Journal of Peptide Science
J. Peptide Sci. **8**: 144–150 (2002)
DOI: 10.1002/psc.375

Identification of Single Amino Acid Residues Essential for the Binding of Lipopolysaccharide (LPS) to LPS Binding Protein (LBP) Residues 86–99 by Using an Ala-scanning Library

O. REYES[a*] , M. G. VALLESPI[a], H. E. GARAY[a], L. J. CRUZ[a], L. J. GONZÁLEZ[a], G. CHINEA[a], W. BUURMAN[b] and M. J. ARAÑA[a]

[a] Division of Physical Chemistry, Center for Genetic Engineering and Biotechnology, Havana, Cuba
[b] Department of General Surgery, University of Maastricht, The Netherlands

Received 21 June 2001
Accepted 28 November 2001

Abstract: Lipopolysaccharide binding protein (LBP) is a 60 kDa acute phase glycoprotein capable of binding to LPS of Gram-negative bacteria and facilitating its interaction with cellular receptors. This process is thought to be of great importance in systemic inflammatory reactions such as septic shock. A peptide corresponding to residues 86–99 of human LBP (LBP_{86-99}) has been reported to bind specifically with high affinity the lipid A moiety of LPS and to inhibit the interaction of LPS with LBP. We identified essential amino acids in LBP_{86-99} for binding to LPS by using a peptide library corresponding to the Ala-scanning of human LBP residues 86–99. Amino acids Trp91 and Lys92 were indispensable for peptide–LPS interaction and inhibition of LBP–LPS binding. In addition, several alanine-substituted synthetic LBP-derived peptides inhibited LPS–LBP interaction. Substitution of amino acids Arg94, Lys95 and Phe98 by Ala increased the inhibitory effect. The mutant Lys95 was the most active in blocking LPS binding to LBP. These findings emphasize the importance of single amino acids in the LPS binding capacity of small peptides and may contribute to the development of new drugs for use in the treatment of Gram-negative bacterial sepsis.
Copyright © 2002 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: lipopolysaccharide; lipopolysaccharide binding protein; Ala-scanning; peptide analogues

INTRODUCTION

The recognition of pathogenic bacteria and their toxic products is very important for survival of the host during infection and bacteraemia [1]. Lipopolysaccharide (LPS, endotoxin) is an integral part of the outer membrane of all Gram-negative bacteria and is centrally involved in the recognition of bacteria by infected hosts. LPS elicits innate immune reactions, including cytokine release, up-regulation of adhesion molecules, phagocytosis, oxygen radical production and some other effects in diverse cell types [2]. Cell responses to LPS are dependent on LPS-binding soluble proteins and membrane receptors. In particular, LPS-binding protein (LBP) and CD14 play a critical role in mediating responses to LPS [3]. Binding of LPS to LBP enhances the cellular effects of LPS by facilitating subsequent binding to CD14. Recent studies have also identified members of

Abbreviations: Abbreviations: BPI, bactericidal permeability increasing protein; DIC, 1,3-diisopropylcarbodiimide; DMF, *N,N*-dimethylformamide; Fmoc, fluorenylmethoxycarbonyl; HOBt, 1-hydroxybenzotriazole; LALF, *Limulus* anti-LPS factor; MBHA, 4-methylbenzhydrylamine; rp-HPLC, reverse phase high performance liquid chromatography; tBu, tert-butyl; TFA, trifluoroacetic acid; TIS, triisopropylsilane

* Correspondence to: Dr O. Reyes, Centro de Ingeniería Genética y Biotecnología, División de Química-Física, Apartado Postal 6162, Ciudad de La Habana 10600, Cuba; e-mail: oreyes@cigb.edu.cu

Copyright © 2002 European Peptide Society and John Wiley & Sons, Ltd.

the Toll-like receptor family as potential components of the signalling unit of the LPS receptor, which transduce responses that are dependent on LBP and CD14 [4]. Thus diverse evidence indicates that LPS complexing with LBP is a major event in mediating toxic responses triggered by LPS [5].

The region between amino acids 82–108 of LBP seems to be involved in LPS binding, as ascertained by studies with synthetic LBP peptides [6,7]. Taylor *et al.* identified two overlapping peptides, corresponding to residues 91–108 of human LBP, that specifically bound the lipid A moiety of LPS with high affinity and inhibited LPS binding to LBP. On the other hand, Battafarano *et al.* demonstrated that a synthetic peptide, comprising residues 82–108 of human LBP, inhibited the LPS-induced TNF-α secretion by macrophages *in vitro*. In particular, the peptide corresponding to residues 86–99 of human LBP retained a significant binding to LPS and inhibited the binding of LPS to LBP [8].

In order to design small molecules with increased ligand-binding and neutralizing capacity it is often necessary to prior identify peptides with specific folds and proper functions [9]. Although through the analysis of peptide structure one could determine the contribution of each amino acid residue to a specific function, this could also be approached by additional studies. A valuable procedure is the study of an alanine-scanning synthetic library based on the parental sequence [10,11]. This may provide extensive data from one screening step: for example the residues decisive for the LPS binding capacity, which could be changed without any impact on the activity: the influence of the amphipathic character or charge distribution in the bioactivity of the peptide, among others.

In the context of the whole protein, single or double mutations of basic residues (Arg 94 and Lys 95) within the proposed LPS binding motif of LBP dramatically reduced the LPS binding activity of LBP [12]. Nevertheless, no analysis of single mutations of the small LBP_{86-99} peptide has been reported so far. We constructed and screened an Ala-scanning synthetic library restricted to the LBP region 86–99 to identify indispensable amino acid residues for peptide binding to LPS. This study could contribute to the development of peptides or derived molecules of potential prophylactic or therapeutic value in the management of sepsis and other LPS-associated disorders.

MATERIALS AND METHODS

Materials

All Fmoc-protected amino acids, HOBt and Rink Amide MBHA resin were obtained from Bachem (Switzerland). Trifluoracetic acid, piperidine, *N*,*N*-dimethylformamide (peptide grade), tert-butyl methyl ether, diethyl ether and acetonitrile (HPLC grade) were purchased from Caledon (Canada); reagents for the Kaiser test, DIC and TIS from Merck (Germany). All commercial reagents and solvents were used as received with the exception of DMF, which was stored over activated 0.4 nm molecular sieves (Merck, Germany).

Peptide Synthesis

Peptides were synthesized using manual parallel Fmoc/tBu solid phase chemistry on Rink Amide MBHA resin (0.54 mmol/g, 0.1 mmol scale). Side-chain protecting groups were as follows: Arg(Pmc), Gln(Trt), Lys(Boc), Ser(OtBu) and Trp(Boc). Syntheses were carried out in 15 polypropylene syringes (10 ml) fitted with a polyethylene porous disc. Solvents and soluble reagents were removed by suction. DIC/HOBt activation used at least a threefold molar excess of Fmoc-amino acids in DMF for each coupling cycle. In general no more than 2 h was needed for the completion of the coupling reaction, which was indicated by a negative Kaiser test [13]. Removal of the Fmoc group was carried out with 20% piperidine in DMF (1×1 min, 2×10 min). Washings between coupling and deprotection steps were carried out with DMF (5×1 min) using 5–10 ml/g resin for each wash. All coupling and washing steps were performed under vigorous shaking. After final deprotection each peptide-resin was washed with DMF (5×1 min), methanol (5×1 min), diethyl ether (5×1 min) successively and then dried under vacuum overnight.

Cleavage from the resin and deprotection of side chain protecting groups were performed by a treatment with 10 ml of TFA/TIS/water (95/2.5/2.5) for 2 h. Cleavage solutions were filtered into 15 50 ml centrifuge tubes containing 30 ml of cold tert-butyl methyl ether. After centrifugation (5 min at 3000 rpm) and decanting, the precipitates were washed four times by addition of fresh ether. The peptides were finally dissolved in 10 ml of acetonitrile/water (1/1, v/v) and lyophilized.

Copyright © 2002 European Peptide Society and John Wiley & Sons, Ltd.

Purification and Characterization of the Peptides

Analytical rp-HPLC was carried out on a Pharmacia LKB (Sweden) instrument comprising two solvent delivery pumps (model 2150) and a system controller (model 2152). A linear gradient from 0 to 60% of solvent B (0.05% TFA in acetonitrile) in solvent A (0.1% TFA in water) in 40 min was run on a Vydac C-18 column (25 × 250 mm, 10 µm) at 0.8 ml/min flow rate. UV detection was performed at 226 nm in a Knauer variable wavelength detector (model K-2500). Peptides were purified up to 99% purity on the same instrument on a Vydac C-18 column (25 × 250 mm, 10 µm). A linear gradient from 15% to 50% of solvent B in solvent A during 50 min at a flow rate of 4 ml/min was used. The absorbance was monitored at 226 nm. The peptide molecular mass was verified by mass spectrometry. The low-energy MS/MS spectra were acquired using a hybrid quadrupole orthogonal acceleration tandem mass spectrometer QTOF from Micromass (Manchester, UK) fitted with a Z-spray nanoflow electrospray ion source. The mass spectrometer was operated with a source at 80 °C and a drying gas flow of 50 l/h. Peptides were dissolved in a solution of 50% water/acetonitrile (v/v) containing 1% acetic acid to reach an approximate concentration of 5 pmol/µl. The rp-HPLC peptide solutions were directly infused into MS by using a syringe pump 74900 (Cole Palmer) at 5 µl/min. Voltages applied to the capillary and cone were 3 kV and 35 V, respectively. Data acquisition and processing were performed using a MassLynx system from Micromass.

Blocking LBP–LPS Interaction Using Synthetic LBP Peptides

Equimolar solutions of each peptide were prepared and stored in water for injection. Immuno-Maxisorb plates (Nunc, Roskilde, Denmark) were coated overnight at 4 °C with 10 µg anti-human LBP monoclonal antibody (1E9 MAb) in PBS. Plates were blocked by incubation with BSA 1% in PBS for 1 h at room temperature. Washing steps, and standard, samples and subsequent reagent dilutions were performed with BSA 0.1% in PBS. Two-fold serial dilutions of the standard titration curve of rhLBP (recombinant human LBP) were added in duplicate wells and plates were incubated for 1 h at room temperature. The highest concentration of rhLBP was 100 ng/ml and the lowest 50 pg/ml. Biotinylated LPS from *E. coli* 055:B5 preincubated (1 h at room temperature) in the absence or presence of different amounts of peptides were added to the plates and further incubated for 1 h. The optimal working dilution of biotinylated-LPS was previously determined in independent experiments. LPS bound to LBP was detected using streptavidin–peroxidase conjugate and TMB substrate [14]. The absorbance was measured in a microplate ELISA reader at 450 nm. Reduced OD values indicated the blockage of LPS–LBP interaction. The significance of differential

Table 1 Peptides Derived from the Ala-scanning of the Parental Sequence LBP_{86-89}

Code	Sequence	Theoretical mass	Experimental mass	Z	Error
LBP_{86-99}	RVQGRWKVRKSFFK	607.70	607.68	3	+0.02
A99	RVQGRWKVRKSFF**A**	588.68	588.66	3	+0.02
A98	RVQGRWKVRKSF**A** K	582.36	582.33	3	+0.03
A97	RVQGRWKVRKS**A** FK	582.36	582.34	3	+0.02
A96	RVQGRWKVRK**A** FFK	602.37	602.36	3	+0.01
A95	RVQGRWKVR**A** SFFK	588.68	588.65	3	+0.03
A94	RVQGRWKV**A** KSFFK	579.35	579.33	3	+0.02
A93	RVQGRWK**A** RKSFFK	598.36	598.35	3	+0.01
A92	RVQGRW**A** VRKSFFK	588.68	588.67	3	+0.01
A91	RVQGR**A** KVRKSFFK	569.35	569.33	3	+0.02
A90	RVQG**A** WKVRKSFFK	579.35	579.32	3	+0.03
A89	RVQ**A** RWKVRKSFFK	612.37	612.34	3	+0.03
A88	RV**A** GRWKVRKSFFK	588.69	588.67	3	+0.02
A87	R**A** QGRWKVRKSFFK	598.36	598.35	3	+0.01
A86	**A** VQGRWKVRKSFFK	579.35	579.32	3	+0.03

Copyright © 2002 European Peptide Society and John Wiley & Sons, Ltd.

binding was analysed by Student's *t*-test with the Bonferroni correction, using the estimated parameters of the different inhibition curves.

RESULTS AND DISCUSSION

A library of 15 *N*-terminal free peptides (Table 1) was synthesized including the parental peptide LBP_{86-99}. All peptides were obtained with more than 99% purity according to analytical rp-HPLC (Figure 1). A good correspondence between theoretical and experimental mass was obtained.

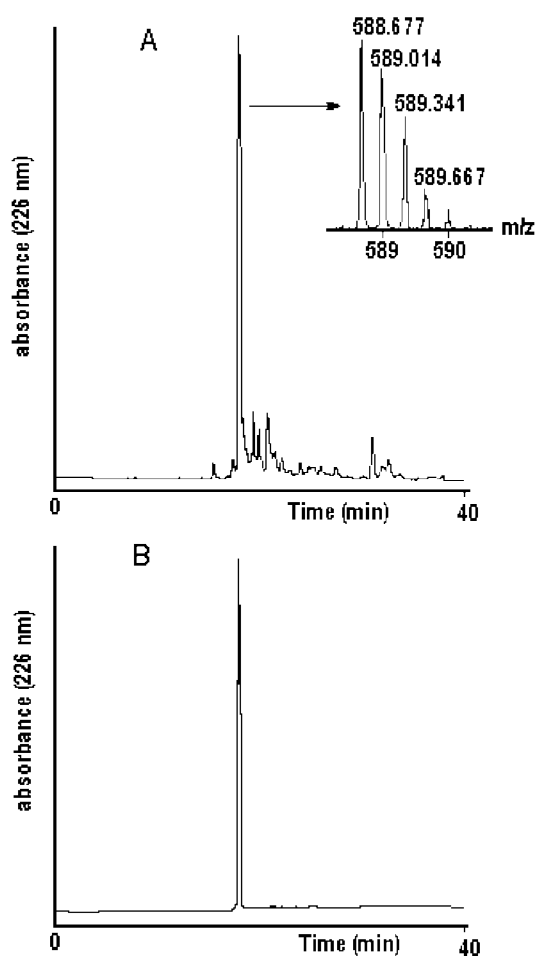

Figure 1 Analytical rp-HPLC chromatograms of A95 peptide. (A) crude and lyophilized A95; (B) purified A95. A linear gradient from 0 to 60% of solvent B (0.05% TFA in acetonitrile) in solvent A (0.1% TFA in water) in 40 min was run on a Vydac C-18 column (25 × 250 mm, 10 μm) at 0.8 ml/min flow rate. The absorbance was monitored at 226 nm. The inset of rp-HPLC profile of crude A95 (A) shows an expanded region of the ESI-MS spectra.

In a previous study we showed that the peptide corresponding to the LBP amino acid region 86–99 (LBP_{86-99}) was able to interfere with the LBP–LPS interaction and to inhibit the toxic effects of LPS [8]. The contribution of each amino acid residue to the LPS binding capacity of LBP_{86-99} was examined by using the single substituted peptides conforming to the Ala-scanning synthetic library. Peptides were pre-incubated with LPS–biotin, and these mixtures were subsequently added to rhLBP-coated surfaces to verify the ability of each synthetic peptide to interfere with LBP–LPS interaction. In initial experiments all peptides were used at 12 μg/ml, a concentration that was clearly inhibitory for LBP_{86-99} in this assay. In parallel experiments it was demonstrated that peptides did not bind to 1E9 MAb or interfere with the recognition of rhLBP by this monoclonal antibody (data not shown). A non-related peptide, B6-1 (KSVIIGSTGGSPEKHHSTVQL), which does not bind to LPS, was used as a negative control in all experiments.

The results are shown in Figure 2. The peptides could be classed in five major groups according to their relative ability to block LPS:LBP interaction, with respect to the parental peptide LBP_{86-99}. Peptides corresponding to A94, A95 and A98 mutations showed a strong ability to block the interaction of LPS–LBP, as ascertained by a sustained abrogation of LPS-binding at every concentration of hLBP tested. A second group of peptides, including A86, A90 and A96 was more inhibitory than LBP_{86-99}, but less potent than the previous group of mutant peptides.

Peptides A87, A88 and A89 showed an interference rather similar to LBP_{86-99}. Mutants A93, A97 and A99 affected LBP:LPS binding marginally only at LBP concentrations over 25 ng/ml, while peptides A91 and A92 did not interfere with LBP–LPS interaction. It was remarkable that alanine substitutions of Trp91 and Lys92 completely abrogated the capacity of these peptides to interfere with LBP:LPS interaction. Thus, it seems that these two amino acids are crucially involved in the binding of the peptides to LPS and consequently in determining an efficient inhibition of LBP–LPS interaction.

To further characterize the contribution of single alanine-substitutions to the strength of LPS-inhibitory activity, we measured the capacity of mutant peptides of the first group (A94, A95 and A98) to block the LBP–LPS interaction, using a minimum concentration of 1 μg/ml of these peptides. Peptides were preincubated for 1 h with 2 ng/ml

Copyright © 2002 European Peptide Society and John Wiley & Sons, Ltd.

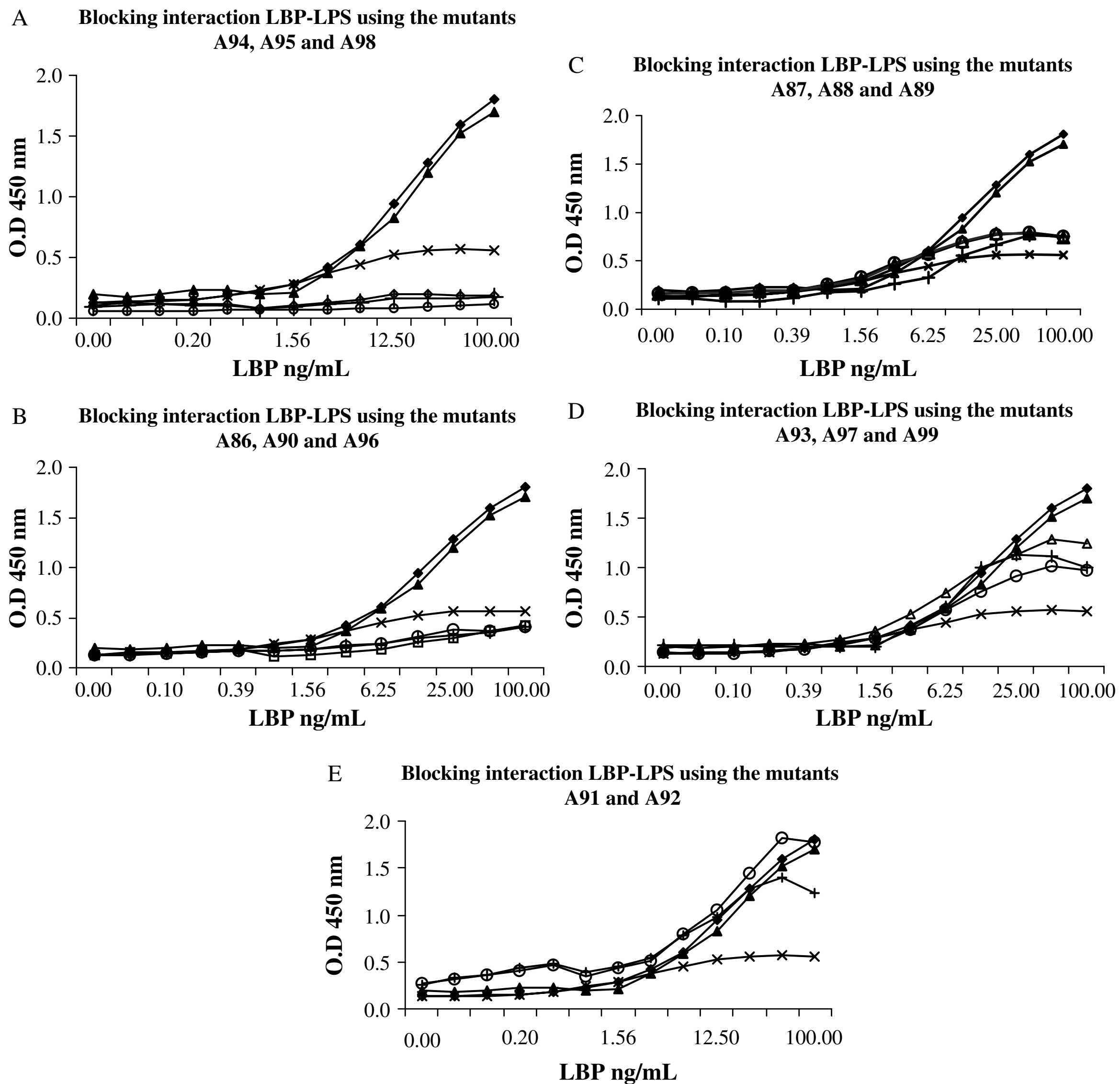

Figure 2 Blocking of LPS–LBP interaction using synthetic peptides from the LBP_{86-99} restricted Ala-scanning library. Two-fold serial dilutions of the standard titration curve of rhLBP (recombinant human LBP) were added in duplicate to wells pre-coated with 10 μg of anti-human LBP monoclonal antibody (1E9 MAb), and plates were incubated for 1 h at room temperature. Biotinylated LPS from *E.coli* 055:B5 preincubated (1 h at room temperature) in the absence or presence of 12 μg/ml of peptides were added to the plates and further incubated for 1 h. LPS bound to LBP was detected using streptavidin-peroxidase conjugate and TMB substrate. Absorbance was measured in a microplate ELISA reader at 450 nm. Representative data of one of three independent experiments are shown. A, •LPS, ×LBP_{86-99}, ▲B6-1, ◊A94, +A95, ◯A98. B, •LPS, ×LBP_{86-99}, ▲B6-1, +A86, □A90, ◯A96. C, •LPS, ×LBP_{86-99}, ▲B6-1, ◯A87, +A88, △A89. D, •LPS, ×LBP_{86-99}, ▲B6-1, +A93, △A97, ◯A99. E, •LPS, ×LBP_{86-99}, ▲B6-1, +A92, ◯A91.

of LPS–biotin and the mixtures were then added to rhLBP-coated wells. As shown in Figure 3, A95 peptide displayed the strongest blocking effect on LPS:LBP interaction. The binding of LPS to LBP was more than 50% inhibited at LBP concentrations higher than 25 ng/ml. Interestingly, these results indicated that the inhibition displayed by the A95 mutant peptide was not affected by the concentration of LBP, at least within the evaluated range. A similar effect was previously described for the parental peptide LBP_{86-99}[8], although as demonstrated herein more than 12 μg of LBP_{86-99} was necessary to similarly block the LPS–LBP interaction. It should be pointed out that by mutating Lys95

Copyright © 2002 European Peptide Society and John Wiley & Sons, Ltd.

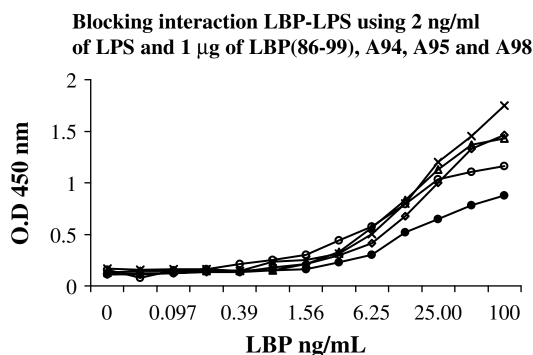

Figure 3 Blocking of LPS–LBP interaction using peptides LBP_{86-99}, A94, A95 and A98 at a lower concentration. In this experiment peptides at 1 μg/ml were preincubated with biotinylated LPS from *E. coli* 055:B5 (1 h at room temperature). Other experimental conditions were as described in Figure 2. Data from a representative experiment out of three are presented. LPS, ×LBP_{86-99}, ▲A-94, ♦A-95, ○A98.

into alanine the alternating pattern of hydrophilic and hydrophobic residues within the peptide was extended up to Phe97, in addition to eliminating a positive-charged amino acid.

The region between amino acids 86 to 104 was first proposed as the LPS binding domain of LBP based on similarities of the amphipathicity pattern of this region with the protruding loop of the *Limulus* anti-LPS factor (LALF), an LPS-binding protein from the *Limulus* horseshoe crab [15]. A similar discrete region in BPI was also described as a presumed LPS binding site by these authors. Subsequent studies have demonstrated that synthetic peptides corresponding to these putative LPS-binding sites were able to block LPS-mediated responses both *in vitro* and *in vivo* [6,7,12,16]. However, the contribution of each amino acid residue within these sequences to the observed inhibitory effects has not been previously described. Using this Ala-scanning based approach we determined that, within the peptide sequence corresponding to the human LBP region 86–99, amino acids Trp91 and Lys92 are indispensable for the efficient interaction of peptides with LPS, in terms of the inhibition of LPS binding to LBP. These residues are included centrally in the region between Arg90 and Arg94, which seems to constitute a core of higher amphipathicity within the peptide sequences. On the contrary, a single changes of Arg94, Lys95 or Phe98 into alanine potentiated the interference of peptides with LBP–LPS interaction, indicating that these residues in the original sequence, in the context of the peptides, are detrimental for their efficient interaction with LPS. This effect was maximal for the alanine-substitution at position 10 (Lys95), which also marks the apparent importance of an alternating hydrophobic-hydrophilic sequence for peptides to interfere with LBP–LPS binding. Other single residues, which seem to be important for the inhibitory effect, are Val93, Phe97 and Lys99; the alanine-substitutions of the two formers affected the extended amphipathic sequence previously assumed. The last residue, Lys99 could be involved in electrostatic interactions with lipid A phosphate groups, as could be the case for the only other positive charged amino acid essential for the blocking effect of these peptides, Lys92.

CONCLUSIONS

Single indispensable amino acids for the binding of a synthetic peptide, LBP_{86-99}, to LPS were described through a one step screening of a restricted Ala-scanning library. Simultaneously, single substitutions that increase the inhibitory activity on LPS–LBP interaction of the peptides were also defined throughout this procedure. Alanine at positions 9 or 10 of the 14 residue-peptide sequence, instead of arginine or lysine respectively, improved the interaction of these small peptides with LPS, as indirectly revealed by a decrement of the binding of LPS to LBP in the presence of the peptides. In contrast, Trp6 and Lys7 were essential for an effective binding of peptides to LPS. In addition, this study provided preliminary data on the structural features of these peptides that seem to be important for their activity, such as amphipathicity and the positioning of charged residues. The present investigation illustrates the convenience of this type of procedure for obtaining information potentially useful for the development of endotoxin antagonists for clinical use.

REFERENCES

1. Schumann RR, Rietschel ET, Loppnow H. The role of CD14 and lipopolysaccharide-binding protein (LBP) in the interaction of different cell types by endotoxin. *Med. Microbiol. Immunol.* 1994; **183**: 279–297.
2. Ulevitch RJ, Tobias PS. Receptor-dependent mechanism of cell stimulation by bacterial endotoxin. *Annu. Rev. Immunol.* 1995; **13**: 437–457.

Copyright © 2002 European Peptide Society and John Wiley & Sons, Ltd.

3. Karima R, Matsumoto S, Higashi H, Matsushima K. The molecular pathogenesis of endotoxic shock and organ failure. *Mol. Med. Today* 1999; **5**: 123–132.
4. Kirschning CJ, Wesche H, Ayres Y, Rothe M. Human Toll-like receptor confers responsiveness to bacterial lipopolysaccharide. *J. Exp. Med.* 1998; **188**: 2091–2097.
5. Jack RS, Fan X, Berheiden M, Rune G, Ehlers M, Weber A, Kirsch G, Mentel R, Furll B, Freudenberg M, Schmitz G, Stelter F, Schutt C. Lipopolysaccharide-binding protein is required to combat a murine Gram-negative bacterial infection. *Nature.* 1997; **389**: 742–745.
6. Taylor AH, Heavner G, Nedelman M, Sherris D, Brunt E, Knight D, Ghrayeb J. Lipopolysaccharide neutralizing peptides reveal a lipid A binding site of LPS binding protein. *J. Biol. Chem.* 1995; **270**: 17934–17938;4.
7. Battafarano RJ, Dahlberg PS, Ratz CA, Johnston JW, Gray BH, Haseman JR, Mayo KH, Dunn DL. Peptide derivatives of three distinct lipopolysaccharide binding proteins inhibit lipopolysaccharide-induced tumor necrosis factor-alpha secretion *in vitro*. *Surgery* 1995; **118**: 318–324.
8. Araña MJ, Vallespi MG, Chinea G, Vallespi GV, Rodríguez-Alonso I, Buurman W, Reyes O. Inhibition of LPS-responses by synthetic peptides derived from LBP associates with the ability of peptides to block LBP/LPS interaction. *J. Endotoxin Res.* 2001; *submitted.*
9. Mayo KH. Solution nuclear magnetic resonance characterization of peptide folding. *Methods Enzymol.* 1997; **289**: 646–672.
10. Sikorski JA, Devadas B, Zupec ME, Freeman SK, Brown DL, Lu HF, Nagarajan S, Mehta PP, Wade AC, Kishore NS, Bryant ML, Getman DP, McWherter CA, Gordon JI. Selective peptidic and peptidomimetic inhibitors of Candida albicans *myristoylCoA*: protein *N*-myristoyltransferase: a new approach to antifungal therapy. *Biopolymers.* 1997; **43**: 43–71.
11. Veale CA, Alford VC, Aharony D, Banville DL, Bialecki RA, Brown FJ, Damewood JR Jr, Dantzman CL, Edwards PD, Jacobs RT, Mauger RC, Murphy MM, Palmer W, Pine KK, Rumsey WL, Garcia-Davenport LE, Shaw A, Steelman GB, Surian JM, Vacek EP. The discovery of non-basic atrial natriuretic peptide clearance receptor antagonists. *Bioorg. Med. Chem. Lett.* 2000; **10**: 1949–1952.
12. Lamping N, Hoess A, Yu B, Park TC, Kirschning C-J, Pfeil C, Reuter D. Wright SD, Herrmann F, Schumann RR Effects of site directed mutagenesis of basic residues (Arg 94, Lys 95, Lys 99) of LPS-binding protein on binding and transfer of LPS and subsequent immune cell activation. *J. Immunol.* 1996; **157**: 4648–4656.
13. Kaiser E, Colescott RL, Bossinger CD, Cook PI. Color test for detection of free terminal amino groups in solid-phase synthesis of peptides. *Anal. Biochem.* 1970; **34**: 595–598.
14. Madersbacher S, Berger P. Double wavelength measurement of 3,3′,5,5′-tetramethylbenzidine (TMB) provides a three-fold enhancement of the ELISA measuring range. *J. Immunol. Methods* 1991; **138**: 1121–1124.
15. Hoess A, Watson S, Siber GR, Liddington R. Crystal structure of an endotoxin-neutralizing protein from the horseshoe crab, *Limulus* anti-LPS factor, at 1.5 Å resolution. *EMBO J.* 1993; **12**(9): 3351–3356.
16. Ried C, Wahl C, Miethke T, Wellnhofer G, Landgraf C, Schneider-Mergener J, Hoess A. High affinity endotoxin-binding and neutralizing peptides based on the crystal structure of recombinant *Limulus* anti-LPS factor. *J. Biol. Chem.* 1996; **271**(45): 28120–28127.

Copyright © 2002 European Peptide Society and John Wiley & Sons, Ltd.