Bericht zur Max-Buchner-Forschungsarbeit

„Multi-Clickable Biocompatible Hydrogels“ (MBFSt-Kennziffer: 3798)

Dr. Aurelien Forget, Institute for Macromolecular Chemistry, University of Freiburg, Germany

1. Aim and Motivation

Carboxylation of agarose has been used by different research group as a mean to introduce reactive functional groups to the inert agarose polysaccharide backbone and to modulate the mechanical properties of the resulting hydrogel.[1] By oxidizing the primary alcohol on the C6 position into a carboxylic acid a reactive site is created which disturbs the secondary structure of the polysaccharide, which in turns impact its capacity in forming crosslinking points, thus modifiying its mechanical properties. Now, the created carboxylic acid can react with amine throught a carbodiimide coupling strategy.[2] However, this coupling requires several steps and lead to byproducts which needs to be remove. This means that the reaction has a low yield, is inefficient and require intensive manpower to create functionalised biopolymers.
For an efficient functionalisation, “click chemistry”, chemical reactions that are high yielding are of high interest. There is now a broad library of click reagents. But these are often limited to imaging probes, while beeing extremely useful for the functionalization of polymers of natural origin to increase their range of application.
Recently, several click reactions have been considered for biofunctionalization such as the Inverse Electron Demand Diels-Alder (iEDDA)[3] and thiol-ene Michael Addition[4]. In this project, we aim to evaluate the reaction kinetic of click reagents as functional moieties on carboxylated agarose (CA), and test their coupling with peptides.

2. Performed investigations

The first coupling chemistry that was tested was the iEDDA. Several studies have reported the use of the tetrazine-norbonene pair.[5] We hypothesize that this chemistry can be used to efficiently couple molecules onto CA as an alternative to the carbodiimie chemistry. To test the compatiblity of this chemistry with CA hydrogels for its functionalisation under physiological condition, we test the reaction kinetic of the tetrazine-norbornen system under different solubilization conditions.
Another promising strategy for click chemistry under physiological conditions are the thiol-ene reactions specifically with maleimide. Thiol-ene reactions are rapid reactions and chemo selective for thiols, and they proceed without catalyst. Currently, it is one of the most popular methods for site-selective modification of cysteine residues in bioconjugation technology.

3. Results

We examine the reaction rates between the synthesized 4-(6-methyl-1,2,4,5-tetrazin-3-yl)aniline and 5-norbornene-2-carboxylic acid to demonstrate that the synthesized tetrazine undergoes an iEDDA reaction even at room temperature and with low concentrations. We followed the decrease of tetrazine concentration by UV-Vis spectrophotometry to determine the reaction rates of iEDDA. The UV-Vis absorption spectrum of 4-(6-methyl-1,2,4,5-tetrazin-3-yl)aniline shows a maximum of 540 nm which was used to determine the reaction rate. A series of kinetic measurements in DMSO and in water was performed. Since 1,2,4,5-tetrazines and norbornene acid are very hydrophobic, the substrates have been dissolved in little amounts of DMSO and then mixed with water to give a final concentration of 0.2 mmol/L for the tetrazine and 20 mmol/L for 5-norbornene-2-carboxylic with a final water volume percentage of 93.75 %. The pseudo first order- and second order rate constants were calculated for a reaction in DMSO and in water (**Figure 1**). The reported acceleration of iEDDA reactions by addition of water could be verified by increasing the second order reaction rate by 17 %. But these challenges are

limited to the transfer of this chemistry to CA functionalization and an alternative chemistry was sought of.

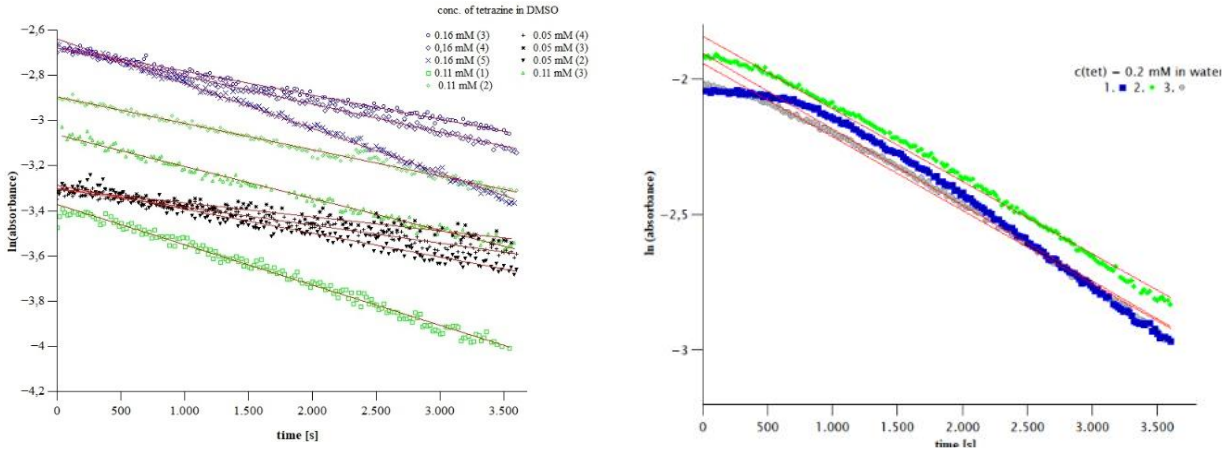

Figure 1. Kinetic measurements of iEDDA reaction in DMSO with different concentrations of tetrazine(***left)***. Kinetic measurements of iEDDA reaction in water with tetrazine concentration of 0.2 mM (***right***).

Next, we investigate the potential of Thiol-ene reagent for the functionalization of CA. However, it is well known that the chemistry of maleimide can be affected easily by hydrolysis of its ring compound and thus lost reactivity towards thiols.[6] As a consequence, optimising the specific conditions for kinetic is crucial to successfully attach peptides through click chemistry. Thus, maleimide was coupled to CA and its stability under different aqueous conditions was investigated.

The degradation of maleimide was monitored by measuring the absorbance of maleimide at pH 7.5 in phosphate buffered saline. We observed the loss of absorbance is 3% in the first 5 minutes. This study demonstrates that maleimide is unstable at pH 7.5. However, the time of reaction between maleimide and thiol group in phosphate buffered saline is quicker than the degradation of maleimide under these conditions (**Figure 2**). Finally, the functionalised CA with maleimide was reacted with a thiol-terminated peptide and the reaction kinetic was measured (**Figure 2**) and it was found that the coupling is occurring less than five minutes.

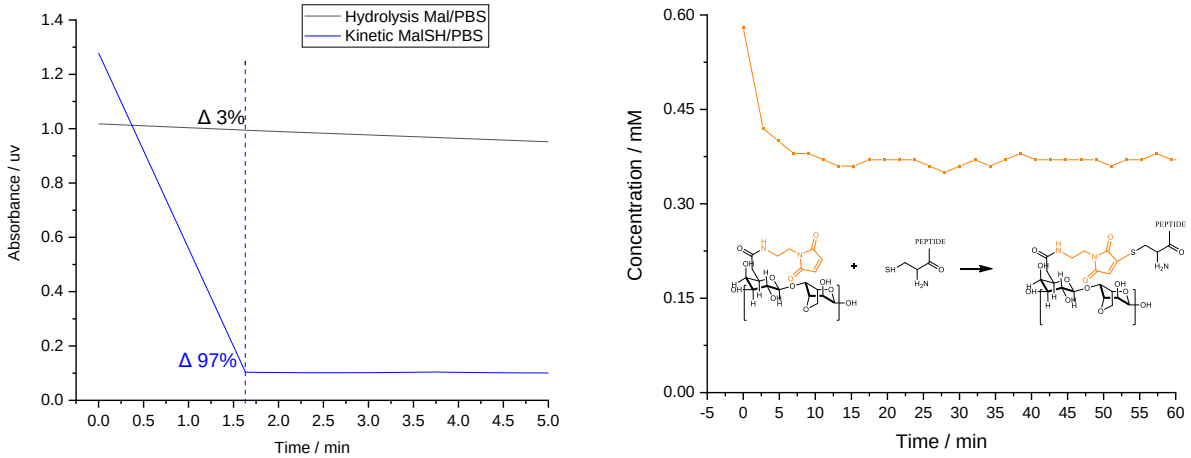

Figure 2. Hydrolysis and reaction of maleimide under physiological conditions (***left***). Kinetics – UV/vis. Thiol-ene kinetic reaction between carboxylated agarose functionalized with maleimide and thiol-terminated peptide (***right***).

4. Conclusion

Two click chemistry functionalities were tested to achieve a simple and efficient coupling of peptides onto carboxylated agarose a hydrogel used in several tissue engineering The first Diels-Alders reagent was found to be challenging to produce and with limited

solubilities could have been difficult to apply to hydrogels. It was decided to switch to maleimide function for thiol-ene conjugation. This was found to be suited for the click – coupling of peptides terminated by cysteine amino-acid (-SH group). The thermal and pH stability of maleimide was investigated to insure the potential transfer of this innovation into tissue engineering applications.[7]

5. Literature

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