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# Lysophosphatidic acid and lipopolysaccharide bind to the $PIP_2$-binding domain of gelsolin

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**Abstract**

The binding of the gelsolin P2 peptide (residues 150–169) with lysophosphatidic acid (LPA) and lipopolysaccharide (LPS) was investigated by isothermal titration calorimetry. P2 binds to LPS with higher affinity than to LPA. For the interaction of 1-oleoyl-LPA with P2 in the absence of salt, $K_d$ and ΔH° were 920 nM and −2.07 kcal/mol, respectively, at pH 7.4 and 25 °C. For the interaction of lipopolysaccharide (LPS) from *P. aeruginosa* with P2 under the same conditions, $K_d$ was 177 nM and ΔH° was −7.6 kcal/mol.


*Keywords:* Binding; Gelsolin; Lipopolysaccharide; Lysophospholipid; Thermodynamics

## 1. Introduction

Lysophosphatidic acid (LPA) is structurally the simplest of the glycerophospholipids. Intracellularly, LPA is produced via de novo synthesis from glycerol-3-phosphate or by deacylation of phosphatidic acid, and extracellularly it is generated by a lysopholipase known as autotaxin through cleavage of the headgroup of lysophosphatidylcholine (LPC). LPA is a well-known endogenous ligand of several members of the EDG (endothelial differentiation genes) family of G-protein-coupled receptors (GPCR) genes [1]; in addition, some forms of LPAs activate the lipid-regulated transcription factor PPARγ [2,3]. This phospholipid growth factor displays a myriad of cellular functions. Examples of activities that are controlled by the interactions of LPA with GPCRs [4,5] are cell survival, cell proliferation, $Ca^{2+}$ homeostasis, cell motility, angiogenesis, and embryo implantation. The discovery that LPA is as efficient as $PIP_2$ in dissociating related actin-binding protein complexes [6] suggests an additional role for this lipid, i.e., as a regulator of cellular structural reorganization via interactions with gelsolin. [7,8]. Recent research has revealed that lipopolysaccharide (LPS), the major cell wall component in the outer membrane of Gram-negative bacteria, also binds to gelsolin [9].

Gelsolin is an approximately 85-kDa protein present in plasma and in extracellular fluids at concentrations of 100–250 μg/ml [10]. This protein regulates cellular morphology and motility by severing actin filaments, binding actin monomers, and nucleating actin polymerization, resulting in cytoskeletal modifications [7,11]. It is mainly regulated by phosphatidylinositol 4,5-bisphosphate ($PIP_2$) [12,13] through binding at two sites near the *N*-terminus of the protein [7,14], denoted as P1 (residues 135–149) and P2 (residues 150–169: KHVVPNEVVVQRLFQVKGRR-OH). P2 was shown to compete with full-length gelsolin for binding to $PIP_2$ [14].

In this paper, the thermodynamics of LPA and LPS interactions with the P2 domain of gelsolin have been characterized and compared by isothermal titration calorimetry (ITC).

## 2. Materials and methods

### 2.1. Gelsolin P2 peptide

Gelsolin P2 was synthesized by solid-phase peptide synthesis on an Applied Biosystems 433A peptide synthesizer (Foster City, CA) starting with preloaded *N*-α-Fmoc-Arg(Pmc)-Wang resin. The 0.1 mmol FastMoc chemistry (Applied Biosystems) was used for elongation of the peptide chain with an

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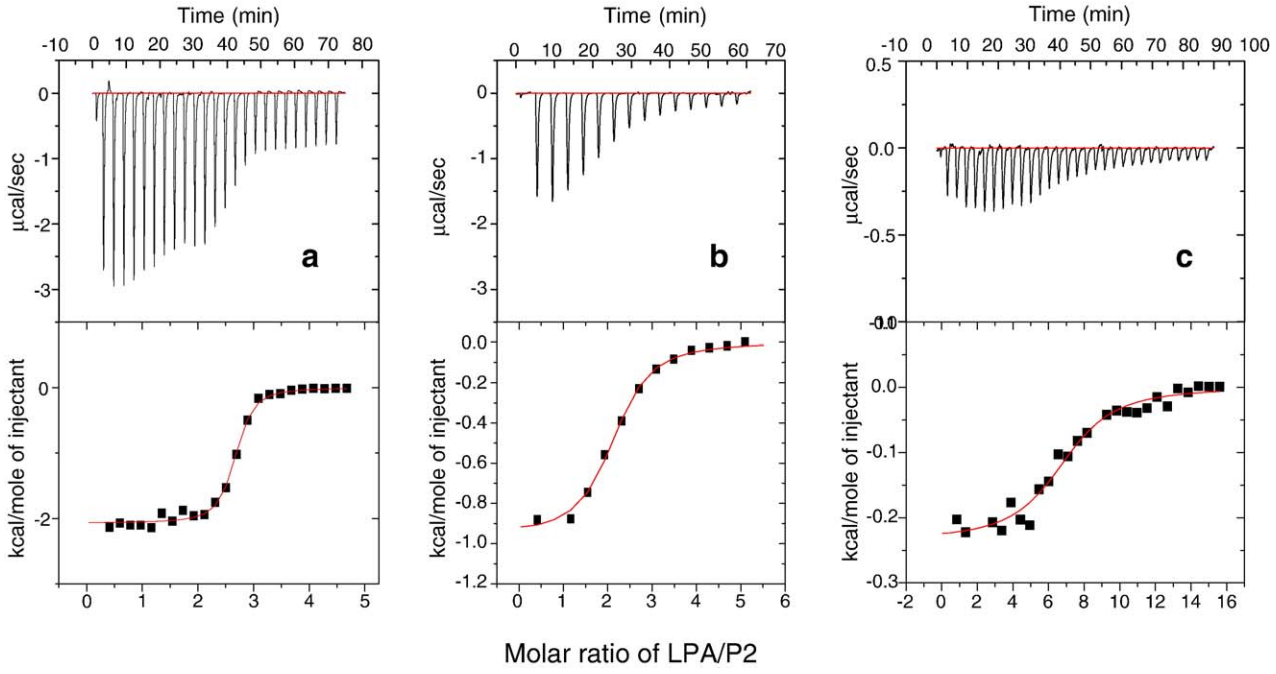


Fig. 1. Upper panels: ITC traces of binding of 74 µM gelsolin P2 with 4.9 mM 1-oleoyl-LPA in (a) the absence of NaCl, and in the presence of (b) 100 mM NaCl, and (c) 150 mM NaCl at 25 °C. Lower panels: integrated data from top showing best fit curves. See Materials and methods for experimental details.

*O*-benzotriazolyl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU), 1-hydroxybenzotriazole (HOBt), and diisopropylethylamine-catalyzed single coupling step using 10 equiv of the protected Fmoc-amino acids. Cleavage from the resin was accomplished with a mixture of trifluoroacetic acid, phenol, thioanisole, and water. After precipitation with cold diethyl ether, the crude peptide was purified by C18-HPLC (elution with a water/acetonitrile gradient containing 0.1% trifluoroacetic acid, detection at 220 nm). ESI-MS: *m*/*z* calcd., 2388.9; found, 2390.0.

Gelsolin P2 was dissolved in 10 mM HEPES buffer, pH 7.4, at concentrations of 74 to136 µM. The solution was vortexed gently for 1 min and sonicated in a water bath at room temperature for 5 min to aid in the dissolution of the peptide. Buffers were prepared with ultra-pure water (distilled and passed through a Milli-Q water purification system (Millipore, Bedford, MA) to a resistivity of 18 MΩ cm).

### 2.2. Lipids

1-Oleoyl-LPA, 1-myristoyl-LPA, and 1-palmitoyl-lysophosphatidylcholine (LPC) were purchased from Avanti Polar Lipids (Alabaster, AL) and were used without further purification. LPS from *P. aeruginosa* was purchased as the lyophilized powder from Sigma-Aldrich (St. Louis, MO).

LPA was dissolved in buffer consisting of 10 mM HEPES, 0.1 mM EDTA, pH 7.4, with or without NaCl, to a concentration of ~5 mM (see figure captions for concentrations) and vortexed vigorously for ~2 min at room temperature. Mixtures of LPC and LPA were prepared by dissolving LPC and LPA in $CHCl_3$, followed by evaporation under a stream of $N_2$ until a thin lipid film remained. The film was dried further under vacuum overnight and hydrated with buffer, vortexed vigorously for ~5 min, and sonicated in a water bath at room temperature. LPS was dissolved in salt-free 10 mM $NaH_2PO_4$ buffer, pH 7.4.

### 2.3. Isothermal titration calorimetry

High-sensitivity ITC was conducted in a Microcal MCS calorimeter (Northampton, MA) at 25 and 37 °C. All solutions were degassed with stirring for at least 30 min prior to experiments. The 250-µl injection syringe was loaded with LPA solution and 8–10 µl aliquots were injected into the ITC sample cell containing P2, after temperature equilibration, at 4-min intervals. The contents of the sample cell were stirred at a rate of 400 rpm throughout the experiment.

For the LPS–P2 binding experiments, the titration was carried out by filling the syringe with P2 and the sample cell with LPS. P2 and LPS were dissolved in 10 mM $NaH_2PO_4$, pH 7.4 (in the absence of NaCl). Aliquots of 6 µl of 136 µM P2 were injected into 2.7 µM LPS at 4-min intervals and stirred as described above.

Data were collected and analyzed using Origin 5.0. Best-curve fit routines of the integrated area under the peaks of the raw data were performed using a one-binding-site model, from which the values of ΔH° and ΔS° were obtained. The dissociation constant $K_d$ and Gibbs free energy change were obtained from the fundamental equations of thermodynamics, $\Delta G^\circ = -RT\ln K = \Delta H^\circ - T\Delta S^\circ$.

## 3. Results

### 3.1. LPA–P2 interactions

Fig. 1a shows the results of a titration of 74 µM P2 with 4.9 mM 1-oleoyl-LPA at 25 °C in the absence of salt. To determine the contributions of ionic interactions, the binding was studied with increasing concentrations of NaCl (Fig. 1b, c). The interactions in all of the titrations are exothermic, and the results are summarized in Table 1. Increasing the salt concentration caused the reaction to become less exothermic, from −2.1 kcal/mol in the absence of salt to −0.23 kcal/mol in the presence of

Table 1
Thermodynamic data for the interaction of 1-oleoyl-LPA with gelsolin P2 at 25 °C

| [NaCl] (mM) | $\Delta H^\circ$ (kcal/mol) | $T\Delta S^\circ$ (kcal/mol) | $\Delta G^\circ$ (kcal/mol) | $K_d$ (µM) |
|---|---|---|---|---|
| 0 | −2.1 | 6.1 | −8.2 | 0.92 |
| 100 | −0.96 | 5.9 | −6.9 | 9.0 |
| 150 | −0.23 | 6.1 | −5.8 | 24 |

The values reported are averages from 2 to 3 trials and were within ±15%.

Table 2
Thermodynamic data for 1-oleoyl-LPA/gelsolin P2 interactions in 150 mM NaCl, HEPES buffer, pH 7.4, at 25 °C and 37 °C

| Temperature (K) | $\Delta H°$ (kcal/mol) | $K_d$ (μM) |
|---|---|---|
| 298 | −0.23 | 24 |
| 310 | −0.59 | 14 |

The values reported are averages from 2 to 3 trials and were within ±20%.

Table 3
Thermodynamic data for the interaction of 1-oleoyl-LPA/1-palmitoyl-LPC mixed micelles with gelsolin P2 in 100 mM NaCl at 25 °C

| LPA/LPC (mol/mol) | $K_d$ (μM) | $\Delta H°$ (kcal/mol) | $T\Delta S°$ (kcal/mol) |
|---|---|---|---|
| 1:1 | 40 | −0.64 | 5.4 |
| 1.6:1 | 27 | −0.87 | 5.4 |

Errors from 2 to 3 trials were within a standard deviation of ±5%.

150 mM NaCl. The $K_d$ decreased with lower salt concentration, from 24 μM in the presence of 150 mM NaCl to 0.92 μM in the absence of salt.

The structural specificity with respect to the acyl chain of LPA and P2 was examined by titrating P2 with 1-myristoyl-LPA. An exothermic heat of binding was detected but did not reach saturation, indicating non-specific interactions (data not shown). Ionic interactions appear to be important since no discernible binding (i.e., no heat change detected; data not shown) was observed when the buffer contained 150 mM NaCl.

The binding of LPA to P2 was also studied at 37 °C and 150 mM NaCl. The interaction was more exothermic at the higher temperature, and $K_d$ decreased (Table 2). The difference in $\Delta H°$ observed at the two temperatures allowed an approximate estimation to be made of the change in heat capacity ($\Delta C_p$) according to the Kirchoff equation [15–17]. The value for $\Delta C_p$ was negative (−29.5 cal/K), which suggests hydrophobic dehydration on binding [15,18].

### 3.2. Lysophosphatidylcholine does not bind to P2

When P2 was titrated with 1-palmitoyl-LPC no binding was detected in buffer containing 100 mM NaCl (Fig. 2, top panel, curve a). When the LPA/LPC ratio was increased from 1:1 to 1.6:1 (mol/mol), $\Delta S°$ was unchanged and $\Delta H°$ became slightly more negative (Fig. 2, curve c, and Table 3), and the binding affinity increased slightly ($K_d$ decreased from 40 μM to 27 μM). To confirm that the heat signal detected was a result of binding of LPA to P2 and not to demicellization of the LPA/LPC aggregate, the same solution of LPA/LPC was injected into the buffer, and an insignificant heat change was observed (Fig. 2, top panel, curve b).

### 3.3. LPS–P2 interactions

Fig. 3 shows the data from the calorimetric titration in which aliquots of P2 were injected into the sample cell filled with 2.7 μM LPS in 10 mM $NaH_2PO_4$ buffer, pH 7.4, devoid of sodium chloride, at 25 °C. Analysis of the binding isotherm shows that P2 binds with a higher affinity to LPS ($K_d$=177 nM) than to 1-oleoyl-LPA ($K_d$=0.92 μM) under the same experimental conditions, and that the binding is considerably more exothermic ($\Delta H°$=−7.6 kcal/mol). These results are consistent with recent work showing strong interactions between LPS and a rhodamine-labeled 10-residue peptide (gelsolin residues 160–169) derived from P2 [9]. The binding was considered to be highly specific because no interactions were detected when

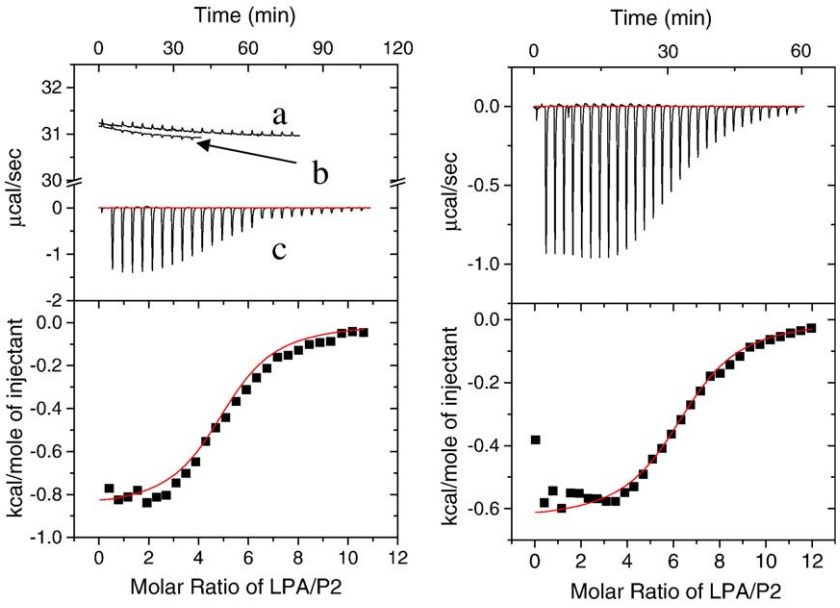


Fig. 2. Binding isotherms of 1-oleoyl-LPA/1-palmitoyl-LPC mixtures with P2 at 25 °C. Top panels represent raw ITC data; bottom panels represent integrated heats/mol injectant. Curve a, LPC injected into a solution of P2; curve b, LPA/LPC mixture injected into a solution of pure buffer; curve c, LPA/LPC mixture (left panel, 1.6/1; right panel, 1/1 (mol/mol)) injected into P2. Titrations were performed in buffer containing 100 mM NaCl. The concentration of 1-oleoyl-LPA in the syringe was 5 mM; the P2 concentration was 100 μM.

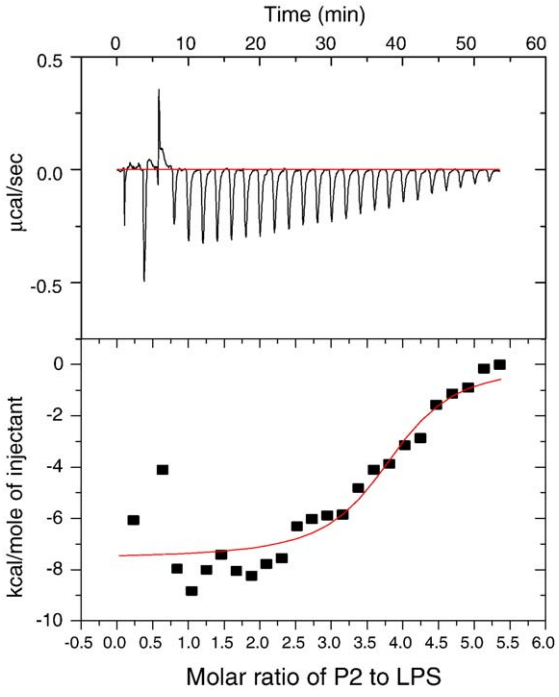


Fig. 3. Titration isotherm of binding of LPS with P2 in $NaH_2PO_4$ buffer, pH 7.4, at 25 °C. The spurious data point at the third injection is apparently a result of an air bubble.

P2 was replaced with a peptide in which the sequence of amino acids was scrambled.

## 4. Discussion

### 4.1. LPA–P2 interactions

It has been shown previously that LPA binds to full-length, plasma gelsolin [10,19], and very recently the binding of gelsolin to LPS from *Salmonella minnesota* was also demonstrated [9]. The goal of the studies described here was to compare the interactions of LPA and LPS with one of the two known peptides that comprise part of gelsolin's binding site for polyphosphoinositides.

The equilibrium binding of 1-oleoyl-LPA with P2 is sensitive to the presence of NaCl (Table 1), indicative of the role of ionic interactions. The peptide bears 5 positive charges and LPA bears ~1.5 negative charges at the experimental pH; therefore, the finding of an electrostatic component of the binding is not surprising. The addition of salt to the buffer shields the charged groups on P2 and LPA, decreasing affinity. Although $K_d$ and ΔH° both became more favorable as the salt concentration was decreased, binding was not abolished even in 150 mM NaCl, and $K_d$ remained in the low micromolar range. Additional evidence for the existence of specificity for LPA-P2 binding is provided by the facts that we did not detect binding of LPA to the second $PIP_2$-binding domain, P1, by calorimetric titration (data not shown), and that $PIP_2$ binds with high affinity to an engineered P2 analog bearing a net negative charge [11]. Moreover, replacing 1-oleoyl-LPA with 1-myristoyl-LPA resulted in an exothermic but unsaturable isotherm, which also suggests that charge attraction is insufficient to fully account for the high affinity binding observed in Fig. 1.

Intestinal fatty acid binding protein interacts with lipids via electrostatics to create a "collisional complex," after which hydrophobic forces appear to drive the high affinity binding [20]. A similar phenomenon may occur with P2, with the ionic forces between P2 and LPA bringing the species into close proximity prior to hydrophobic binding.

### 4.2. LPA/LPC interaction with P2

LPC, which is structurally similar to LPA, also appears to interact with various GPCRs [21]. LPC and LPA differ in their headgroup structure, which is apparently sufficient to prevent LPC from binding to P2. The absence of a net negative charge in LPC may prevent the formation of the collisional complex that appears to be required for binding with P2. LPA retains its ability to bind with high affinity to P2 in mixed micelles with LPC, suggesting that the affinity of LPA for P2 is greater than that between LPA and LPC in the mixed micelles.

### 4.3. LPS–P2 interaction

The negative heat capacity obtained from these studies[1] suggests that hydrophobic dehydration of the charges occurs on binding of LPA to P2 [15,16]. This has been observed for the binding of LPS with the polycationic drug polymyxin B [15]. That P2 interacts with LPS is not surprising since the polycationic character of P2 is one of the structural requirements found to be important for LPS-binding properties, the others being cationic protonatable groups residing approximately 15 Å apart and a hydrophobic moiety [22]. The high affinity of P2 for LPS (indicated by $K_d$ in the nanomolar range; see Fig. 3) and the high degree of specificity of the peptide for LPS found by Bucki et al. [9] suggest a role for gelsolin in the transport of the toxin through the bloodstream. Gelsolin levels decrease during trauma, and low levels of the protein coincide with greater susceptibility to shock [23]. Thus, LPS appears to be an additional lipid, in addition to polyphosphoinositides and LPA, that interacts with and affects the levels of free gelsolin in plasma.

### 4.4. Conclusions

ITC was employed to study the interactions of the $PIP_2$-binding domain of gelsolin with LPA and LPS. The equilibrium constant for binding of P2 to 1-oleoyl-LPA was in the micromolar range, and $\Delta H°$ and $\Delta S°$ were both thermodynamically favorable and salt- and temperature-dependent; the affinity remained high whether or not NaCl was present at both temperatures used in this study. Gelsolin P2 did not bind to LPC, and LPC did not interfere with LPA binding with P2. The binding of P2 to LPS from *P. aeruginosa* was tighter than to 1-oleoyl-LPA and 1-myristoyl-LPA.

[1] A rigorous application of the Kirchoff relationship requires additional experiments at other temperatures.

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## References

[1] E.J. Goetzl, S. An, Diversity of cellular receptors and functions for the lysophospholipid growth factors lysophosphatidic acid and sphingosine 1-phosphate, FASEB J. 12 (1998) 1589–1598.
[2] T.M. McIntyre, A.V. Pontsler, A.R. Silva, A. St Hilaire, Y. Xu, J.C. Hinshaw, G.A. Zimmerman, K. Hama, J. Aoki, H. Arai, G.D. Prestwich, Identification of an intracellular receptor for lysophosphatidic acid (LPA): LPA is a transcellular PPARγ agonist, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 131–136.
[3] C. Zhang, D.L. Baker, S. Yasuda, N. Makarova, L. Balazs, L.R. Johnson, G.K. Marathe, T.M. McIntyre, Y. Xu, G.D. Prestwich, H.S. Byun, R. Bittman, G. Tigyi, Lysophosphatidic acid induces neointima formation through PPARgamma activation, J. Exp. Med. 199 (2004) 763–774.
[4] W. Siess, K.J. Zangl, M. Essler, M. Bauer, R. Brandl, C. Corrinth, R. Bittman, G. Tigyi, M. Aepfelbacher, Lysophosphatidic acid mediates the rapid activation of platelets and endothelial cells by mildly oxidized low density lipoprotein and accumulates in human atherosclerotic lesions, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 6931–6936.
[5] C. Pagès, M.F. Simon, P. Valet, J.S. Saulnier-Blache, Lysophosphatidic acid synthesis and release, Prostaglandins Other Lipid Mediat. 64 (2001) 1–10.
[6] J. Chun, J.J. Contos, D. Munroe, A growing family of receptor genes for lysophosphatidic acid (LPA) and other lysophospholipids (LPs), Cell Biochem. Biophys. 30 (1999) 213–242.
[7] F.X. Yu, H.Q. Sun, P.A. Janmey, H.L. Yin, Identification of a polyphosphoinositide-binding sequence in an actin monomer-binding domain of gelsolin, J. Biol. Chem. 267 (1992) 14616–14621.
[8] H.L. Yin, J.H. Albrecht, A. Fattoum, Identification of gelsolin, a Ca2+-dependent regulatory protein of actin gel–sol transformation, and its intracellular distribution in a variety of cells and tissues, J. Cell Biol. 91 (1981) 901–906.
[9] R. Bucki, P.C. Georges, Q. Espinassous, M. Funaki, J.J. Pastore, R. Chaby, P.A. Janmey, Inactivation of endotoxin by human plasma gelsolin, Biochemistry 44 (2005) 9590–9597.
[10] E.J. Goetzl, H. Lee, T. Azuma, T.P. Stossel, C.W. Turck, J.S. Karliner, Gelsolin binding and cellular presentation of lysophosphatidic acid, J. Biol. Chem. 275 (2000) 14573–14578.
[11] W. Xian, P.A. Janmey, Dissecting the gelsolin-polyphosphoinositide interaction and engineering of a polyphosphoinositide-sensitive gelsolin C-terminal half protein, J. Mol. Biol. 322 (2002) 755–771.
[12] T.P. Stossel, On the crawling of animal cells, Science 260 (1993) 1086–1094.
[13] P.A. Janmey, Phosphoinositides and calcium as regulators of cellular actin assembly and disassembly, Annu. Rev. Physiol. 56 (1994) 169–191.
[14] P.A. Janmey, J. Lamb, P.G. Allen, P.T. Matsudaira, Phosphoinositide-binding peptides derived from the sequences of gelsolin and villin, J. Biol. Chem. 267 (1992) 11818–11823.
[15] K. Brandenburg, A. David, J. Howe, M.H. Koch, J. Andra, P. Garidel, Temperature dependence of the binding of endotoxins to the polycationic peptides polymyxin B and its nonapeptide, Biophys. J. 88 (2005) 1845–1858.
[16] H. Heerklotz, R.M. Epand, The enthalpy of acyl chain packing and the apparent water-accessible apolar surface area of phospholipids, Biophys. J. 80 (2001) 271–279.
[17] K. Gallagher, K. Sharp, Electrostatic contributions to heat capacity changes of DNA–ligand binding, Biophys. J. 75 (1998) 769–776.
[18] P. Garidel, A. Blume, Interaction of alkaline earth cations with the negatively charged phospholipid 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol: a differential scanning and isothermal titration calorimetric study, Langmuir 15 (1999) 5526–5534.
[19] K. Meerschaert, V. De Corte, Y. De Ville, J. Vandekerckhove, J. Gettemans, Gelsolin and functionally similar actin-binding proteins are regulated by lysophosphatidic acid, EMBO J. 17 (1998) 5923–5932.
[20] B. Corsico, G.R. Franchini, K. Hsu, J. Storch, Fatty acid transfer from intestinal fatty acid binding protein to membranes: electrostatic and hydrophobic interactions, J. Lipid Res. 46 (2005) 1765–1772.
[21] N. Murakami, T. Yokomizo, T. Okuno, T. Shimizu, G2A is a proton-sensing G-protein-coupled receptor antagonized by lysophosphatidylcholine, J. Biol. Chem. 279 (2004) 42484–42491.
[22] S.A. David, Towards a rational development of anti-endotoxin agents: novel approaches to sequestration of bacterial endotoxins with small molecules, J. Mol. Recognit. 14 (2001) 370–387.
[23] B. Dahl, F.V. Schiodt, P. Ott, R. Gvozdenovic, H.L. Yin, W.M. Lee, Plasma gelsolin is reduced in trauma patients, Shock 12 (1999) 102–104.