

Highly functional polypeptides (HiPEP) - Publishable summary

Project objectives

The main objective of HiPEP is the development of a functional materials platform based on polypeptides for advanced applications.

To accomplish this, two specific objectives were identified in the project proposal:

- (1) *Fundamental objective:* Develop an understanding of copolymerisation behaviour in N-carboxyanhydride (NCA) polymerisation and mapping of structure-property relationship of polypeptide copolymers.
- (2) *Biomaterials objective:* Synthesis and biomedical activity tests of Glatiramer-like polypeptides with defined structures.

Work performed

The first stage of the work exclusively dealt with the challenge to understand and better control random copolymerisations of NCA amino acid derivatives. Even though random NCA copolymerisation had been carried out before, analysis was restricted to the final polypeptide obtained. No information on the kinetics and relative behaviour of different amino acid NCAs was available to date.

We therefore first worked on establishing a method to identify and quantify amino acid NCAs in a binary mixture of monomers. Currently infrared spectroscopy (IR) is used to analyse and quantify amino acid NCAs; however, this method measures the intensity of the C=O bond of the NCA and is therefore insensitive to different side chains of the amino acid and unable to distinguish between two different monomers. Informed by the fact that in other scientific areas amino acid derivatives are routinely identified, separated and quantified via HPLC methods, we endeavoured to use the same approach to analyse mixtures of amino acid NCAs.

Amino acid NCAs are used as monomers for the preparation of polypeptides because of their high reactivity with simple chemical groups. They are thus difficult to analyse directly. We developed a protocol based on acidic hydrolysis to rapidly return the activated (unreacted) amino acid NCAs into the original amino acid derivative without inducing polymerisation of the monomers. Different procedures (acid concentration and volume and solvent mixtures) were explored to optimise this method.

To analyse the amino acid derivatives obtained after acidic hydrolysis, we developed an HPLC

method using a gradient of water and acetonitrile to separate the analytes. For detection, a UV detector was used. This approach relies on the fact that several amino acids require side protection groups that strongly absorb UV light for the NCA polymerisation process.

Using the approach outlined above, we were able to monitor the consumption of two and three amino acid NCAs simultaneously during NCA copolymerisation. The overall consumption was verified by comparison with the established IR method. Our technology allowed us to access extensive kinetic data on the polymerisation of three amino acid NCAs, Glu(Bzl), Lys(Z) and Tyr(Bzl).

Main results

1. Monitoring amino acid NCA consumption simultaneously

To monitor and ultimately understand the kinetics of random amino acid NCA copolymerisation, a method was established that enables the qualitative and quantitative analysis of more complex amino acid NCA mixtures. The method consists of two steps; (i) unreacted NCA monomers are converted back to the original amino acid derivatives by acidic hydrolysis and (ii) the obtained samples were analysed by HPLC. The quenching procedure was verified by LC-MS and the HPLC procedure was calibrated using quenched NCA solutions of known concentrations. The validity of the HPLC measurements was confirmed with homopolymerisations of the respective amino acids and comparison with data obtained from IR measurements (Fig 1).

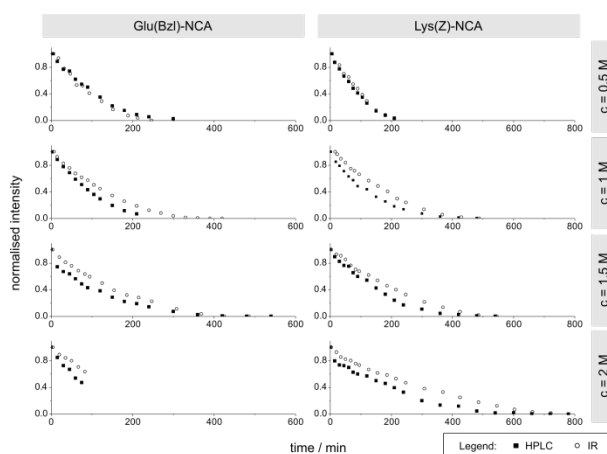


Figure 1 Comparison of normalised HPLC and IR data for homopolymerisation of two amino acid NCAs.

2. Kinetics of a random copolymerisation of two NCAs

The method developed above was subsequently employed to determine the copolymerisation kinetics of a mixture of two amino acid NCAs, Glu(Bzl)-NCA and Lys(Z)-NCA. Kinetic curves for

various monomer ratios were determined and the copolymerisation parameters were obtained by NMR measurements of the polymers obtained at low conversion (<10%). Glu(Bzl)-NCA was identified to be the more reactive monomer, resulting in preferential incorporation of Glu(Bzl) into the polypeptide at the beginning of the polymerisation. Conversion of the kinetic data into the amount of NCA incorporated into the polypeptide allowed estimations of the copolymer composition over time (Fig 2), indicating that the copolymer composition remained largely constant after approximately 30% conversion.

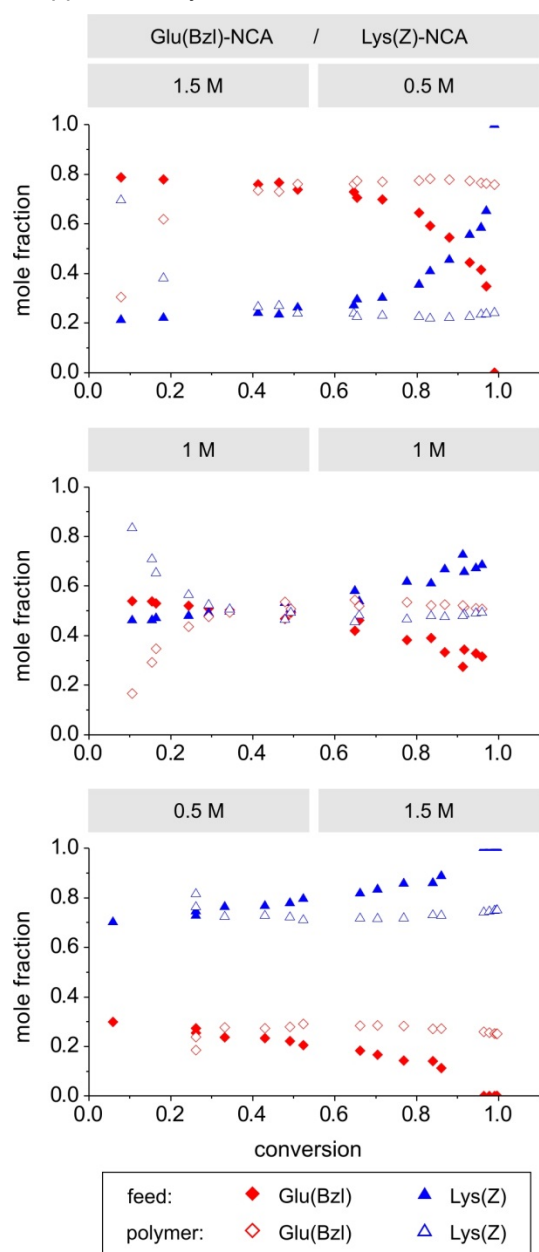


Figure 2 Copolymer composition as a function of monomer conversion for three different monomer ratios.

3. Kinetics of a more complex (ternary) NCA copolymerisation

Glatiramer acetate, the biological target of objective 2 of this proposal, consists of four amino acids. To work towards the objective of preparing

better defined Glatiramer acetate related copolypeptides, the complexity of the copolymerisation system was increased to three amino acids that are present in Glatiramer acetate, Glu, Lys and Tyr. Side chain protected NCA analogues of these amino acids were copolymerised and monitored as described for the binary system. Copolymerisation parameters from binary reactions were used to predict the behaviour of the ternary system, showing excellent agreement with the experimental data. A ternary diagram was obtained that indicated the initial copolymer composition and the reactivity of the three monomers (Fig 3). The copolymer composition was determined on the basis of the kinetic data, elucidating how the copolymer composition evolves over time.

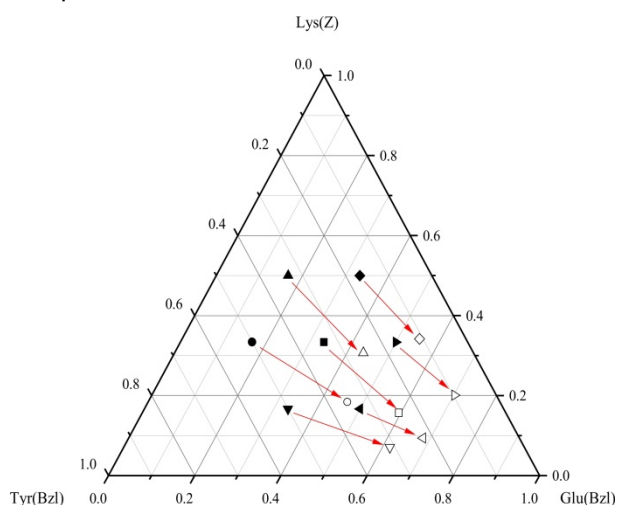


Figure 3 Ternary diagram showing the change in composition between the feed (solid symbols) and the terpolymer (open symbols) after 20 min polymerisation.

Potential impact

The work has laid a solid foundation for the characterisation and subsequent understanding of random copolypeptide synthesis. With the methods and data obtained to date, more accurate prediction of the behaviour of amino acid NCAs during the copolymerisation can be made, allowing better control over the sequence – and hence the properties – of the polymer.

This work, together with subsequent efforts in the field of polypeptide sequence control, will make better defined amino acid based polymeric materials accessible for more sophisticated applications while still maintaining their advantages of ready synthesis and biologically based building blocks. Research building on this work may provide the materials necessary to improve our understanding of the mechanisms of action of polypeptide based drugs.