and closing [9]. Such a novel mechanism is different than L-PYR binding in the conventional substrate-binding site of DnaK like a competitive inhibitor.

In this report, the chemistry of L-PYR binding to DnaK was studied using complementary pre-steady-state kinetic and single turnover ATPase assays. The results indicate that L-PYR binds to DnaK, even lidless DnaK, and stimulates the ATPase activity of each DnaK species. On this basis, we thus conclude that L-PYR binds primarily to the conventional substrate-binding site of DnaK, much like other substrates, and, by competing with natural substrates, L-PYR effectively decreases the cellular concentration of DnaK.

## 2. Materials and methods

### 2.1. Protein and reagents

All reagents were of the highest purity and were purchased from Sigma, unless stated otherwise. Wild-type and the lidless variant, DnaK(2-517), were isolated and made free of bound nucleotide as previously described [10,11] and were maintained in a HEPES buffer (25 mM *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid/50 mM KCl/5 mM $MgCl_2$/5 mM 2-mercaptoethanol at pH 7.0). Protein was stored in the HEPES sample buffer containing 10% glycerol at −80 °C prior to use. The p5, L-PYR, and D-PYR peptides were purchased from Genemed Synthesis Incorporated (S. San Francisco, CA), purified to >95% by high performance liquid chromatography, and peptide masses were verified by electrospray mass spectroscopy.

* Corresponding author. Fax: +1-318-675-5180.
*E-mail address:* switt1@lsuhsc.edu (S.N. Witt).

*Abbreviations:* HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; p5, *all*-L synthetic peptide CLLLSAPRR; ap5, acrylodan-labeled p5 peptide; L-PYR, *all*-L amino acid active form of pyrrhocoricin; D-PYR, *all*-D amino acid inactive pyrrhocoricin analog

0014-5793/$22.00 
doi:10.1016/j.febslet.2004.03.075

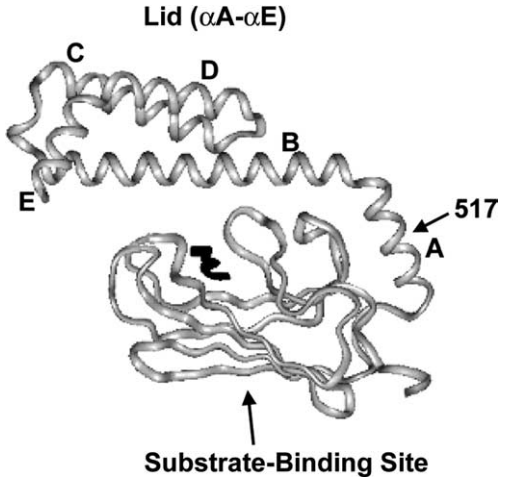


Fig. 1. Structure of the substrate-binding domain of DnaK (residues 394–607) [19]. The NR peptide (NRLLLTG), bound in the conventional substrate-binding pocket, is depicted in black. The five α-helices that comprise the lid are labeled A–E. The image was constructed from PDB file 1DKX.

*2.2. Stopped-flow fluorescence*

A SX-18MV stopped-flow fluorescence spectrometer (Applied Photophysics Ltd, Leatherhead, UK) was used to monitor two different reactions involving DnaK. 'Reverse' reactions, which involved mixing ATP-bound DnaK with unlabeled peptide, were conducted with 295 nm excitation and a 320 nm long-pass filter [12]. Peptide dissociation reactions, which involved mixing preformed ADP·DnaK · ap5 complexes with excess unlabeled peptide (100 μM), were conducted with 370 nm excitation and a 470 nm long-pass filter [13]. Preformed complexes were prepared by incubating DnaK (2 μM) and ap5 (0.1 μM) in 1 mM ADP for 30 min at room temperature. All stopped-flow traces are the average of 6–10 individual traces. Temperature control of both the jacketed reactants and the jacketed mixing chamber was achieved with a circulating external water bath ($\Delta T = \pm 0.2$ °C). Reagent concentrations in the text refer to after mixing.

*2.3. Single turnover ATPase assay*

The single turnover ATPase assay was performed as described by Karzai and McMacken [14]. Briefly, the reaction mixture (30 μl) contained 2.5 μM DnaK or DnaK(2-517), 51 nM ATP (0.05 μCi of [α-$^{32}$P]-ATP; 3000 Ci/mmol) in a HEPES buffer (25 mM HEPES/KOH, 10 mM $MgCl_2$, and 150 mM KCl, pH 7.6). Note that $K^+$ is an essential co-factor for Hsp70-mediated ATP hydrolysis [15,16]. To measure the peptide-stimulated activity, peptide (p5, L-PYR, or D-PYR) concentration was 300 μM. Samples were pre-incubated for 2 min at 25 °C. The reaction was initiated by the addition of ATP. Aliquots (3 μl) were removed periodically, quenched with 1 μl of 0.2 M HCl, and then 2 μl aliquots were subjected to polyethyleneimine (PEI)–cellulose thin layer chromatography using a 1 M formic acid and 0.5 M lithium chloride mobile phase. Radio-labeled ATP and ADP were visualized and quantified using a Phosphorimager (STORM 860). ADP formation traces followed single exponential kinetics, where the first-order rate constant is $k_{cat}$ ($min^{-1}$). Non-enzymatic hydrolysis of ATP was determined for each experiment and did not exceed $8 \pm 1.5\%$ after 4 h incubation ($n = 17$).

*2.4. Curve fitting*

Stopped-flow data were analyzed using a curve-fitting program that used a Marquardt algorithm based on the program Curfit given in [17]. Least squares fitting of data and determinations of standard errors of the fitted parameters were conducted using the program KaleidaGraph (Synergy Software, Reading, PA).

## 3. Results

According to recent reports, L-PYR binds to wild-type DnaK at two sites, the conventional substrate-binding site and the αD and αE helices of the multi-helical lid [4,18]. In the experiments described below the binding of L-PYR to the conventional substrate-binding site of DnaK was characterized.

Short peptides that bind in the conventional substrate-binding site of DnaK, such as NR (NRLLLTG) [19], p5 (CLLLSAPRR), and Cro (MQERITLKDYAM), rapidly bind to ATP-bound DnaK (ATP · DnaK*) and yield an increase in the tryptophan fluorescence of the protein [11,12] (Eq. (1)). We recently discovered that L-PYR, when mixed with ATP · DnaK*, also increases the tryptophan fluorescence of DnaK. Because of this finding, the reaction between ATP · DnaK* and L-PYR was investigated in detail. (The asterisk indicates that ATP · DnaK* is less fluorescent than ATP·DnaK·P.)

$$\mathrm{ATP \cdot DnaK^* + P \underset{k_{off}}{\overset{\Delta F\uparrow\ k_{on}}{\rightleftharpoons}\ _{\Delta F\downarrow}} ATP \cdot DnaK \cdot P} \quad (P = \text{peptide}) \qquad (1)$$

The kinetics of L-PYR binding to low-affinity DnaK (ATP·DnaK)* were studied by stopped-flow fluorescence. The experiments were carried out under pseudo-first-order conditions in which L-PYR concentration was varied at a fixed concentration of ATP · DnaK* (1 μM). Upon mixing L-PYR with ATP · DnaK* the intrinsic tryptophan fluorescence of DnaK rapidly increased, and the traces followed single exponential kinetics over a range of L-PYR concentrations (Fig. 2A). Note that control experiments demonstrated that no signal increase occurred when ATP-bound DnaK was mixed with a negative control peptide (polyglutamic acid or D-PYR). The plot of $k_{obs}$ versus L-PYR concentration yielded $k_{on}$ and $k_{off}$ values of 2.3 ($\pm 0.1$) $\times 10^5$ $M^{-1}$ $s^{-1}$ and 6.7 ($\pm 0.9$) $s^{-1}$, respectively (Fig. 2B). Interestingly, these constants are quite similar to the kinetic constants for short synthetic peptides that bind to the conventional substrate-binding site of DnaK (Table 1).

We were curious to see whether L-PYR binds to a lidless variant of DnaK. To this end, rapid mixing experiments, like those described above, were conducted using DnaK(2-517), a variant that lacks 1/2 of the A helix, the αB–αE helices, and the 30 or so residues that constitute the flexible C-terminal tail. In general, peptides bind to DnaK(2-517) with about a 10-fold lower affinity than to the wild-type protein, and the lowered affinity is due almost entirely to a 10-fold increase in the rate of peptide dissociation [11]. To achieve a reasonable fluorescence signal, a DnaK(2-517) concentration of 5 μM was used, L-PYR concentration was varied from 100 to 400 μM, and experiments were conducted at 5.6 °C (to slow down peptide dissociation). This experimental set up enabled us to estimate $k_{on}$ and $k_{off}$ values for the reaction between L-PYR and ATP-bound DnaK(2-517).

Fig. 3A shows representative formation traces obtained upon mixing ATP·DnaK(2-517)* with L-PYR. Significantly, no signal change occurred when the inactive *all*-D isomer, D-PYR, was used instead of the active *all*-L isomer. Each formation trace was fit to a single exponential function, yielding a value for $k_{obs}$. The plot of $k_{obs}$ versus L-PYR concentration yielded $k_{on}$ and $k_{off}$ values of 7.6 ($\pm 0.8$) $\times 10^4$ $M^{-1}$ $s^{-1}$ and 11.6 ($\pm 2.3$) $s^{-1}$ ($K_d = k_{off}/k_{on} = 153$ μM), respectively (Fig. 3B). For comparison, the p5 and the NR peptide bind to ATP-bound DnaK(2-517) at 5 °C with $K_d$ equal to 73 μM (20.4 $s^{-1}/2.8 \times 10^5$ $M^{-1}$ $s^-$) and 1455 μM (32 $s^{-1}/2.2 \times 10^4$ $M^{-1}$ $s^-$) [11], respectively. The results reveal that L-PYR specifically binds to lidless DnaK.

Most peptides that bind to DnaK stimulate DnaK-mediated ATP hydrolysis. In contrast, it has been reported that

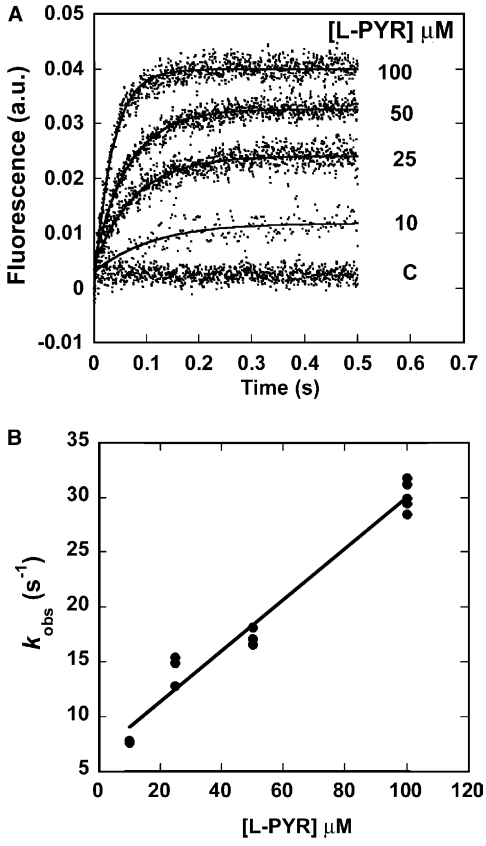


Fig. 2. L-PYR binding to ATP-bound DnaK monitored by tryptophan fluorescence. (A) ATP·DnaK·L-PYR complex formation followed single exponential kinetics (solid line). Trace "C" was obtained by mixing the negative control peptide, polyglutamic acid, with ATP-bound DnaK. Conditions: concentrations after mixing, 1 μM DnaK, 1 mM ATP, and 10–100 μM L-PYR; temperature = 25 °C. (B) Observed rate constants plotted against L-PYR concentration. Data were fit to the equation $k_{obs} = k_{on}$ [L-PYR] + $k_{off}$ (solid line, $R = 0.984$), yielding $k_{on}$ and $k_{off}$ values of 2.3 (±0.1) × $10^5$ $M^{-1}$ $s^{-1}$ and 6.7 (±0.9) $s^{-1}$, respectively.

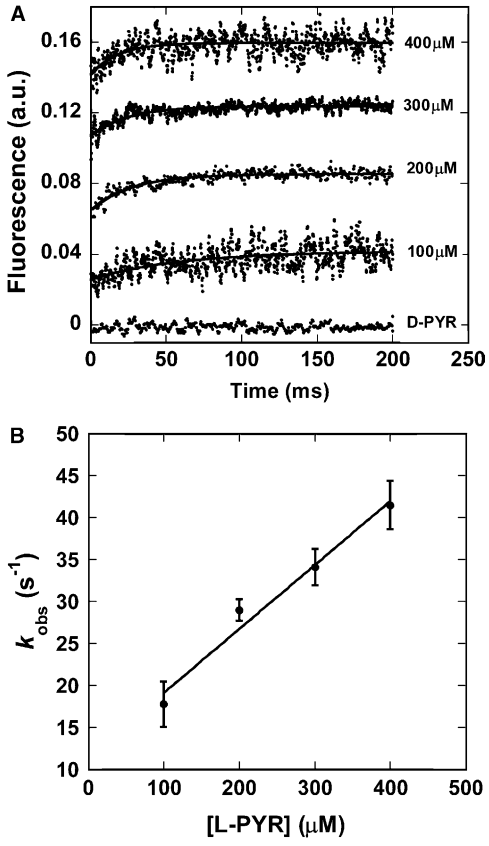


Fig. 3. L-PYR binding to ATP-bound DnaK(2-517) monitored by tryptophan fluorescence. (A) ATP·DnaK(2-517)·L-PYR complex formation followed single exponential kinetics (solid line). For clarity, traces are offset from each other. Conditions: concentrations after mixing, 5 μM DnaK(2-517), 1 mM ATP, 100–400 μM L-PYR or 300 μM D-PYR; temperature = 5.6 °C. (B) Observed rate constants plotted against L-PYR concentration. Data were fit to the equation $k_{obs} = k_{on}$ [L-PYR] + $k_{off}$ (solid line, $R = 0.988$), yielding $k_{on}$ and $k_{off}$ values of 7.6 (±0.8) × $10^4$ $M^{-1}$ $s^{-1}$ and 11.6 (±2.3) $s^{-1}$, respectively.

L-PYR inhibits DnaK's ATPase activity [20]. Because L-PYR binds to ATP-bound wild-type and lidless DnaK like other short peptides, we tested whether L-PYR stimulates the ATPase activity of both forms of DnaK using a single turnover assay ([DnaK or DnaK(2-517)] ≫ [ATP]).

Fig. 4A shows representative single turnover ADP formation curves obtained from experiments using DnaK(2-517) with or without L-PYR obtained at 25 °C. In the absence of peptide, DnaK(2-517) hydrolyzed ATP with a first-order rate constant, $k_{cat}$, equal to 0.016 (±0.001) $min^{-1}$, whereas in the presence of 300 μM L-PYR $k_{cat}$ increased approximately 4-fold to 0.061 (±0.003) $min^{-1}$. Identical experiments were conducted on wild-type DnaK. The combined results in Fig. 4B show that on an average p5 produced a ~6-fold stimulatory effect on wild-type and lidless; whereas, on an average L-PYR produced a ~4-fold stimulatory effect on wild-type and lidless.

It has been hypothesized that proline-rich antibacterial peptides kill bacteria by preventing the frequent movements of the multi-helical lid over the substrate-binding site [20]. We sought to test this hypothesis using the following postulate as a basis for the experimental design: If DnaK possesses two peptide binding sites, then it is possible that DnaK forms a two-peptide complex in which a short fluorescently tagged peptide molecule, such as aNR or ap5, is bound in the conventional substrate-binding site and an L-PYR molecule is bound to the lid. If L-PYR inhibits opening of the lid, then L-PYR should *decrease* the rate of ap5 dissociation from DnaK. This hypothesis was thus tested by measuring the rate of ap5 dissociation from preformed

Table 1
Kinetic constants for the reverse reaction (1) at 25 °C

| Peptide (P) | $k_{on}$ ($M^{-1}$ $s^{-1}$) | $k_{off}$ ($s^{-1}$) | $K_d$ (μM) | Reference |
|---|---|---|---|---|
| NR | 1.1 (±0.1) × $10^5$ | 9.4 (±1.0) | 86 ± 6 | [11] |
| p5 | 9.4 (±0.4) × $10^5$ | 7.0 (±3.1) | 7.4 ± 3.8 | [11] |
| Cro | 2.4 (±0.4) × $10^4$ | 2.9 (±0.5) | 121 ± 29 | [12] |
| L-PYR | 2.3 (±0.1) × $10^5$ | 6.7 (±0.9) | 29 ± 4 | This work |

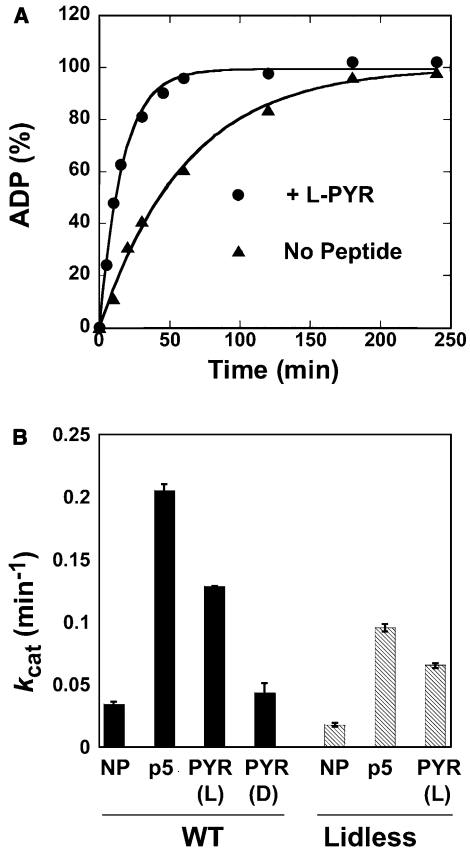


Fig. 4. Single turnover ATPase assay. (A) DnaK(2-517)-mediated ATP hydrolysis with (●) and without (▲) L-PYR. Data were fit to the equation $ADP(t) = ADP_{max}(1 - \exp[-k_{cat}\, t])$ (solid line), yielding $k_{cat}$(−PYR) and $k_{cat}$(+PYR) equal to 0.016 (±0.001) and 0.061 (±0.003) min$^{-1}$, respectively. Conditions: 2.5 μM DnaK(2-517), 300 μM PYR, and 51 nM ATP (0.05 μCi [α-$^{32}$P] ATP/reaction); temperature = 25 °C. (B) ATPase activity ($k_{cat}$) of wild-type and lidless DnaK. Shown are results from the single turnover ATPase assay with no peptide (NP), L-PYR, or D-PYR. Conditions: 300 μM p5, L-PYR or D-PYR; temperature = 25 °C. Each value is an average of at minimum three experiments.

ADP · DnaK·ap5 complexes in the absence or presence of excess L-PYR. As a baseline experiment, we found that upon mixing preformed ADP·DnaK·ap5 complexes with excess unlabeled p5 peptide (100 μM), the ap5 peptide dissociated with $k_{off}$ equal to $4.6\,(\pm 0.1) \times 10^{-4}\ s^{-1}$. When the same experiment was conducted with a large excess of unlabeled PYR (100 μM) instead of unlabeled p5, the ap5 peptide dissociated with $k_{off}$ equal to 4.3 $(\pm 0.1) \times 10^{-4}\ s^{-1}$ (data not shown). That L-PYR did not appreciably decrease the rate of ap5 dissociation indicates that L-PYR does not inhibit opening of DnaK's lid.

## 4. Discussion

The results from this study show that L-PYR binds to DnaK like a conventional substrate and that L-PYR does not inhibit DnaK-mediated ATP hydrolysis. There were several factors that lead us to hypothesize that L-PYR binds in the conventional substrate-binding site of DnaK. (i) PYR (VDKGSYLPRPTPPRPIYNRN) contains a classic DnaK-binding site, i.e., a leucine residue flanked by positively charged residues. (A DnaK-binding motif contains a hydrophobic core flanked by positively charged residues [21]. Leucine residues within the hydrophobic core are particularly important, because the substrate-binding site of DnaK contains a leucine-pocket [19].) (ii) DnaK has a propensity to bind polycations like PYR. For example, protamine, which is a polycationic peptide with antimicrobial activity, binds to DnaK in a manner similar to short synthetic peptides [22]. (iii) The inactivity of D-PYR to DnaK agrees with previous findings that DnaK does not bind peptides composed of *all*-D amino acids [5]. Overall, our results are consistent with L-PYR being a pseudo-substrate of DnaK, i.e., a competitive inhibitor.

The main findings from previous studies [4,20] of PYR–DnaK interactions are summarized as follows: (i) L-PYR inhibits the ATPase activity of DnaK; (ii) biotinylated-L-PYR specifically binds to DnaK (590–615), which is a fragment containing the αD–αE helices; (iii) biotinylated-L-PYR non-specifically binds to both DnaK (397–439), which is a fragment of the substrate-binding domain, and to DnaK (596–637), which constitutes the αE helix and the 30-residue flexible C-terminus; and (iv) the N-terminal residues 1–9 of L-PYR tightly bind to an 'allosteric ATPase site' on DnaK [20], which explains the ability of L-PYR to inhibit DnaK-mediated ATP hydrolysis. Aspects of these earlier findings and their relation to our findings are discussed below.

It is important to define what is meant by the term 'allosteric ATPase site'. Human and other eukaryotic Hsp70s contain a highly conserved tetrapeptide (EEVD) carboxyl terminus. (*E. coli* Hsp70 DnaK contains KDKK as the carboxyl terminal residues.) Mutating EEVD to AAAA in the human Hsp70 resulted in a ~2-fold increase in the basal ATPase activity, decreased tryptophan fluorescence, and loss of ATPase stimulation by the DnaJ-like co-chaperone HDJ-1 [23]. On this basis, Freeman et al. concluded that EEVD is a regulatory motif that couples substrate recognition with ATPase activity by binding to an allosteric site on the ATPase domain. The question is whether DnaK contains such an allosteric ATPase site. On the basis of dot blot binding experiments and computer modeling, Kragol et al. [20] concluded that PYR binds to the αD + αE helices, and because binding down regulated DnaK's ATPase activity, they hypothesized that the αD + αE helices are the allosteric ATPase site. Their hypothesis was reinforced by a recent study showing that the ATPase activity of DnaK is allosterically modulated by the C-terminal lid domain, particularly by the αD + αE helices [24]. However, in this study no evidence was found that L-PYR inhibits DnaK-mediated ATP hydrolysis. Presently, we cannot rule out that L-PYR binds to the lid, although such an interaction is likely to be quite weak.

The discrepancy between this study and earlier studies of PYR–DnaK interactions is probably due to the different buffers and different ATPase assays employed in the respective studies. The single turnover assay used in this study was conducted in a HEPES buffer with 150 mM potassium salt; [α-$^{32}$P] ATP was separated from [α-$^{32}$P] ADP via PEI–cellulose thin layer chromatography. This assay is widely used to measure the rate of ATP hydrolysis by DnaK [14,25,26]. In contrast, the spectrophotometric assay used in a previous study, which detected the release of inorganic phosphate, was conducted in a TRIS buffer without an essential potassium salt [20]. These two assays probably give different results because they utilized different buffers and measured different phenomena.

In summary, on the basis of this study, we propose that the primary binding site for L-PYR on DnaK is the conventional substrate-binding pocket. By binding in the conventional substrate-binding pocket of DnaK, L-PYR competitively inhibits DnaK, which thereby prevents natural substrates from binding. Because of the high concentration of antimicrobial peptides in the site of infection, organisms defend themselves with high efficiency. Utilizing the knowledge that PYR binds to the conventional substrate-binding site of DnaK might enable more effective antimicrobial agents to be designed.

*Acknowledgement:* This work was supported in part by a grant from the National Institutes of Health (GM 51521).

## References

[1] Bukau, B. and Horwich, A.L. (1998) Cell 92, 351–366.
[2] Slepenkov, S.V. and Witt, S.N. (2002) Mol. Microbiol. 45, 1197–1206.
[3] Hartl, F.U. and Hayer-Hartl, M. (2002) Science 295, 1852–1858.
[4] Otvos Jr., L., O, I., Rogers, M.E., Consolvo, P.J., Condie, B.A., Lovas, S., Bulet, P. and Blaszczyk-Thurin, M. (2000) Biochemistry 39, 14150–14159.
[5] Feifel, B., Schonfeld, H.J. and Christen, P. (1998) J. Biol. Chem. 273, 11999–12002.
[6] Anderson, R.M. (1999) Nat. Med. 5, 147–149.
[7] Tan, Y.T., Tillett, D.J. and McKay, I.A. (2000) Mol. Med. Today 6, 309–314.
[8] Zasloff, M. (2002) Nature 415, 389–395.
[9] Otvos Jr., L. (2000) J. Pept. Sci. 6, 497–511.
[10] Slepenkov, S.V. and Witt, S.N. (1998) Biochemistry 37, 1015–1024.
[11] Slepenkov, S.V. and Witt, S.N. (2002) Biochemistry 41, 12224–12235.
[12] Slepenkov, S.V. and Witt, S.N. (1998) Biochemistry 37, 16749–16756.
[13] Buczynski, G., Slepenkov, S.V., Sehorn, M.G. and Witt, S.N. (2001) J. Biol. Chem. 276, 27231–27236.
[14] Karzai, A.W. and McMacken, R. (1996) J. Biol. Chem. 271, 11236–11246.
[15] Palleros, D.R., Reid, K.L., Shi, L., Welch, W.J. and Fink, A.L. (1993) Nature 365, 664–666.
[16] O'Brien, M.C. and McKay, D.B. (1995) J. Biol. Chem. 270, 2247–2250.
[17] Bevington, P.R. (1969) Data Reduction and Error Analysis for the Physical Sciences. McGraw-Hill, New York.
[18] Kragol, G. et al. (2002) Eur. J. Biochem. 269, 4226–4237.
[19] Zhu, X., Zhao, X., Burkholder, W.F., Gragerov, A., Ogata, C.M., Gottesman, M.E. and Hendrickson, W.A. (1996) Science 272, 1606–1614.
[20] Kragol, G., Lovas, S., Varadi, G., Condie, B.A., Hoffmann, R. and Otvos Jr., L. (2001) Biochemistry 40, 3016–3026.
[21] Gragerov, A., Zeng, L., Zhao, X., Burkholder, W. and Gottesman, M.E. (1994) J. Mol. Biol. 235, 848–854.
[22] Witt, S.N., Galiano, F.J. and Slepenkov, S.V. (1997) Cell Stress Chaperon. 2, 110–118.
[23] Freeman, B.C., Myers, M.P., Schumacher, R. and Morimoto, R.I. (1995) EMBO J. 14, 2281–2292.
[24] Slepenkov, S.V., Patchen, B., Peterson, K.M. and Witt, S.N. (2003) Biochemistry 42, 5867–5876.
[25] Russell, R., Karzai, A.W., Mehl, A.F. and McMacken, R. (1999) Biochemistry 38, 4165–4176.
[26] Montgomery, D.L., Morimoto, R.I. and Gierasch, L.M. (1999) J. Mol. Biol. 286, 915–932.