

Structural determination and aggregation of Ace-Gln-Gln-NH₂ using solid-state NMR

Corey A. Rice*

**Institut für Physikalische Chemie, Universität Göttingen, Tammannstr. 6, D-37077 Göttingen*

This project week was done in the group of Dr. Marc Baldus under supervision of Robert Schneider, who is also a member of the graduate school. Solid-state NMR was used to make a structural determination of a protected dipeptide, acetylglutamylglutamylamide (Ace-Gln-Gln-NH₂, Figure 1). From the assortment of 2D-NMR experiments, we have come to the conclusion that the protected dipeptide is β -sheet-like.

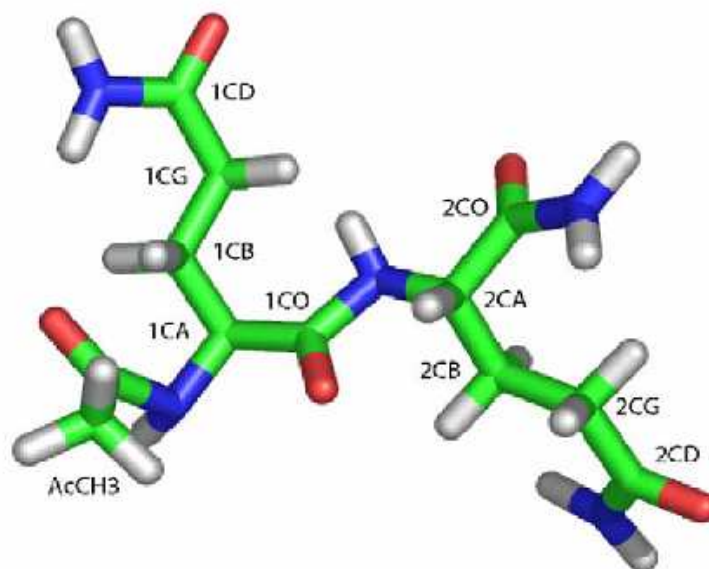


Figure 1: Structure of acetylglutamylglutamylamide.

Background

Poly-L-glutamine side-chains are known to aggregate and make fibrils, which have been known to cause neurodegenerative diseases, such as Huntington's disease. By structural determination, one can observe how chain-length assists in the aggregation. We have chosen to start with the capped dipeptide (Ace-Gln-Gln-NH₂), which makes the data easier to analyze. By using a ground-up approach, we start from small protected peptides and enlarge the systems to determine the effects of longer chains on the structure of larger peptide units.

Results

The project week involved the use of a solid-state NMR spectrometer (Bruker 400MHz). Solid-state NMR (ssNMR) differs from that of liquid-state NMR (lsNMR). In lsNMR, molecules can re-orientate on a nanosecond timescale; however, this is not the case in ssNMR where anisotropic interactions are present (chemical shift anisotropy and dipolar coupling are present in the solid-phase sample). In difference to lsNMR, the sample in ssNMR is turned at a high repetition rate (5–20 kHz, normally) and held at the magic angle to the magnetic field. The combination of spinning the sample and holding it at an angle is called Magic Angle Spinning (MAS). The angle, at which the sample is held, is 54.7°, at which angle the term $3 \cos^2 \theta - 1$ in the dipolar coupling and chemical shift anisotropy formulae equals zero, such that these interactions are averaged out best at this angle. If MAS was not used, a powder spectrum would be obtained which contains information about all different orientations of the molecule in space. We have used MAS during the experiments which reduces the anisotropy of the sample and reduces the linewidth.

During the week many experiments were run on the Ace-Gln-Gln-NH₂, which was synthesized using ¹³C and ¹⁵N labelled glutamine and a synthesizer. The protecting groups were not labelled. The signals from the 1D N and C NMR spectra were compared to a data base of NMR spectra (Biological Magnetic Resonance Data Bank (BMRB), <http://www.bmrw.wisc.edu/>). The secondary chemical shift of ^αC and ^βC in diglutamine were calculated by

$$(\alpha C(\text{measured}) - \alpha C(\text{BMRB})) - (\beta C(\text{measured}) - \beta C(\text{BMRB})). \quad (1)$$

The calculated values are -5.24 ppm for the first glutamine and -7.53 ppm for the second. Since the values are negative, this supports the β -sheet-like structure; however, if the calculated values were positive, this leans toward an α -helical structure.

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