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Probing the ionisation state of functional groups by chemical shift tensor fingerprints

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Contents

1. Introduction

Acid/base reactions are of tremendous importance in organic and inorganic chemistry, as they are also in biochemistry. The concept of acidity is based on the degree of protonation of a given basic site B into its conjugate acid BH, defined by the molar ratio $r = [BH]/[B]$, from which practical pH and pK values can be derived, ignoring activity coefficients in a first approximation. Starting from the end of 1960s, it was demonstrated that liquid-state NMR can be used efficiently for the determination of acid-base equilibrium constants (pK values) in compounds of biological interest such as amino acids [1–3], peptides [4,5], ligands and proteins [6–8], polyamines [9,10], antibiotics [11,12] or other molecules containing acidic or basic groups [13–18]. Solution-state NMR-pH titra-

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tions rely on the plots of the isotropic chemical shifts against pH in conditions of fast exchange where a single resonance signal is observed for the protonated and deprotonated species. This leads to a characteristic sigmoid shape of the plots with an inflection point at $pH = pK$.

This article reviews an attractive possibility of using solid-state NMR spectroscopy for a deeper insight into the ionisation state of different functional groups as compared with liquid-state NMR measurements. An obvious advantage of solid-state over liquid-state NMR for such purpose arises from a dramatic slowing down of inter and intramolecular proton exchanges on the NMR time scale. This frequently leads to separate resonances from protonated and unprotonated species and from the different tautomeric and conformational forms. Another, may be even more important benefit of solid-state measurements results from an easy access to the principal values of the chemical shift anisotropy (CSA) tensors, which, by virtue of their nature, are much more sensitive to the changes in the ionisation state and concurrent hydrogen-bonding interactions than the isotropic chemical shifts. Consequently, we will pay here special attention to emphasise the solid-state NMR studies of the ionisation state through the use of chemical shift tensor fingerprints.

More generally, we wish to underline the fact, that the solid-state NMR data obtained so far strongly suggest the pH and pK 'memory' of lyophilised compounds containing ionisable functional groups, supporting the suggestion of protein 'pH memory' [19,20]. A knowledge of the pK_a values of ionisable groups is indeed crucial in relation to the structure–function correlation of biological molecules including the functions of proteins. A straightforward assignment and characterisation of charges, and the hydrogen bonding neighbourhood, for ionisable species like carboxyl, amino, phenolic and imidazole groups found in proteins remains a challenging problem in the elucidation of a mechanism for proton transfer reactions in solids and membrane proteins, including the bacteriorhodopsin photocycle and ATP synthetase.

The issue of whether ionisable systems of biological interest retain their ionisation state upon dehydration is also important with regard to their solid-state stability and use as catalysts or drugs in anhydrous media. Many of the interactions between drugs and receptors are indeed known to depend on the ionisation state of the functional groups involved in the interactions [21]. The solid-state NMR suitability for a straightforward determination of the ionisation state of the functional groups reviewed below opens up a new and attractive perspective.

2. Acid-base equilibria of lyophilised L-histidine

2.1. ^{15}N CP/MAS studies

Solid-state CP/MAS NMR studies on powders of ^{15}N-enriched histidine and imidazole [22], and of α-lytic protease specifically ^{15}N-enriched at His-57 [23], lyophilised at various pH values, represent probably the first examples of an NMR 'pH titration' of a functional group in the solid-state where separate NMR signals of the various ionic and tautomeric states were observed. These studies showed that the ^{15}N chemical shift anisotropy is strongly influenced by the protonation state of the nitrogen and was found to double in magnitude upon the deprotonation of the π nitrogen [22]. It has also been demonstrated that the N^{π}–H tautomer of His-57 incorporated into the catalytic triad of α-lytic protease predominates in powders prepared at high pH and that $N^{\pi}(H)$ participates in a strong hydrogen bond [23]. This suggests that the active site catalytic triad structure of Asp^{102}-His^{57}- Ser^{195} is maintained in these lyophilised powders, so that the tertiary structures of α-lytic protease in the powder and in solution are very similar. However, in these studies, the intensity ratio of the ^{15}N resonance peaks from protonated and unprotonated sites was found to differ from the population ratio r of species B and BH. This is because the acidic proton is directly attached to the observed ^{15}N nucleus, which strongly enhances cross polarisation efficiency in the protonated species BH as compared to that in the unprotonated conjugate base B. This fact prevented a definite answer to be obtained on a basic issue of whether there is conservation of pK values from parent solutions to their lyophilisates. Indeed, one may wonder whether hydroxonium ions contained in the initial aqueous solution remain attached to residual water molecules or to the basic solute(s) B, possibly altering the acid-to-base ratio on solvent removal. One may also suspect the acid-base properties of solutes to be modified after desolvation of species in the solid-state, as is observed when going from an aqueous solution to a gas phase.

2.2. The pH dependence of isotropic ^{13}C chemical shifts

Besides the preliminary report of the effect of titration of the imidazole group on solid-state ^{13}C NMR spectra of histidine [24], the systematic comparisons of acid-to-base ratios r with those encountered in the parent solutions, have been done in lyophilised L-histidine only very recently [25]. The ^{13}C nuclei are not directly linked to the acidic protons of histidine, so it is always possible to achieve complete and uniform magnetisation transfers from the proton reservoir to all ^{13}C nuclei of histidine. Another advantage lies in the possibility of observing several sets of non-equivalent nuclei in ^{13}C spectra, which allows several independent determinations of population ratios r This permits in turn a checking of the validity of the experimental procedure, and for improving the precision of the measurements.

Histidine has four protonation sites, the carboxylate and amino ends and the two tautomeric nitrogen atoms in the imidazole ring. As shown in Scheme 1, one can expect to detect the presence of four different forms following three successive deprotonations, namely those of the carboxylic