Biophysical Chemistry 238 (2018) 16–21

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: www.elsevier.com/locate/biophyschem

Conformational studies of Gram-negative bacterial quorum sensing acyl homoserine lactone (AHL) molecules: The importance of the n → π* interaction

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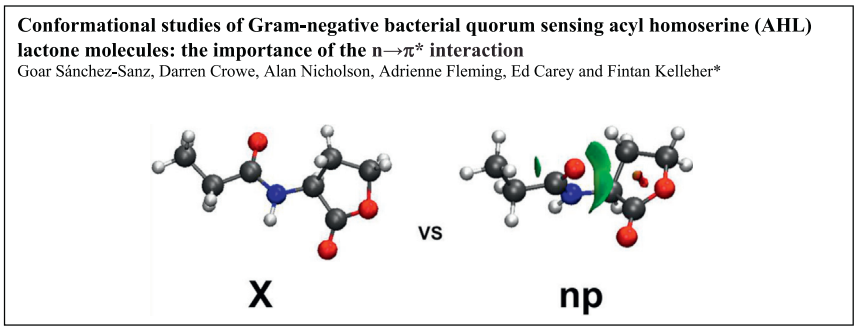
HIGHLIGHTS

- AHLs show intermolecular hydrogen-bond in $CDCl_3$ solution.
- DFT calculations of extended and n → π* interaction conformations of AHLs in the gas phase.
- DFT calculations of extended and n → π* interaction conformations of AHLs in the solvent models.
- The n → π* interaction becomes more important with increased dielectric constant of the solvent.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Acyl homoserine lactones
Quorum sensing
n → π* interaction
DFT calculations
Non-covalent interactions, solvent effects

ABSTRACT

A ^{1}H NMR study shows the presence of intermolecular hydrogen bonds for AHLs in $CDCl_3$ solution. A detailed computational study of the structure of AHLs and the relative stability between the extended conformations (**X**) and those showing n → π* interactions (**np**) have been carried out by means of DFT calculations. Solvent effects have been shown to be very important when stabilising **np** conformations, particularly with polar solvents. This was shown by the shortening of C···O intramolecular distances and the increase in the relative energies favouring the **np** conformation with the dielectric constant of the solvent. The charge transfer between the O donor and the acceptor carbonyl group, assessed by second order perturbation energies, E(2), also shows an increase in the E(2) values with the dielectric constant of the solvent.

1. Introduction

According to the World Health Organisation, Antimicrobial Resistance (AMR) is one of the biggest Global challenges facing mankind [1]. Bacteria which are resistant to all currently used antibiotics are appearing so the requirement for the discovery of new antibiotic classes and compounds has never been more important. Of particular concern is the rise in AMR of Gram-negative species such as *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Eschericia coli*, since many of the current antibiotics in development only target Gram-positive species. Bacteria in general have the ability to communicate with each other using molecular signals and when the bacteria reach a threshold

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https://doi.org/10.1016/j.bpc.2018.04.002
Received 8 March 2018; Received in revised form 10 April 2018; Accepted 10 April 2018
Available online 17 April 2018

concentration (quorum) their behaviour can change to that of a multi-celled organism from being single-celled. This ability to detect the numbers of other bacteria in their environment is known as quorum sensing (QS) [2]. Alterations in gene expression ultimately lead to an increase in virulence factors of the bacteria, and one outcome can be the generation of biofilms, which can be highly resistant to antibiotics as the biofilm behaves as a physical barrier to antibiotics. In a number of Gram-negative species the communication molecules secreted are acyl homoserine lactones (AHLs), along with their 3-oxo analogues (OHLs) [3]. Significant effort has gone in to trying to develop analogues of these molecules to inhibit the communication process thus switching off the QS process. Importantly, inhibitors of QS would be bacteriostatic in nature rather than bactericidal, which would mean that there would be less likelihood for the development of resistance [4]. Both AHLs and OHLs contain a γ-lactone head group which is attached, by an amide linker, to an alkyl chain, usually of four to fourteen carbons in length. Therefore, in order design new QS inhibitors a greater understanding of the overall structures and properties of AHLs and OHLs is important.

We recently reported on conformational studies of OHLs by NMR and computational methods [5]. Significantly it was found that there are a number of possible low energy conformations depending on whether the OHLs were studied in the gas phase or in solvent models (computational), in solution (NMR), in the solid state (x-ray crystallography) or bound to their cognate receptor (x-ray crystallography). The 3-oxo group of the side-chain was found to form a hydrogen-bond (HB) with the amide N—H, stabilising a compact conformation. We were next interested in studying in detail the relative stabilities of the AHLs, which lack the 3-oxo group, in terms of their possible conformations.

2. Experimental

The C8 AHL was purchased from Sigma-Aldrich (Irl) Ltd. NMR spectra were obtained in $CDCl_3$, at 25 °C, on a Bruker Avance III 500 spectrometer operating at 500 MHz for ^{1}H NMR. The typical resolution of this machine is 0.11 Hz or 0.00022 ppm. Solutions of the C8 AHL were prepared to a final concentration of 1, 2.5, 5 and 10 mM, and were allowed to equilibrate at ambient temperature, for 7 days before measurement of the spectra.

All systems were optimised using the Gaussian16 package at the M06–2 × [6] computational level with the 6–311 + G(2d,p) basis set. [7] The M06–2 × functional has been shown to properly describe weak interactions, taking into account dispersion forces where other traditional functionals fail. Effects of water solvation have been included, by means of the SCFR–PCM approaches implemented in the Gaussian16 package, including dispersing, repulsing, and cavitating energy terms of the solvent, by starting from the gas–phase geometries and then re-optimising. The electron density of the complexes has been analysed within the Atoms in Molecules (AIM) [8] theory using AIMAll software [9]. The Natural Bond Orbital (NBO) [10] method has been used to analyse the interaction of the occupied and unoccupied orbitals with the NBO–3 program [11] since this kind of interaction is of utmost importance in any charge transfer. The Non-Covalent Interactions (NCI) index, based on the reduced gradient of the electron density, has been calculated to identify attractive and repulsive interactions with the NCI program [12] and plotted with the VMD program [13]

Cartesian coordinates and molecular graphs of all compounds studied at the M06-2x/6–311 + G(2d,p) computational level in the gas phase and PCM solvent models can be found in the electronic supporting information.

3. Results and discussion

3.1. NMR study

NMR solution studies were useful in showing the presence of an intramolecular HB between the 3-oxo carbonyl group and the amide N—H in the OHLs, which would be absent in the AHLs [5]. This absence of a HB leads to a general upfield move in the chemical shift of amide N—H in the AHLs when compared to the OHLs. Since the AHLs contain a number of HB acceptors it was of interest to see whether intermolecular HBs between the amide N—H and any of the HB acceptors are present in solution. Fig. 1 shows a stack of the amide N—H chemical shift of the AHL with a C8 side-chain, as the concentration was increased from 1 mM to 10 mM. The corresponding chemical shifts measured were 5.899 ppm, 5.905 ppm, 5.911 ppm and 5.925 ppm, respectively. Although the overall change in chemical shift is only 0.026 ppm downfield, nonetheless, it is a measurable change, which

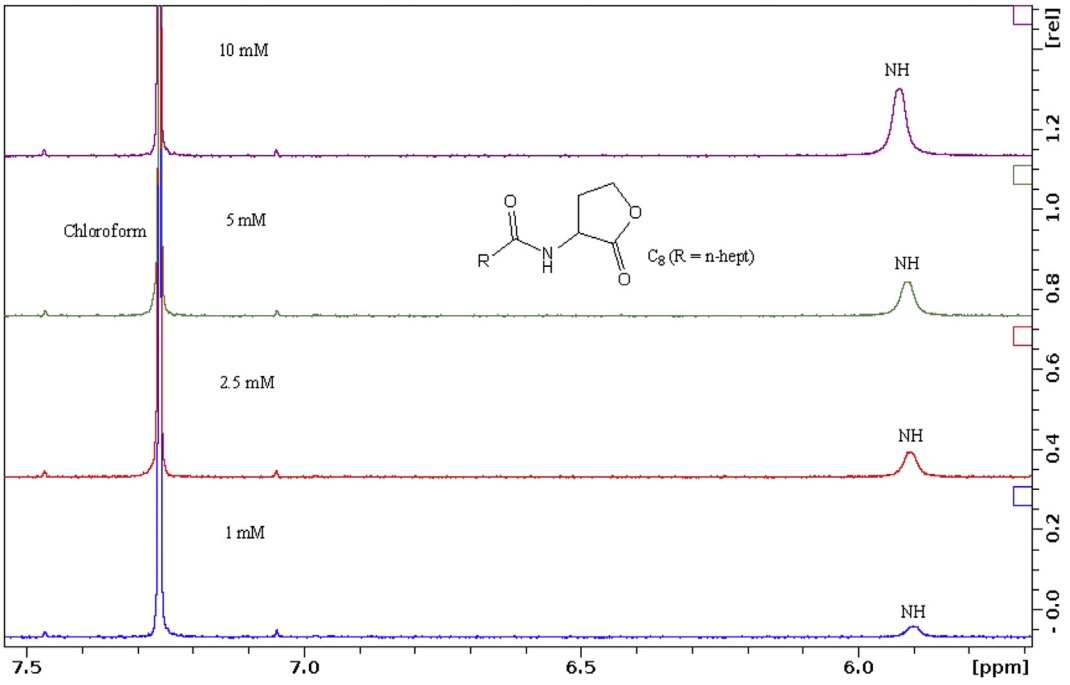

Fig. 1. Stacked ^{1}H NMR spectra of C_8 AHL at 1–10 mM, in $CDCl_3$ at 7 days.