Abstract

This thesis deals with the employment of nuclear magnetic resonance (NMR) spectroscopy in the studies of non-covalent interactions of biologically active compounds. The introduction serves as a brief overview of the literature, pointing out the milestones in determining and studying the most common non-covalent interactions – hydrogen bonding, π-π stacking and halogen bonding – by NMR spectroscopy. This part also includes the basics of this method to ensure that the results presented and the principles used will also be understandable for readers outside the field. Similarly, the employment and limitations of theoretical calculations are shortly discussed.

The thesis aimed to determine the role of non-covalent interactions in biologically active compounds, including hydrogen bonding of modified nucleobases, interactions influencing ketoenol-diketo tautomerism of pharmacologically active curcuminoids, and the role of hydrogen bonding and amino acid sequence in the adoption of secondary structure in short peptides.

Methylation and oxidation are nucleobases' most common natural modifications, causing changes in their hydrogen bonding pattern and preferences. We found that N-methylation of adenine residue stabilises its hydrogen-bonded base pairs with thymine and that these base pairs tend to adopt Hoogsteen-like geometry. Interestingly, we observed the greatest stabilisation in the 2-(methylamino)adenine derivative, which offers three hydrogen bonds on the Watson-Crick side. We hypothesised that this stabilisation might be driven by hydrogen bond count or more suitable bonding geometry. We extended our study to 8-oxopurine and 8-aminoadenine derivatives offering potent hydrogen bonding patterns on the Hoogsteen side. We employed NMR spectroscopy and theoretical calculations to determine whether base pairs' bonding geometry (Watson-Crick or Hoogsteen) significantly contributes to their stability.

We studied the ketoenol-diketo tautomerism of curcuminoids, which are a class of natural drugs. This study involved the FDA-approved orphan drug ASC-JM17, its congeners and curcumin. The previously established NMR studies of such equilibria are full of controversies. We recognised the main factors influencing the equilibration rates, such as solvent, water content or the presence of impurities, and further studied equilibrium constitutions, which were not significantly affected by the environmental effects. We focused on the effect of substitution of curcumin moiety on the equilibrium population in curcuminoids. Diketo tautomer was slightly predominant in most cases. However, we also found an analogue existing almost exclusively (97%) in the ketoenol form.

We employed NMR spectroscopy as one of the experimental methods in a comprehensive study of structural adaptation of short peptides. This work originates in the predictions made by computational chemists recognising specific amino acid triplets tending to adopt helical or extended secondary structures. NMR spectroscopy is a powerful tool for conformational studies of short peptides at high (atomic) resolution. We distinguished amino acid triplets tending to adopt helical (α or PPII) and extended secondary structures based on the temperature dependencies of vicinal $^{3}J_{NH,H\alpha}$ coupling values. The computational and experimental data confirmed that the tendency to adopt a specific secondary structure is already imprinted in protein sequence at the tripeptides level.

This thesis brings new insights into the non-covalent interactions of small bioactive molecules, resulting in four articles published in impacted scientific journals.