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Burkholderia pseudomallei Capsular Polysaccharide Recognition by a Monoclonal Antibody Reveals Key Details toward a Biodefense Vaccine and Diagnostics against Melioidosis

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S Supporting Information

ABSTRACT: Burkholderia pseudomallei is the bacterium responsible for melioidosis, an infectious disease with high mortality rates. Since melioidosis is a significant public health concern in endemic regions and the organism is currently classified as a potential biothreat agent, the development of effective vaccines and rapid diagnostics is a priority. The capsular polysaccharide (CPS) expressed by B. pseudomallei is a highly conserved virulence factor and a protective antigen. Because of this, CPS is considered an attractive antigen for use in the development of both vaccines and diagnostics. In the present study, we describe the interactions of CPS with the murine monoclonal antibody (mAb) 4C4

using a multidisciplinary approach including organic synthesis, molecular biology techniques, surface plasmon resonance, and nuclear magnetic spectroscopy. Using these methods, we determined the mode of binding between mAb 4C4 and native CPS or ad hoc synthesized capsular polysaccharide fragments. Interestingly, we demonstrated that the O-acetyl moiety of CPS is essential for the interaction of the CPS epitope with mAb 4C4. Collectively, our results provide important insights into the structural features of B. pseudomallei CPS that enable antibody recognition that may help the rational design of CPS-based vaccine candidates. In addition, our findings confirm that the mAb 4C4 is suitable for use in an antibody-based detection assay for diagnosis of B. pseudomallei infections.
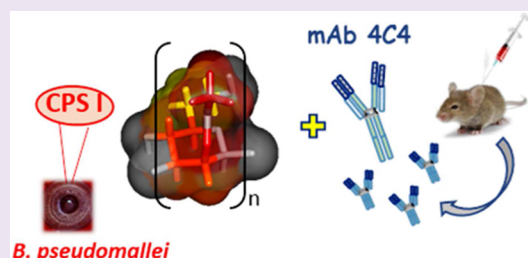
Burkholderia pseudomallei, the etiologic agent of melioidosis, is a facultative intracellular, Gram-negative pathogen that causes severe, debilitating, and often fatal diseases in humans and animals.[1] This environmental saprophyte can be isolated from moist soils, stagnant waters, and rice paddies in endemic regions that typically border 20° north and south of the equator.[2,3] Routes of infection include inhalation, ingestion, and percutaneous inoculation.[4] Clinical symptoms of melioidosis are multifaceted, and disease may manifest as chronic or acute localized infections, acute pulmonary infections, or fulminating septicemias.[1,3] Treatment of these diseases can be challenging, and even with appropriate antibiotic therapy, mortality rates are unacceptably high.[5,6] At present, there are no human or veterinary vaccines available for immunization against melioidosis. Due to the potential misuse of B. pseudomallei as an agent of biological warfare and terrorism, as well as its impact on public health in endemic regions, there is a significant interest in developing effective vaccines and rapid diagnostics to combat disease caused by this CDC (Centers for Disease Control and Prevention) Tier 1 select agent.[7,8]

Capsular polysaccharides (CPS) are a major component of bacterial cell envelopes. Structurally diverse in nature, these highly hydrated antigens exhibit a variety of important biological functions including resistance to desiccation, adherence to host tissues, and protection against innate/acquired immune defenses.[9] Previous studies have demonstrated that the predominant CPS antigen expressed by B. pseudomallei is an unbranched homopolymer consisting of monosaccharide repeats having the structure [→3)-2-O-acetyl-6-deoxy-β-D-manno-heptopyranose-(1→].[10] Interestingly, similar to Burkholderia mallei, all virulent isolates of B. pseudomallei express this CPS antigen.[11]

Several studies have shown that CPS-deficient mutants of *B. pseudomallei* are avirulent in small animal models of infection.[12−14] It has also been shown that CPS production by *B. pseudomallei* contributes to resistance to phagocytosis by reducing C3b deposition on the surface of the bacterium, again implicating CPS as an important virulence determinant.[13,15,16] More recently, it has been shown that CPS-specific mouse monoclonal antibodies (mAbs) and CPS-based glycoconjugates are capable of passively/actively immunizing mice against lethal challenges of *B. pseudomallei*.[17−19] Such findings confirm the protective capacity of this surface-exposed antigen and support the rationale for exploring the use of CPS-based vaccines and CPS-specific mAbs to prevent/treat melioidosis. In addition, during active infections, CPS has been identified as a circulating bacterial antigen that represents an attractive diagnostic target for melioidosis.[20] The purpose of our study was to investigate, at a molecular level, the interaction between a specific murine monoclonal antibody, mAb 4C4, and the CPS from *B. pseudomallei* in order to support their therapeutic and diagnostic potential against melioidosis. To accomplish this, we employed a multidisciplinary approach including organic synthesis, molecular biology techniques, and biophysical methods. The isolation and purification of both the murine monoclonal antibody and *B. pseudomallei* CPS allowed the use of SPR (surface plasmon resonance) and NMR (nuclear magnetic resonance) techniques to gain insights into the mAb 4C4 affinity for CPS, as well as on the structural requirements for epitope binding. Furthermore, *ad hoc* synthesized fragments of CPS were used to investigate in depth the structural features of the interaction with the mAb 4C4, with the aim to provide a detailed picture of the pathogen recognition process in solution.

RESULTS AND DISCUSSION

Binding of CPS to mAb 4C4. *Immunoblot Analyses.* The first evidence we gained on the binding between the CPS from *B. pseudomallei* and mAb 4C4 derived from immunoblot analyses (see Supporting Information). In order to demonstrate that the monoclonal antibody was able to recognize the pathogen capsular polysaccharide, we first carried out a dot blot experiment. Once spotted onto the nitrocellulose filter, the purified CPS and the lipopolysaccharide (LPS) from *E. coli* chosen as a reference, we incubated it with the mAb 4C4. As expected, data showed that CPS was reactive with the monoclonal antibody, unlike the LPS from *E. coli* (Figure S1). These results were then confirmed by a Western blot analysis performed on the native capsule, the deacetylated CPS, and the LPS from *B. pseudomallei*. All the samples were probed with mAb 4C4 (Figure S1), and the results suggested that there was an interaction only between the monoclonal antibody and the native CPS, while neither the deacetylated CPS nor the LPS were recognized by the mAb 4C4.

NMR Analysis. The system CPS−mAb 4C4 also underwent NMR analysis, specifically by Saturation Transfer Difference (STD) NMR spectroscopy,[21,22] with the aim of identifying the region of the polysaccharide closer to the mAb binding pocket. First attempts to investigate the ligand−antibody interaction by STD NMR spectroscopy were unsuccessful. In fact, despite the use of different irradiation frequencies, the capsule, even in the absence of the antibody, became inherently saturated and thus gave rise to signals in the STD NMR spectra, due to its high molecular weight. Accordingly, we decided to carry out STDD (saturation transfer double difference) NMR experiments[23] with the aim to qualitatively assess the occurrence of binding

between the antibody and the CPS. In detail, we performed a reference STD NMR spectrum of the capsule in the absence of the antibody, and then we subtracted the resulting STD spectrum from that of CPS in the presence of the mAb 4C4 (Figures S2,S3). The observed signals in the STDD spectrum were further proof of the binding between the antibody and the capsular polysaccharide (Figure S2), in accordance with the immunoblot analyses.

SPR Analysis. The binding of mAb 4C4 to CPS was also investigated using SPR, which is a method that allows for analysis of bimolecular interactions in real-time and without chemical reporters. Biotin-labeled CPS was immobilized on the surface of a streptavidin-coated sensor chip, after which the binding of several serial dilutions (330−1.3 nM) of mAb 4C4 was analyzed. Each concentration of mAb was injected over the sensor surface for 60 s, after which it was allowed to passively dissociate for 120 s.

The association (0−60 s) and dissociation (60−180 s) phases of mAb 4C4 at each concentration were recorded and plotted as a sensorgram, which shows the changes in refractive index (binding/dissociation) in arbitrary response units (RU) as a function of time (s) (Figure 1, colored lines). The
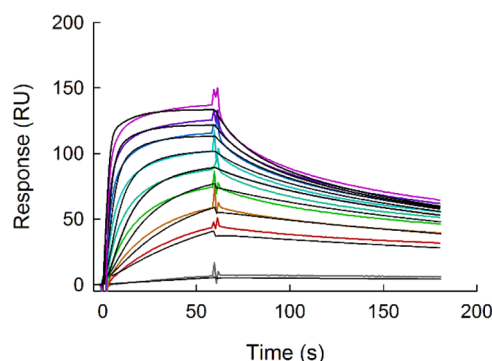

Figure 1. SPR analysis of mAb 4C4 binding to CPS. Biotinylated CPS was immobilized on the surface of a streptavidin-coated sensor chip. Samples (2-fold serial dilution of mAb 4C4 [330−1.3 nM]) were injected over the sensor surface for 60 s, after which the mAb was allowed to passively dissociate for 120 s. Apparent kinetic k_{on} and k_{off} values were determined using a bivalent analyte kinetic algorithm. Both the experimental (colored lines) and fitted (black lines) data are shown.

sensorgrams that were produced by each concentration of mAb 4C4 appeared to show biphasic kinetics—both k_{on} and k_{off} were composed of a "fast" phase and "slow" phase. This kinetic model is expected to be complex due to the multivalency of the CPS antigen and the bivalency of mAb 4C4. A bivalent analyte kinetic algorithm was used to fit the binding data and determined the apparent kinetic constants (Figure 1, black lines): $k_{on} = 1.2 \times 10^6$ M^{-1} s^{-1}, $k_{off} = 0.035$ s^{-1}.

Binding of ad hoc Synthesized CPS Fragments (1−3) to mAb 4C4. In order to acquire further information on the binding mode of the mAb 4C4 and to accurately map the interacting epitope, synthetic fragments (monosaccharide **1**, disaccharide **2**, and deacetylated disaccharide **3**) related to the CPS of *B. pseudomallei* were investigated.

As depicted in Scheme 1, the target compounds **1** and **2** were synthesized from a thioheptoside donor according to a sequence involving a *β*-stereoselective naphthyl-mediated intramolecular aglycon delivery (NAP-IAD) followed by C2-acetylation and global deprotection.[24] Ammonolysis of

Scheme 1. Synthetic Mono- and Disaccharidic Fragments (1−3) of CPS from *B. pseudomallei* (Ac = acetyl; All = allyl; Bn = benzyl; IAD = intramolecular aglycon delivery; NAP = 2-naphthylmethyl; SPh = thiophenyl)
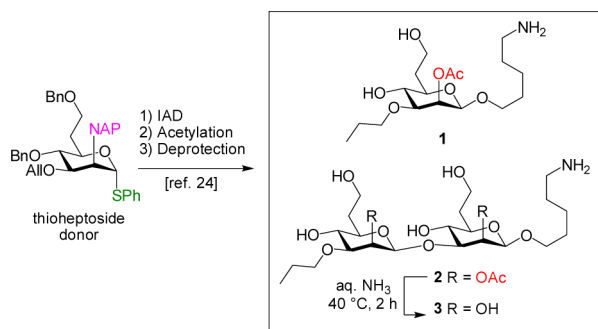
disaccharide **2** led to the formation of the deacetylated disaccharide **3**, which was also investigated for binding with mAb 4C4.

STD NMR Analysis. As a first step of the analysis, STD NMR experiments were performed on both acetylated ligands (**1** and **2**) in the presence of the antibody, once the experimental conditions optimized. The STD NMR spectrum obtained on the monosaccharide **1**-mAb 4C4 mixture did not show any signal (Figure S4), likely due to unfavorable binding kinetics, which impaired an efficient NOE buildup and the observation of STD-NMR effects.[25,26]

Interesting results were achieved by the analysis of the interaction between the antibody and synthetic disaccharide **2**, which allowed the direct characterization of the ligand binding epitope (Figure 2). A qualitative evaluation of the STD effects for the sugar proton signals showed that the strongest enhancement was observed for the *O*-acetyl group located at C2, indicating that the OAc moiety was in close contact with the antibody, experiencing the highest transfer of saturation.
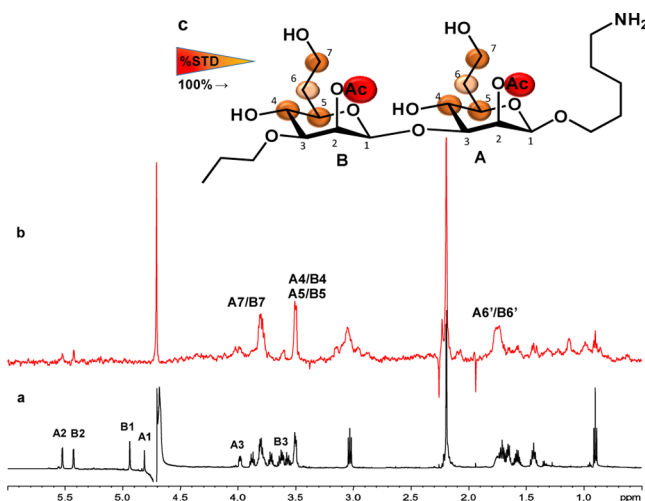

Figure 2. STD NMR analysis of disaccharide **2** when bound to mAb 4C4. (a) Reference ^{1}H NMR spectrum and (b) STD 1D NMR spectrum of a 1:60 mAb 4C4/disaccharide **2** mixture. (c) Chemical structure and epitope binding of disaccharide **2** bound to mAb 4C4. The irradiation frequency was set at −1 ppm, and a saturation time of 2 s was used. The sugar protons more involved in the interaction are indicated with the following color coding. Red circle: STD 100%. Orange circle: STD 90−70%. Yellow circle STD < 70%.

These data were in accordance with the above results obtained for the binding of mAb to the native capsule (Figures S1,S2). Furthermore, STD effects were also observed for proton signals at positions 7, 5, 4, and 6; on the other hand, proton signals 1, 2, and 3 were not boosted, emphasizing their distance from the antibody surface binding site.

In order to quantitatively investigate the CPS recognition from the antibody mAb 4C4, STD buildup curves were determined at different saturation times.[27,28] This method allowed minimization of possible bias in the calculation of STD effects due to the different proton longitudinal relaxation times (T1), as well as the intramolecular spin diffusion within the bound state (see Supporting Information, Figure S5).

Table 1 summarizes the STD percentages (STD epitope fit) derived from the fitting of experimental data to the monoexponential equation $STD = STD_{max}[1 - \exp(-k_{sat}t)]$.

From the quantitative results, it was confirmed that the protons H7, H5, H4, and H6 were recognized by the antibody since they exhibited an STD epitope$_{fit}$ above the 70%.

Particularly, the epitope map of disaccharide **2** showed also that the highest degree of enhancement was ascribable to the acetyl moiety of the 6-deoxyheptose. These outcomes were in agreement with Western blotting data which assessed the loss of reactivity of the deacetylated CPS with mAb 4C4 (Figure S1), confirming the importance of the acetyl group for the interaction with the antibody.

Given the previous results and in order to understand if the acetyl group was strictly required for the recognition event, we chose to investigate, by STD NMR, the interaction between the deacetylated disaccharide **3** and mAb 4C4 (Figure 3). It is noteworthy that no STD NMR signal was observed from the whole disaccharide, indicating that the acetyl moiety was definitely essential for the recognition and binding of the CPS epitope with the antibody. We could then conclude that the binding was deeply hampered by the absence of this ligand portion.

Tr-NOE Analysis. The transferred NOE method allows acquisition of key information about the bioactive conformation of a ligand bound to its receptor; therefore, it represents a suitable approach to complement interaction studies gained by STD NMR spectroscopy. In order to assess the ability of mAb 4C4 to recognize the disaccharide **2** and to analyze its possible conformational changes upon binding, we carried out 2D NOESY experiments on the ligand in the presence and in the absence of the monoclonal antibody. As shown in Figure 4, the change in the sign of NOE signals, between the free ligand and the bound form in solution, confirmed the physical binding between mAb 4C4 and disaccharide **2**.[29]

A quantitative analysis based on the construction of NOE buildup curves[30,31] was performed, and the consequent evaluation of the pattern of specific interglycosydic NOE contacts allowed the description of the mAb binding mode. The cross peak intensity, measured at different mixing times, was used to extract the ^{1}H−^{1}H cross relaxation rate (σ) and to calculate the proton−proton *inter*-residual distances. These values were then compared with the calculated distances obtained for the main energetic minimum, predicted for the disaccharide **2** (Figure 5a,b).

Although slight differences in the cross peak volumes were observed between the free and the bound state, the quantitative results indicated that the glycosydic linkage, and thus the disaccharide whole conformation, was barely affected upon binding to the antibody. The comparison of the experimental

Table 1. Experimental STD Intensities of Disaccharide 2 Bound to mAb 4C4 at Different Saturation Times[a]

	STD exp						
[1]H	1s	3s	5s	STD max	Ksat	STD (fit)	STD epitope (fit)
H7	77.2	67.2	57.3	0.0094	0.4586	0.00431	84
H4	90.9	89	80	0.0147	0.3191	0.00469	91.4
H5	90.9	89	80	0.0147	0.3191	0.00469	91.4
H6	68.1	63.6	53.3	0.0091	0.4134	0.00376	73.2
OAc	100	100	100	0.0197	0.2607	0.00513	100

[a]STDmax were calculated by fitting the data to a monoexponential equation: $STD(t_{sat}) = STD_{max}(1 - \exp(-k_{sat} \times t_{sat}))$.
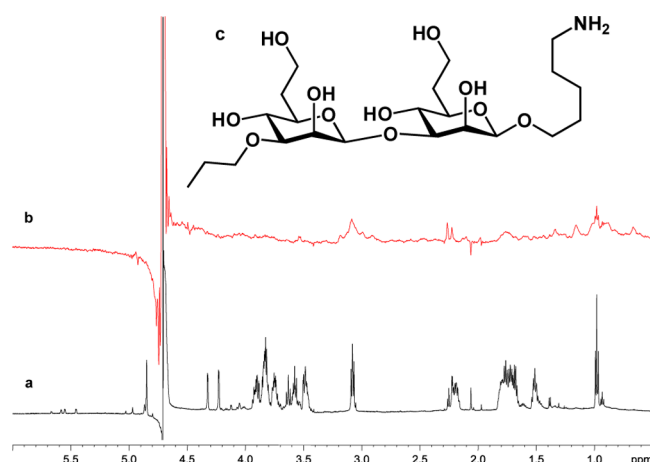

Figure 3. STD NMR analysis of disaccharide **3** when bound to mAb 4C4. (a) Reference ^{1}H NMR spectrum and (b) STD 1D NMR spectrum of a 1:100 mAb 4C4/deacetylated disaccharide mixture. (c) Chemical structure of the deacetylated disaccharide.
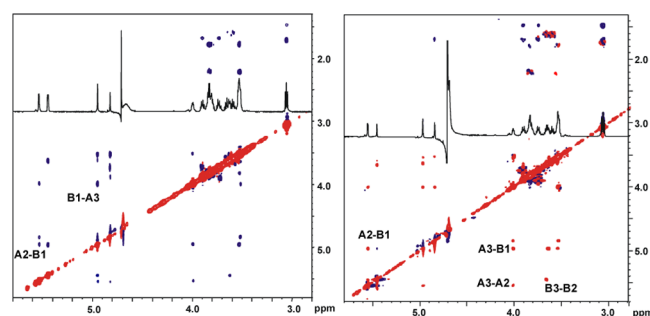

Figure 4. NOESY spectra of the disaccharide **2**. On the left: NOESY of the disaccharide **2** in the free state. On the right: tr-NOESY of the disaccharide **2** bound to mAb 4C4. A mixing time of 300 ms was used.

and calculated distances (Table S1), indeed, demonstrated that, both in the free and bound state, the disaccharide preferentially populates the energetic minimum characterized by positive values of the dihedral angles, Φ (∼50°) and Ψ (∼10°) (Figure 5c).

Pulsed-field Gradient (PFG)-NMR Analysis. An alternative approach, based on PFG NMR measurements, was employed for studying the recognition of *B. pseudomallei* by the monoclonal antibody mAb 4C4. PFG-NMR is a useful technique to calculate the diffusion coefficient (*D*) of molecules in solution and offers a fast method to characterize molecular interactions.[32] A ligand and its receptor exhibit, in the free state, their own diffusion coefficients, which are dependent on their weight, size, and shape. When the ligand forms a complex with its receptor protein, a variation in the observed *D* may occur. In detail, for the case where the host–guest association is strong,

both the ligand and the protein exhibit the same diffusion coefficient that reflects the tight complex. In the case of weak interaction, no changes in *D* values are observed; for the other cases, when exchange is fast on the NMR time scale, the measured diffusion coefficient is the weighted average of that of the free and bound ligand.[33,34] The possibility to discriminate between the diffusion coefficient of the disaccharide **2** and those of the antibody mAb 4C4 (see Supporting Information, Figures S6–S7) simplified the attempt to investigate the binding by using PFG NMR experiments.[35] In order to achieve the condition of fast exchange, on both chemical shift and diffusion time scales, a molar excess of ligand and sufficiently long values of Δ were used (see Methods).[32] Self-diffusion coefficients of the free and the bound forms were determined by following the decay of signal intensity at 2.18 ppm of the acetyl group (Figure 6). The quantitative analysis of the PFG NMR spectra yielded a diffusion coefficient of 5.2×10^{-10} m²/s for the disaccharide **2** alone in solution, while, upon the addition of the mAb, it skewed toward lower values (3.9×10^{-10} m²/s). Therefore, as we expected, going from the free to the bound form, the ligand showed a change in the measured diffusion coefficient, further highlighting the recognition of disaccharide **2** by the antibody.

The resulting diffusion coefficients are reported at the top right of each panel. The error associated with the measurement was estimated, from repeated calculations of the diffusion coefficients, to be below 5%. Notably, monoexponential behavior was observed also for the decay of other proton signals (data not shown).

Conclusions. Understanding how complex glycans interact with antibodies constitutes a pivotal step toward the design of new carbohydrate-based vaccines or drugs against several diseases and bacterial infections. Currently, disparate polysaccharide derived vaccines, which are able to boost the immune response against carbohydrates exposed on disease-causing bacteria, are commercially available.[37−39] On the other hand, the conjugation of appropriate oligosaccharide to a carrier protein, as well as the use of glycan mimetics to replace or complement them in the triggering of immune response, represents different valuable strategies.[40] In recent years, several studies based on NMR, molecular modeling, and X-ray analysis were published to promote the development of efficient vaccine strategies against pathogens like *Shigella flexneri*,[39−41] for instance. NMR spectroscopic methods, coupled to X-ray crystallography, indeed constitute an exquisite tool to gain molecular level insight into the antibody recognition of bacterial surface glycoconjugates required to design efficient carbohydrate-based vaccines or drugs.[42] To date, only a small number of mAb-glycoconjugate X-ray structures were reported.[43] Thus, whenever the production of complexes for crystallographic studies is not achieved,[42] NMR represents a robust approach to improve the knowledge on the mechanisms
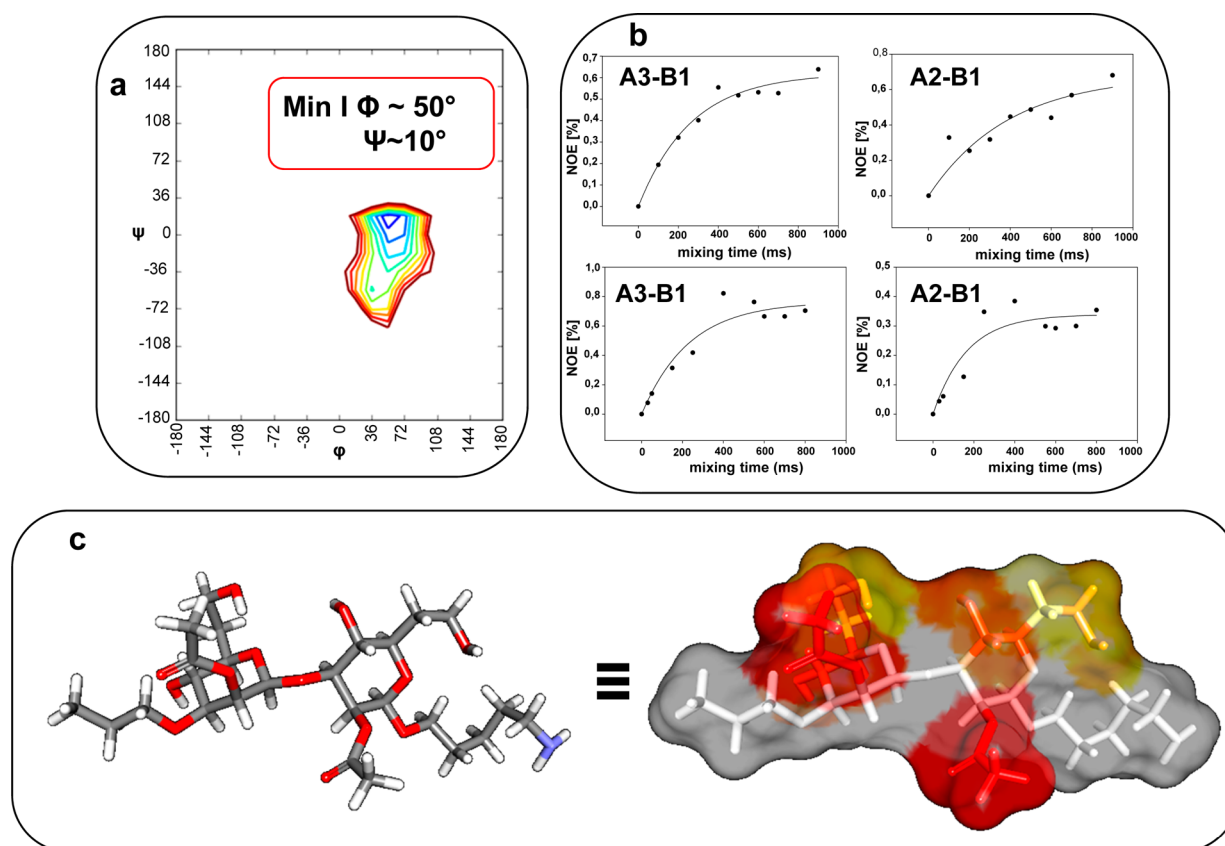
Figure 5. Tr-NOESY analysis. (a) Adiabatic energy map of disaccharide **2** indicating one main global minimum characterized from Φ and Ψ positive values (Min I). (b) In the upper panel, NOE buildup curves of disaccharide **2**, for A3-B1 and A2-B1 contacts, in the absence of mAb 4C4. In the lower panel, the buildup curves of the disaccharide **2**, for the same cross peaks, in the presence of the antibody. The curves have been normalized using the decay of the corresponding diagonal signal as a reference. (c) Representations of the predicted energetic minimum, preferentially populated by the ligand in the free and bound state. STD derived epitope mapping on the molecular envelope of disaccharide **2**, with color coding from the highest (red) to the lowest (yellow) observed STD effect (*cf.* Table 1, Figure 2).

of glycoconjugates recognition by antibodies. Moreover, peptides or glycopeptides that mimic complex bacterial polysaccharide antigens, derived from NMR based analysis, represent alternatives to the classical polysaccharide-based conjugate vaccines developed so far. To this aim, the analysis of the molecular basis of bacterial antigens−antibody recognition and binding constitutes a breakthrough in the development of new conjugate vaccines; the map of the binding epitope, in fact, is a starting point to design peptides or glycopeptides mimicking the binding antigen.

In this context, the present study focused on the application of NMR methods to investigate the key structural requisites governing the recognition of *B. pseudomallei* surface glycans by the murine monoclonal antibody 4C4. In this work, we employed a multidisciplinary approach including organic synthesis, molecular biology, and biophysics. Upon the isolation and purification of the CPS from *B. pseudomallei* and the monoclonal antibody mAb 4C4, we gained the first evidence of binding from immunoblotting experiments. Then, an accurate NMR analysis, carried out on both purified and *ad hoc* synthesized products, permitted the in depth characterization of the binding at the atomic level. Interestingly, STD NMR experiments demonstrated that the acetyl group, located at position 2 of the *B. pseudomallei* CPS repeating unit, played an essential role in the interaction with the antibody surface. Its effect on the recognition event was so significant that the absence of the acetyl moiety resulted in a complete loss of mAb

4C4 affinity for the pathogen. Furthermore, transferred NOESY methods have been employed to improve the knowledge on the conformational behavior of the bacterial CPS. In the case of mAb 4C4−CPS disaccharide fragment recognition, no conformational selection occurred upon binding, unlike some previously described protein−carbohydrate interaction systems.[29]

All these outcomes may have implications in the development of new therapeutics and diagnostics against melioidosis. Indeed, on the one side, our results support the potential use of the mAb 4C4 as an adjunct therapy to antibiotics and, at the same time, indicate that the acetyl moiety is strictly required for optimal mimicry of the CPS epitope. These findings are of particular note in the context of vaccine and therapeutic design because the fine-tuning of selective binders resides at the basis of highly active drugs production.[44]

On the other hand, our data provide a rational basis to improve the early detection of the pathogen *B. pseudomallei* and support the CPS as a potential biomarker for diagnosis of melioidosis. Along with being secreted during infection, the CPS appears to be present in most all *B. pseudomallei* and *B. mallei* isolates and rarely found in near neighbor *Burkholderia* species.[20] This has recently been shown by analytical reactivity and specificity testing with the CPS-specific mAb 3C5.[45] The collected data, indeed, underline the utility of including mAb 4C4 in a point of care microbial detection assay. In fact, due to its superior affinity for CPS, mAb 4C4 has replaced mAb 3C5
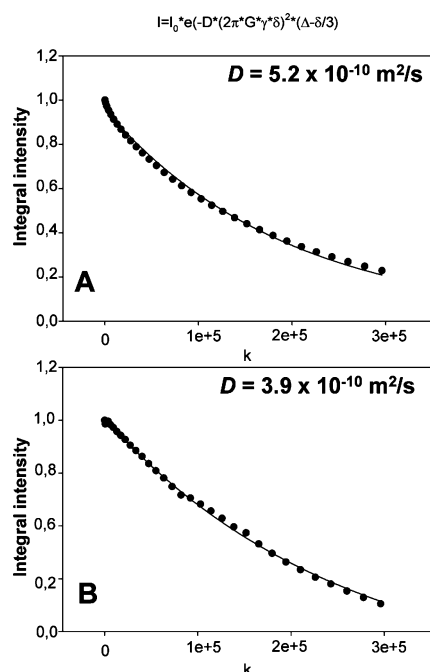
$$I = I_0 * e^{(-D*(2\pi*G*\gamma*\delta)^2*(\Delta-\delta/3)}$$

$D = 5.2 \times 10^{-10}$ m^2/s

A

$D = 3.9 \times 10^{-10}$ m^2/s

B

Figure 6. PFG-NMR analysis. Decay of the proton signal at 2.18 ppm, in the absence (A) and in the presence (B) of the antibody, as a function of the complex abscissa $k = (G\gamma\delta)^2(\Delta - \delta/3 - \tau/2)$. When sinebell gradient pulses are chosen, the gradient amplitude is scaled by a factor of $2/\pi$ with respect to the rectangular pulse.[36]

in the prototype Active Melioidosis Detect Lateral Flow Immunoassay (AMD LFI)[45] In unpublished studies, mAb 4C4 appears to possess similar analytical reactivity and specificity to that of mAb 3C5, while its superior affinity might allow for detection of lower concentrations of CPS within patient samples.

To conclude, the comprehension, at a molecular level, of which structural and conformational elements of the CPS influenced the selectively binding with mAb 4C4, may open a new avenue toward establishing rules for the focused design of carbohydrate-based antigens, diagnostics, and therapeutics against melioidosis; performing passive immunization studies with these CPS specific mAbs may validate the usefulness of a CPS-based vaccine and offer insights as to the affinity and IgG subclass that may need to be generated with such a vaccine.

METHODS

Purification of *B. pseudomallei* CPS and LPS. Purified CPS and LPS antigens were obtained as previously described[46,47] from *B. pseudomallei* RR2683 (O-polysaccharide mutant) and *B. pseudomallei* RR2808 (CPS mutant; see also the Supporting Information).

Immunization of Mice and Production of mAb 4C4. mAb 4C4 IgG$_1$ was produced as described in the Supporting Information.

SDS-PAGE, Dot Blot, Western Immunoblotting Analyses. See the Supporting Information.

SPR. Antibody−antigen binding experiments were performed as previously described.[48] The experimental details are reported in the Supporting Information.

NMR Analysis. All NMR experiments were performed with a Bruker 600 MHz DRX instrument fitted with a cryo probe at 310 K. All the samples were dissolved in a phosphate buffer

(pH 7.4), and spectra were calibrated with internal sodium [d$_4$](trimethylsilyl)propionate (TSP).

Data acquisition and processing were performed with TOPSPIN 3.2 software.

The experimental conditions used in STD NMR, STDD NMR, tr-NOESY, and PFG-NMR analyses are described in detail in the Supporting Information.

Conformational Studies. Molecular mechanics calculations were performed as described in the Supporting Information.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschembio.5b00502.

Reactivity of mAb 4C4 with CPS antigens (Figure S1), STDD NMR analysis of the binding between mAb 4C4 and the CPS (Figure S2), control NMR spectra for STDD analysis (Figure S3), STD NMR analysis of the binding between mAb 4C4 and the monosaccharide 1 (Figure S4), STD buildup curves for protons of disaccharide 2 in the presence of mAb 4C4 (Figure S5), experimental and calculated interproton distances for the disaccharide 2 (Table S1), PFG NMR analysis of disaccharide 2 and mAb 4C4 (Figure S6 and S7), and experimental detail (PDF)

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Notes
The authors declare no competing financial interest.

ABBREVIATIONS

CDC, Centers for Disease Control and Prevention; CPS, capsular polysaccharide; LPS, lipopolysaccharide; mAb, monoclonal antibody; NAP-IAD, naphthyl-mediated intramolecular aglycon delivery; NMR, nuclear magnetic resonance; PFG, pulsed-field gradient; RU, response unit; SPR, surface plasmon resonance; STD, saturation transfer difference; STDD, saturation transfer double difference; Tr-NOE analysis, Transferred Nuclear Overhauser effect

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