**Supplementary information**

**Lysozyme’s Lectin-like Characteristics Facilitates its Immune Defense Function**

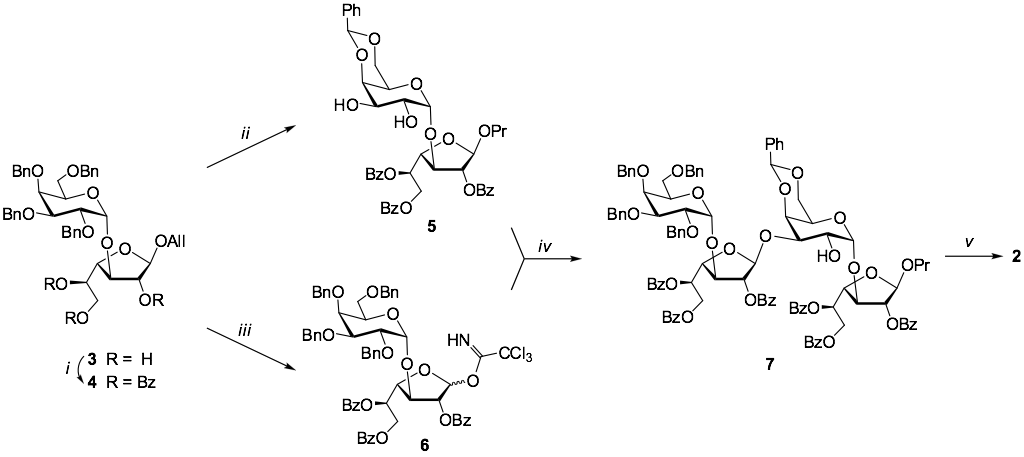
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|  |  |
| --- | --- |
|  | ka (M-1 s-1) = 2.16 ×102  kd (s-1) = 8.86 ×10-2  KD (M) = 4.10 × 10-4 |

**Figure S1.** SPR data of human lysozyme interacting with LPS from *K. pneumoniae*O1. Five channels (A1 - A5) are used with different concentrations of LPS. Channel six was used for the running buffer only. The *K. pneumoniae* LPS concentrations are as follows: A1: 100 μl, A2: 80 μl, A3: 40 μl, A4: 20 μl, A5: 10 μl (ka - association constant, kd – dissociation constants).



**Scheme S1.** The synthesis of disaccharide **1**1 was performed with the use of described1 synthetic block **3** (scheme 1) which was prepared with the use of recently discovered pyranoside-*into*-furanoside rearrangement (Krylov et al. 2014; Krylov et al. 2016). Thus, the benzoylation of **3** (→**4**) and subsequent O-debenzylation, reduction of allyl group and 4’,6’-O-benzylidenation produced diol **5**. On the other hand, removal of allyl-aglycon in **3** and further introduction of imidate group gave the glycosyl donor **6**. TMSOTf promoted coupling of disaccharide derivatives **5** and **6** proceeded with regioselective (1→3)-bond formation to give tetrasaccharide product **7**. Its deblocking afforded to the desire tetrasaccharide **2**. Its structure, and particularly β-(1→3)-structure of newly formed bond was determined on the basis of characteristic (Gerbst et al. 2015) values of *J*H1”,H2” coupling constant (<2.0 Hz) in 1H NMR spectrum and characteristic low-field chemical shift of C3’ (77.52 ppm) in 13C NMR spectrum. Reagents and conditions: i. BzCl, Py, rt, 90%; ii. 1) H2, Pd/C, EtOAc, rt, 2) PhCH(OMe)2, CSA, CH3CN, rt, 70% (2 steps); iii. 1) PdCl2, MeOH, rt, 2) CCl3CN, DBU, CH2Cl2, -30 oC, 59% (2 steps); iv. TMSOTf, CH2Cl2, -40 oC, 68%; v. 1) H2, Pd(OH)2/C, EtOAc-MeOH, rt, 2) NaOH, MeOH-H2O, rt, 85% (2 steps).

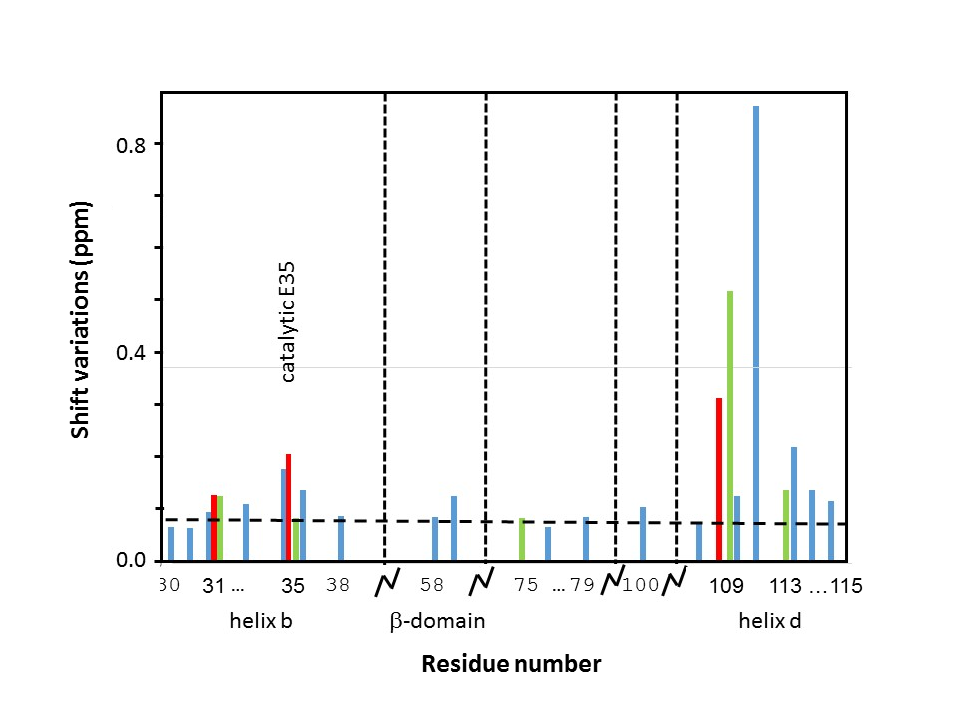
**Table S1**. Titrating resonances in HL upon pH variation in the range 3.8-8.5.

|  |  |  |  |
| --- | --- | --- | --- |
| Residue | Atom | Shift changea (ppm) | Comment |
| *Titrations with pKa 6.8 near Glu 35 and/or on helices b or d* | | | |
| Leu31 | HN | 0.09 | helix b |
| Leu31 | Hβ | 0.13 | helix b |
| Leu31 | Hγ | 0.12 | helix b |
| Lys33 | HN | 0.10 | helix b |
| Glu35 | HN | 0.17 | catalytic residue |
| Glu35 | Hβ | 0.21 | catalytic residue |
| Glu35 | Hβ’ | 0.14 | catalytic residue |
| Glu35 | Hγ | 0.08 | catalytic residue |
| Ser36 | HN | 0.13 | helix b |
| Tyr38 | HN | 0.08 | helix b |
| Gln58 | HN | 0.08 | on β-domain, in Trp 109 plane |
| Ile59 | HN | 0.12 | on β-domain, in Trp 109 plane |
| Val100 | HN | 0.10 | in Trp 109 plane |
| Ala108 | HN | 0.07 | in Trp 109 plane |
| Trp109 | Hβ | 0.32 | Trp side chain in binding cleft |
| Trp109 | H1 | 0.28 | Trp side chain in binding cleft |
| Trp109 | Hε1 | 0.48 | Trp side chain in binding cleft |
| Val110 | HN | 0.12 | helix d, shift change also at pH 3.8 |
| Ala111 | HN | 0.84 | helix d |
| Trp112 | Hε1 | 0.13 | helix d |
| Arg113 | HN | 0.21 | helix d |
| Asn114 | HN | 0.13 | helix d |
| Arg115 | HN | 0.11 | helix d, interacts with helix b |
| *Other titrations* | | | |
| Leu79 | HN | 0.08 | pKa 7.1 |
| Asn75 | Hδ | 0.08 | pKa 7.1 |
| Trp34 | Hε1 | - | pKa >7.5 |
| Asn39 | Hα | - | pKa >7.5 |
| Thr40 | HN | - | pKa >7.5 |
| Gly127 | HN | - | pKa >7.5 |
| Asp91 | HN | - | pKa <4.0 |

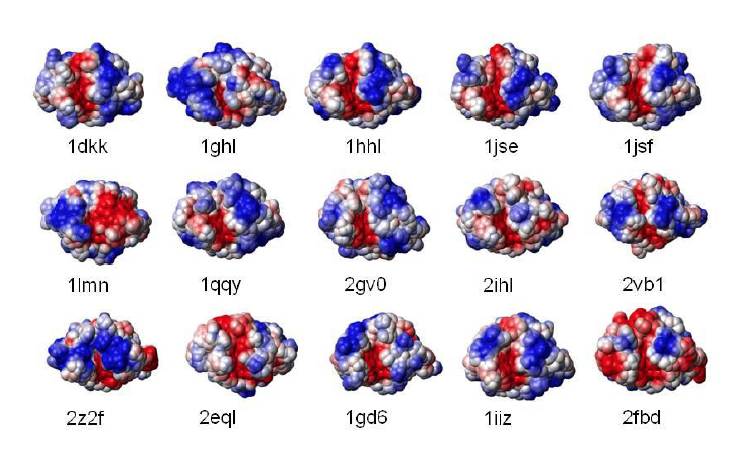
a Shift changes exceeding 0.07 ppm are listed; this corresponds to twice the digital resolution after zero filling.



**Figure S2.** Titration curves for the resonances listed in the top part of Table S1 determined from 2D spectra recorded at various pH values (see Fig. 3). The vertical axis shows normalized chemical shifts Nnorm [Nnorm = (N - N(pH5)) / (N(pH8.1) - N(pH5)), where N(pH5) is the shift measured at pH 5 and N(pH8.1) is the shift measured at pH 8.1, respectively]. Thus, for all curves the chemical shifts are set to unit-less values between 0 (at pH5) and 1 (at pH8.1).



**Figure S3.** Measured shift deviations with a pKa of 6.8 (or 7.1) versus selected sequence fragments in HL. Colour code: blue for HN, red for H and green for other side chain protons. Dotted vertical lines separate sequence fragments; the dashed horizontal line corresponds to the chosen cut-off of 0.07 ppm.



**Figure S4.** Electrostatic surface potentials for 15 c-type lysozyme. Electrostatic potentials where calculated with MOLMOL using charged Asp, Glu, Arg, Lys and uncharged His residues; the surfaces represent the centre positions of a solvent molecule rolling on the protein atoms. Below every figure, the PDB code is given. The different lysozymes are: 1dkk, bobwhite quail; 1ghl, pheasant egg white, 1hhl, guinea fowl; 1jse, turkey; 1jsf, human; 1lmn, rainbow trout; 1qqy, canine milk; 2gv0, soft shell turtle; 2ihl, Japanese quail egg white; 2vb1, hen egg white; 2z2f, bovine stomach; 2eql, equine milk; 1gd6, bombyx mori; 1iiz, tasar silkworm; 2fbd, musca domestica.

**Table S2.** Data collection and refinement statistics.

|  |  |
| --- | --- |
| **PDB entry** | 5LSH |
| **Data collection** |  |
| Synchrotron X-ray source | PETRAIII (DESY) |
| Detector | Pilatus |
| Wave length (Å) | 0.9762 |
| Temperature (K) | 100 |
| Resolution range (Å) | 29.05 - 1.061 (1.099 - 1.061) |
| Space group | P 2(1)2(1)2(1) |
| Unit cell a, b, c (Å) | 33.1, 56.0, 60.5 |
| Total reflections a | 567827 (34483) |
| Unique reflections a | 47721 (3498) |
| Multiplicity a | 11.9 (9.8) |
| Completeness (%) a | 92 (69) |
| Mean(I)/σ(I) a | 17.06 (1.52) |
| Rp.i.m.(%) a, b | 7.6 (108.8) |
| CC(1/2) (%) a, c | 100 (63.3) |
| CC\* (%) a, c | 100 (88.0) |
| Wilson *B*-factor (Å2) | 9.80 |
| **Refinement** |  |
| Reflections used in refinement | 47429 (3498) |
| Reflections used for R-free | 2372 (175) |
| R-work (%) d | 18.1 (32.4) |
| R-free (%) e | 20.4 (33.6) |
| CC-work(%) a, c | 96.9 (69.4) |
| CC-free (%) a, c | 95.2 (70.1) |
| *B*-factors (Å2) (No. of nonhydrogen atoms) |  |
| All | 11.4 (1414) |
| Main chain | 9.3 |
| Side chain | 11.4 |
| Sodium ion | 17.3 (1) |
| Chlorid ion | 13.2 (3) |
| Ligand KTS | 16.6 (90) |
| Water molecules | 20.9 (160) |
| Estimated coordinate error (Å) f | 0.007 |
| rmsd (bonds) (Å) | 0.013 |
| rmsd (angle) (°) | 1.58 |
| Molprobity all-atom clashscore | 7,47 |
| Rotamer outliers (%) | 1.7 |
| Ramachandran plot statistics (%) |  |
| Favoured | 97.0 |
| Allowed | 2.8 |
| Outliers | 0.0 |

|  |  |
| --- | --- |
| a | Values in parentheses are for the high-resolution bin. |
| b |  |
|  | where *Ii(hkl)* is the intensity of the *i*th individual measurement of the reflection with Miller indices *hkl* and *<Ii(hkl)>* is the mean intensity of all measurements of *I(hkl)*, calculated for *I* ≥ *3σ(I); N* is the redundancy or multiplicity of the observed reflection (Weiss 2001; Diederichs et al.1997). |
| c | CC(1/2), percentage of correlation between intensities from random half datasets. Correlation significant at the 0.1 % level. CC\*, the CC of the full dataset against the true intensities (Karplus et al, 2012). |
| d |  |
|  | where *Fobs* and *Fcalc* are the observed and calculated structure-factor amplitudes, respectively. |
| e | *Rfree* is equivalent to *Rcryst* but calculated with reflections (5 %) omitted from the refinement process (Brunger et al. 1992; Tickle et al. 2000). |
| f | Calculated based on a Luzzati plot using the program *SFCHECK* (Vaguine et al. 1999). |

**Table S3. Interactions of tetrasaccharide 2 (KTS) within the binding cleft of human lysozyme.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Residue-Atom** | **Atom (KTS)** | **Ring** | **Distance (Å)** | **Type of bond** |
| **D-Gal*f*-I** |  |  |  |  |
| Ala76-CB | C | 1 | 3.8 | Hydrophobic |
| Arg98-NH2 | O1 | 1 | 3.1 | Hydrogen Bond |
| Arg98-NH2:HOH-O | O | 1 | 2.9 | Bridged H-Bond a |
| Asp102-OD2 | O1 | 1 | 2.8 | Hydrogen Bond |
| Tyr63-OH:HOH-O | O3 | 1 | 3.3 | Bridged H-Bond a |
| **D-Gal*p*-II** |  |  |  |  |
| Asp102-OD2 | O20 | 2 | 2.6 | Hydrogen Bond |
| Tyr63-OH | O7 | 2 | 2.5 | Hydrogen Bond |
| Gly105-O/N/Val99-O:HOH-O | O20 | 2 | 2.7 | Bridged H-Bond a |
| Gln104-NE2 | O9 | 2 | 3.3 | Hydrogen Bond |
| Gly105-O/N/Val99-O:HOH-O | O9 | 2 | 2.8 | Bridged H-Bond a |
| **D-Gal*f*-III** |  |  |  |  |
| Trp64-NE1 | O19 | 3 | 2.9 | Hydrogen Bond |
| Ala108-O:HOH-O | O13 | 3 | 3.3 | Bridged H-Bond a |
| Asn60-OD1/O:HOH-O | O11 | 3 | 2.8 | Bridged H-Bond a |
| Asp49-OD2:HOH-O | O11 | 3 | 3.2 | Bridged H-Bond a |
| Gly105-O/N/Val99-O:HOH-O | O19 | 3 | 2.7 | Bridged H-Bond a |
| Asn60-OD1/Asp49-OD2:HOH-O | O11 | 3 | 3.2 | Bridged H-Bond a |
| **D-Gal*p*-IV** |  |  |  |  |
| Trp109-CA | C20 | 4 | 3.9 | Hydrophobic |
| Trp109-CD1 | C19 | 4 | 3.3 | Hydrophobic |
| Trp109-CE2 | C18 | 4 | 3.7 | Hydrophobic |
| Trp109-CG | C19 | 4 | 3.7 | Hydrophobic |
| Gln58-O | O16 | 4 | 3.1 | Hydrogen Bond |
| Asn60-CB | C16 | 4 | 3.8 | Hydrophobic |
| Asn60-N | O15 | 4 | 2.8 | Hydrogen Bond |
| Trp64-CD1 | C18 | 4 | 3.9 | Hydrophobic |
| Asp53-OD2:HOH-O | O16 | 4 | 2.6 | Bridged H-Bond a |
| Asp53-OD2:HOH-O | O17 | 4 | 2.6 | Bridged H-Bond a |
| Asp53-OD2:HOH-O | O18 | 4 | 3.5 | Bridged H-Bond a |
| Glu35-OE2/Val110-N/Ala111-N:HOH-O | O17 | 4 | 2.6 | Bridged H-Bond a |
| Glu35-OE1:HOH-O | O16 | 4 | 2.6 | Bridged H-Bond a |
| Asn60-OD1:HOH-O | O18 | 4 | 3.1 | Bridged H-Bond a |

1. The given distance for bridged hydrogen bonds is the distance of the bridging water oxygen to the next atom of the tetrasaccharide **2**.

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