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Conformational studies of Gram-negative bacterial quorum sensing 3-oxo *N*-acyl homoserine lactone molecules

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ABSTRACT

In their ^{1}H NMR spectra in $CDCl_3$ 3-oxo-*N*-acyl homoserine lactones (OHLs) show significant downfield chemical shifts of the amide N—H proton when compared to the parent *N*-acyl homoserine lactones (AHLs). NMR spectroscopic and DFT calculation studies have shown that this is most likely due to the presence of a stabilising intramolecular H-bond from the N—H to the 3-oxo group. The ^{1}H NMR spectra also show evidence for the enol tautomers and that the amount of enol present for a range of OHLs is 4.1–4.5% in $CDCl_3$ and 6.5–7.2% in CD_3CN. In contrast, DFT calculations show that the lowest energy enol tautomer and the keto tautomer are of equal energy in the gas phase, but that the keto tautomer is more stable in chloroform, acetonitrile and water solution. The calculations also show that there is no evidence for any n → π* or C5H-bonding interactions being present in either the lowest energy keto or enol tautomer of the OHLs in solution or the gas phase, which is in contrast to the reported solid-state structure.

1. Introduction

Antimicrobial resistance (AMR), particularly among Gram-negative species such as *Klebsiella* species, *E. coli* species and *Pseudomonas aeruginosa*, has been identified by the World Health Organisation as one of the greatest current threats facing the health of mankind.[1] New antibiotics are therefore continuously required to combat the threat of AMR, with as many different approaches as possible being used. Many bacterial species use small molecules or peptides to communicate with each other, either within a bacterial species or between species.[2] The concentration level of these communication molecules (autoinducers) is dependent on the number of bacteria present. When a sufficient number of bacteria are present a "quorum" is reached and the bacteria change their behaviour from that of a single-celled organism to multi-celled. This quorum sensing causes an alteration in gene expression which can lead to changes in the virulence factors of the bacteria, and in some cases leads to biofilm formation.[2] Therefore inhibition of quorum sensing may be an important method for halting the spread of bacterial infections, and very importantly is bacteriostatic rather bactericidal in nature, which may help to reduce incidences of the development of AMR.

In many clinically relevant Gram-negative bacteria these communication molecules are *N*-acyl l-homoserine lactones (AHLs) and 3-oxo *N*-acyl l-homoserine lactones (OHLs), as seen Fig. 1.

Structurally they consist of a γ-lactone head-group linked to a hydrocarbon chain via an amide (AHLs) or β-ketoamide linkage (OHLs). The range of AHLs and OHLs found in nature differ mainly in the length of the hydrocarbon chain and whether it is branched, unsaturated, hydroxylated or indeed combinations of these. However, the amide or β-ketoamide linkage is ubiquitous. A number of groups have studied analogues of both AHLs and OHLs as agonists and antagonists of quorum sensing in a range of clinically important bacterial species, such as *P. aeruginosa* and *A. baumannii*.[3] These analogues have included changes to the lactone head-group, the amide or β-ketoamide linker and the hydrocarbon chains, and some very active inhibitors have been identified.[4] Recently, Blackwell has used receptor mutation studies to detail the important hydrogen bond (HB) interactions for binding of the both native and non-native molecules with the LasR receptor.[5] The Raines group used computational methods *in vacuo* to identify possible n → π* interaction in AHLs,[6] which were recently confirmed by the Prabhakaran group.[7] These reports studied how the n → π* interactions might influence the stability and reactivity of AHL molecules. More recently Raines reported the solid-state X-ray crystal structure of the OHL, *N*-(3-oxobutanoyl)-l-homoserine lactone, which showed evidence for two stabilising n → π* interactions between the ketone carbonyl to amide carbonyl and from

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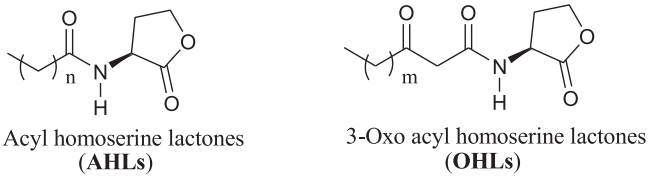

Fig. 1. Structures of AHL and OHL bacterial communication molecules.[2]

the amide carbonyl to the lactone carbonyl (Fig. 2).[8] In addition the crystal packing shows intermolecular H-bonding between the amide carbonyl oxygen of one molecule with the amide N—H of the next molecule in the unit cell. An examination of the published X-ray structures in the Protein Database (PDB)[9] of receptor-bound OHLs shows that the preferred bound conformation is for an extended molecular structure (Fig. 2). The fact that such significantly different conformations are observed would suggest that the molecules are relatively flexible allowing many possible conformations to be achieved. How exactly any of these conformations directly relate to the actual bioactive conformation is not clear, as both the receptor-bound and solid-sate structures might just be the conformations that led to crystallisation under the experimental conditions used.

2. Results and discussion

We were interested in studying the conformational preferences for OHLs, to see how the extra carbonyl group might impact the overall preferred conformations of these molecules. Since Blackwell showed the importance of H-bonding of OHLs to their receptors[5] it would be of interest to see whether there is any evidence for HBs within these molecules *in vacuo* and/or in solution, in the absence of their receptors. If such HBs are possible there is no guarantee that they would be also be present when the OHLs are receptor-bound as the HBs described by Blackwell, between the OHL and the receptor amino acid residues and/or water molecules within the binding site, are likely to be more important. However, a deeper understanding of the overall structural preferences of OHLs may possibly allow for the better design of quorum sensing inhibitors in the future.

2.1. NMR studies of OHL molecules

The ^{1}H NMR spectra, as measured in dilute 1 mM solutions in $CDCl_3$, for a number of commercially available OHLs (C_6-C_{14} side-chains) all showed a downfield chemical shift for the amide N—H protons at 7.6–7.7 ppm (Fig. 3). $CDCl_3$ was chosen as the solvent for the NMR studies because a recent report from the Mavri group used the dielectric constant of the internal protein environment as ~4.0,[10] as previously reported by the Himo group.[11] This value is very close to that of chloroform with a dielectric constant of 4.81, while the dielectric constant of water (biological solvent) is ~78.4.

The chemical shifts obtained for the OHLs were all consistent with those reported in the literature for these compounds.[4,12] The observed downfield chemical shift would be expected if the N—H proton was H-bonded to the 3-oxo group of the side-chain (Fig. 2). Further evidence for the presence, in $CDCl_3$ solution, of the H-bonded keto conformer **C** comes from a comparison of the chemical shift of the amide N—H proton in the 3-oxo AHLs with the values reported (6.0–6.2 ppm) for the analogous AHL compounds which lack the 3-oxo group.[12] The solution concentration used was 1 mM which is in contrast to the solution NMR studies by the Prabhakaran group on the analogous AHL molecules where concentrations of 10 mM for ^{1}H NMR and 60 mM for ^{13}C NMR were used.[7] Raines described that similar peptides to those used in their study of C5 H-bonds were shown to be monomeric at a concentration of 10 mM.[13,14] Although the OHLs being studied here are not peptides there is no reason to think that at a concentration of an order of magnitude lower, i.e. 1 mM, that the molecules would be anything but monomeric in $CDCl_3$ solution. The presence of an intramolecular, rather than intermolecular, HB was confirmed when the ^{1}H NMR spectra of the C_8-OHL at concentrations of 1 mM, 2.5 mM, 5 mM and 10 mM (Fig. 4) were acquired and the observed chemical shift of the N—H proton in each case was consistent at 7.641 ppm, 7.642 ppm, 7.643 ppm and 7.644 ppm, respectively.

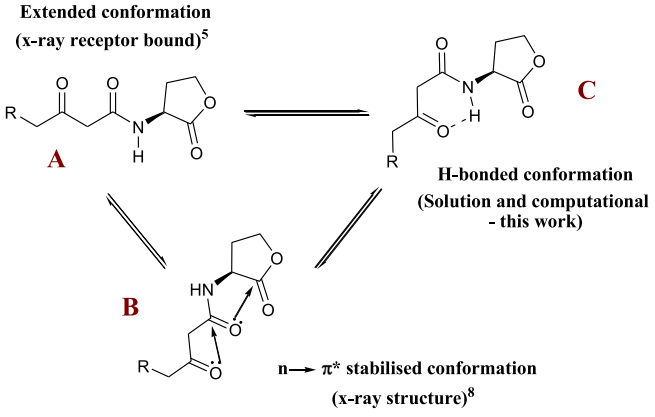

Fig. 2. Some possible conformations of OHLs (R = H or alkyl).[5,8]

2.2. Computational studies of a simple OHL molecule

Density Functional Theory (DFT) calculations, at the B3LYP 6-311++G(d,p) computational level *in vacuo*, of a simple OHL structure (Fig. 2, R = CH_3) gave some unexpected results. A number of repetitions of calculations, involving changes to the starting orientation of the side-chain, by rotation around the amide carbonyl to C_2 carbon bond and lactone α-carbon to amide nitrogen (Fig. 2) were undertaken. The array of starting conformations employed included those seen in the X-ray crystal structure of an isolated OHL,[8] as well as receptor bound conformations.[9] Any attempt to optimise conformation **A** resulted in a distorted structure with the two carbonyl groups pointing to different directions provoked by the repulsion between both groups (Fig. 5). The minimum energy conformation found was quite different to the receptor bound conformation.[9]

Regardless of the starting conformation of the side-chain the lowest energy conformation obtained (**C**) was a compact structure stabilised by one intramolecular HB (see Table S1 for full details). This HB (2.049 Å, *vide infra*) is formed between the amide N—H and the 3-oxo carbonyl group. Although there is the possibility of a C5 type H-bond (between the N—H and the oxygen atom of the lactone), which is now considered to be very important in stabilising peptide and protein structures,[13,14] no evidence of such an interaction was found either by means of Natural Bond Orbital (NBO) calculations[15] or by Atoms In Molecules (AIM)[16] results.

The HB found in conformer **C** is an intramolecular HB forming a 6-membered ring, with the O–N distance being 2.793 Å and an O–H-N angle of 128.2°. The HB is characterised (by AIM) by the presence of a bond critical point (BCP) between both interacting atoms with an electron density (ρ_{BCP}) of 0.0224 a.u., which is in the range of HBs.[16] The Laplacian value ($\nabla^2\rho$) of that HB shows that this interaction is in the closed shell regimen (see Table S1). It has been stated that the negative value of the total electron energy density at the BCP, H_{BCP}, confirms the covalent character of the correspond-