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Structural basis of the binding of fatty acids to peptidoglycan recognition protein, PGRP-S through second binding site

Pradeep Sharma [a], Shavait Yamini [a], Divya Dube [a], Amar Singh [b], Gorakh Mal [c], Nisha Pandey [a], Mau Sinha [a], Abhay Kumar Singh [a], Sharmistha Dey [a], Punit Kaur [a], Dipendra K. Mitra [b], Sujata Sharma [a], Tej P. Singh [a,*]

[a] Department of Biophysics, All India Institute of Medical Sciences, New Delhi, India
[b] Department of Transplantation Immunology and Immunogenetics, All India Institute of Medical Sciences, New Delhi, India
[c] National Research Center on Camel, Bikaner, India

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ABSTRACT

Short peptidoglycan recognition protein (PGRP-S) is a member of the mammalian innate immune system. PGRP-S from *Camelus dromedarius* (CPGRP-S) has been shown to bind to lipopolysaccharide (LPS), lipoteichoic acid (LTA) and peptidoglycan (PGN). Its structure consists of four molecules A, B, C and D with ligand binding clefts situated at A–B and C–D contacts. It has been shown that LPS, LTA and PGN bind to CPGRP-S at C–D contact. The cleft at the A–B contact indicated features that suggested a possible binding of fatty acids including mycolic acid of *Mycobacterium tuberculosis*. Therefore, binding studies of CPGRP-S were carried out with fatty acids, butyric acid, lauric acid, myristic acid, stearic acid and mycolic acid which showed affinities in the range of 10^{-5} to 10^{-8} M. Structure determinations of the complexes of CPGRP-S with above fatty acids showed that they bound to CPGRP-S in the cleft at the A–B contact. The flow cytometric studies showed that mycolic acid induced the production of pro-inflammatory cytokines, TNF-α and IFN-γ by CD3+ T cells. The concentrations of cytokines increased considerably with increasing concentrations of mycolic acid. However, their levels decreased substantially on adding CPGRP-S.

Introduction

Peptidoglycan recognition proteins (PGRPs) are part of the host's innate immune system that forms the first line of defense against the invading microbes [1–8]. These proteins recognize various pathogen-associated molecular patterns (PAMPs)[1] that are present on the bacterial cell surface, bind to them and neutralize the effects of bacterial infections. PGRPs are widely distributed in tissues and are evolutionarily conserved from insects to humans [1]. Insects have been reported to have up to 19 PGRPs [2] whereas in mammals, only four types of PGRPs, long PGRPs (PGRP-L; M.W. ≅ up to 90 kDa), intermediate PGRPs (PGRP-Iα and PGRP-Iβ; M.W. ≅ 40–45 kDa) and short PGRPs (PGRP-S; M.W. ≅ 20–25 kDa) have so far been identified [3]. It has been shown that in humans, the predominant expression of PGRP-L occurs in liver while PGRP-Iα and PGRP-Iβ are located in esophagus [4]. PGRP-S was found in bone marrow and neutrophils [4,5]. So far, PGRP-S has not been reported from human mammary gland while a significant concentration of up to 12 mg/100 ml was reported in the secretions of mammary gland of *Camelus dromedarius* [6]. It has been observed that the quantity of PGRP-S decreases with time during the normal course of lactation. However, its expression was shown to increase 40-fold during mastitis infection [6].

The PGRP-S from the mammary gland of camel (CPGRP-S) was found to be a single chain protein molecule consisting of 171 amino acid residues with six cysteines forming three intramolecular disulfide linkages while human PGRP-S (HPGRP-S) consists of 175 amino acid residues with seven cysteines. In this case also six cysteine residues are involved in three intramolecular disulfide linkages similar to those of CPGRP-S while the role of seventh cysteine residue (Cys8) is so far unclear [7].

So far, crystal structures of full chain camel PGRP-S [8] and a truncated human (residues, 9–175) PGRP-S [9] have been determined. The structure of human PGRP-S was found in monomeric form while that of camel PGRP-S consisted of four crystallographically independent molecules. These were designated as A, B, C and D and formed stable contacts between molecules A and B (A–B contact) and molecules C and D (C–D contact). Since the contacts A–B and C–D corresponded to the opposite sides of the same

* Corresponding author. Address: Department of Biophysics, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110 029, India. Fax: +91 11 2658 8663.
E-mail address: tpsingh.aiims@gmail.com (T.P. Singh).

[1] *Abbreviations used:* CPGRP-S, Camel peptidoglycan recognition protein-S; PAMPs, pathogen associated molecular patterns; PGN, peptidoglycan; MTB, Mycobacterium tuberculosis; BA, butyric acid; LA, lauric acid; MA, myristic acid; MC, mycolic acid; SA, stearic acid.

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protein molecule, it formed a linear chain of protein molecules with alternating A–B and C–D contacts.

Our previous studies have shown that the PAMPs that contained glycan moieties such as lipopolysaccharide (LPS), peptidoglycan (PGN) and lipoteichoic acid (LTA) bound to CPGRP-S at the site located on the C–D contact indicating the role of C–D interface in the binding of cell wall molecules belonging to both Gram-negative and Gram-positive bacteria [10–12].

However, the role of A–B interface was unknown. The examination of the A–B contact clearly indicated a cleft-like structure. It is formed as a result of two parallel α-helices; one of them belonged to molecule A while the other was part of molecule B. The inner sides of these helices contained mainly hydrophobic residues. The structure of the cleft thus formed suggested that fatty acid-like structures including mycolic acid of the *Mycobacterium tuberculosis* cell wall might fit into it. Therefore, the role of A–B contact was examined for the recognition of cell wall fatty acids of *M. tuberculosis*. Hence, the binding studies of CPGRP-S with fatty acids were carried out. Based on the results of binding studies, the complexes of CPGRP-S were prepared with several fatty acids by incubating the protein at protein: fatty acid concentrations of 1:10 M ratios.

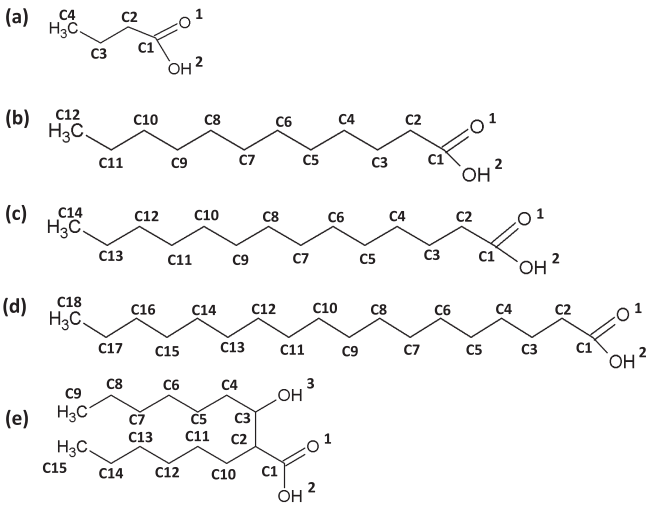

Fig. 1. Schematic representations of chemical structures of fatty acids (a) butyric acid, (b) lauric acid, (c) myristic acid, (d) stearic acid and (e) truncated mycolic acid. The numbering schemes are also indicated.

Table 1
Crystallographic statistics. The values in parentheses correspond to the values in the highest resolution shell.

Ligand	(a) Butyric acid	(b) Lauric acid	(c) Myristic acid	(d) Stearic acid	(e) Mycolic acid
PDB Code	3UMQ	3UIL	3SUX	4FNN	3T2 V
Resolution range (Å)	34.8–2.2	50.0–2.2	47.91–2.3	50.0–2.2	42.6–2.5
Space group	I222	I222	I222	I222	I222
Unit cell parameters					
a (Å)	88.9	89.0	89.6	89.3	89.3
b (Å)	101.5	101.6	101.4	101.4	101.5
c (Å)	162.9	163.3	163.1	163.0	163.3
Scaling					
Total number of measured reflections	104980	210060	120869	221642	162596
Number of unique reflections	34615	33252	34139	31454	25214
Multiplicity	3.0	6.3	3.5	7.0	6.4
Completeness (%)	91.6 (100.0)	94.1 (96.1)	99.6 (99.1)	93 (85)	98.6 (97.6)
I/σ(I)	36.1 (5.6)	9.8 (3.5)	14.7 (5.5)	14.8 (3.3)	5.3 (2.7)
Rsym	0.06(0.32)	0.08(0.46)	0.10(0.42)	0.05(0.24)	0.11(0.33)
Rmeas	0.06(0.27)	0.09(0.52)	0.12(0.50)	0.08(0.58)	0.19(0.45)
Rp.i.m.	0.02(0.11)	0.09(0.21)	0.05(0.21)	0.04(0.38)	0.09(0.24)
Refinement					
R_{cryst} (%)/No. of reflections	22.6/32880	22.5/31478	22.4/32427	21.1/29789	27.7/24008
R_{free} (%)/No. of reflections	24.3/1735	24.6/1774	24.3/1712	24.7/1665	24.1/1206
R.m.s.d in bond lengths (Å)	0.01	0.02	0.02	0.02	0.01
R.m.s.d in bond angles (°)	1.9	1.9	2.0	1.8	2.0
DPI (Å)	0.22	0.23	0.23	0.22	0.29
Ramachandran plot analysis					
Most favoured (%)	91.8	92.9	92.0	94.2	91.5
Additionally allowed (%)	8.2	7.1	8.0	5.8	8.5
Model					
Total amino acids	684	684	684	684	684
Overall B-factors (Å^2)	42.3	33.0	34.0	22.7	40.4
Residues in subunit A	171	171	171	171	171
Residues in subunit B	171	171	171	171	171
Residues in subunit C	171	171	171	171	171
Residues in subunit D	171	171	171	171	171
B-factor, subunit A (Å^2)	40.2	31.1	35.4	21.1	39.0
B-factor, subunit B (Å^2)	47.9	37.4	37.4	24.1	45.5
B-factor, subunit C (Å^2)	39.6	30.8	31.3	22.1	35.7
B-factor, subunit D (Å^2)	38.6	30.9	31.1	16.9	37.8
Additional groups					
Solvent					
No. of water oxygen atoms	581	660	550	550	390
Average B-factor (Å^2)	47.3	46.0	38.4	37.2	49.9
No. of glycerol molecules/atoms	1/6	1/6	1/6	-	1/6
Average B-factor (Å^2)	52.1	43.9	55.0	-	47.2
Ligands occupancies	1.0	1.0	0.7	1.0	1.0
No. of atoms	6	14	16	20	18
Average B-factor (Å^2)	63.6	60.9	41.5	71.5	74.4