From Covalent Protein Mapping to Self-assembly - Michael Reaction Conditions of Acryl Amide Library

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Abstract

InFarmatik experience in fragment design led to the development of a Covalent Protein Mapping Fragment Library based on the reaction of PAM-protein sequencing. After synthesis of the first relevant acryl amide library we checked the covalent binding power of exemplary fragments on small molecule structures modeling protein (AC-Cys).
Based on selectivity of Michael addition of acryl amide library, we assumed a potential application for self-assembly structures. Scope of the chemistry and reaction evaluation data are shown here.

Background

InFarmatik was pioneering the In3D concept in fragment design and we tried to make a similarly approach in the protein-fragment covalent interaction based discovery.
Sunesis developed the tethering technology using a library of fragments attached to a leaving group, which could easily react to the Cys SH via reversible S-S bond formation[1].
Using this anchored group in a further reaction with another mixture of fragments attached to a leaving group, the S-S bond formation could take place between the two fragments at the right position determined by their binding to the protein active site. Using simple analytical methods (MS) the kinetically preferred product could be easily identified and the analogs could be designed for further lead development

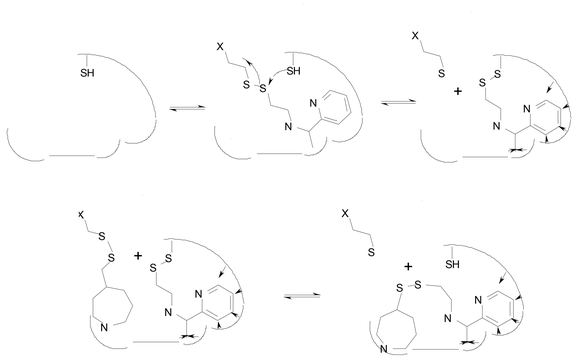

Figure 1

1. Covalent Protein Mapping Fragment Library

While Tethering Technology worked nicely in lead generation, we were interested in development of other libraries which can probe significantly smaller binding sites with appropriate selective reactivity.

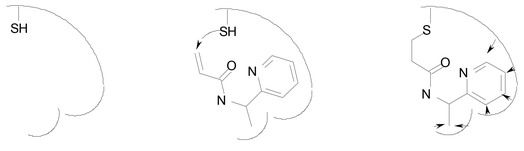

Figure 2

We reviewed the acryl amide-protein cystein SH reaction background in the literature[2,3]. Since the reaction was invented as side-reaction between Cys free SH-groups of the protein sequences and acryl amide residues of the polyamide gel, we found that it must be selective and, due to the nature of the Michael addition, most probably tunable with variety of catalyst.
The acylation reaction with acryl chloride run smoothly, but the stability of the product strongly depends upon the nature of other functional group present in the fragment structures.
On the course of the synthesis we realized that if the Covalent Protein Mapping Fragments contained a nucleophile group, the product started to slowly oligomerize upon standing in solution or in solid form.

The resulted stabile Covalent Protein Mapping Fragment Library statistics were as shown on figure 3. The clgoP values are favorable due to acryl amide group present in all structures. The molecular weight and the hydrogen bond acceptor values of the CMP fragments are shifted to higher values by 60 Da due to the presence of same acryl group.

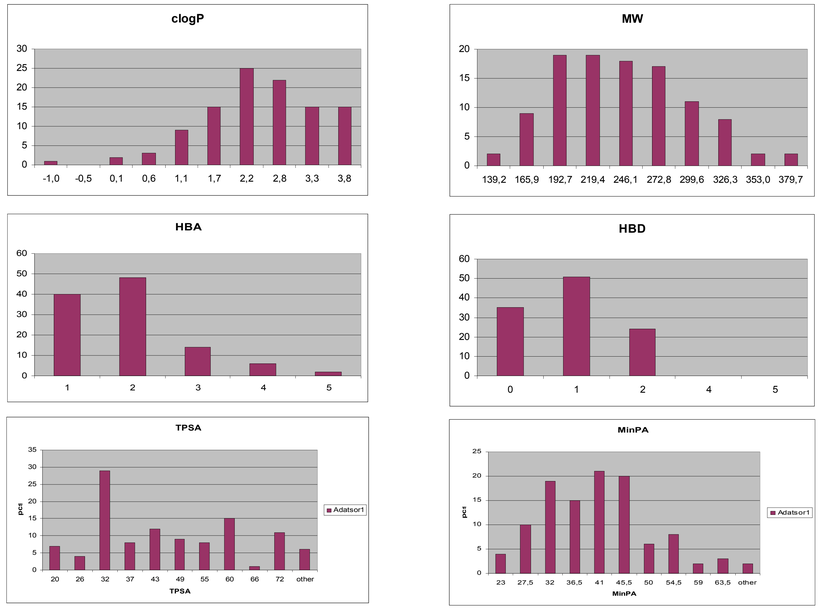

Figure 3

2. Modelling Covalent Protein Reaction of the Library products

We took the following representative structure of the library and tried to model the Covalent Protein Mapping reaction. To model the reaction between residues and acryl amide Covalent Protein Mapping Fragments we use acetyl cystein as model reaction.
The reactions were tested in 500microliter scale in thermal gradient reactor at various temperature between 10 and 51 °C at 37 °C preferably. The reaction between the Ac-Cys and aryl amides could be described by the following equilibrium We have not proved the reversible property of the reaction so far. The Michael addition with C is described definitely as non reversible; however we experienced serious retro-Michael reaction during synthesis and reactions of beta-amino acids.
The elimination of thiolates in basic medium is also well-known phenomenon, but in our system the elimination may occur at different position, stabilized by formation of different “enes”.

2.1. Effect of reaction medium

The Michael reaction between 1 and Ac-Cys was carried out in different solvent mixtures as shown on the below diagram.
As could be seen from the diagram the reaction was optimal at 50% methanol water mixture. Surprisingly in absence of water the yield has dropped dramatically, although the solubility of the materials was very good in this condition.
This is a little bit contradicting to Movassagh and Shaygans[4] NMR based studies performed on α,β-unsaturated ketones; however they did not use mixture of solvent.

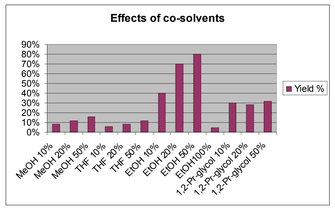

Figure 4

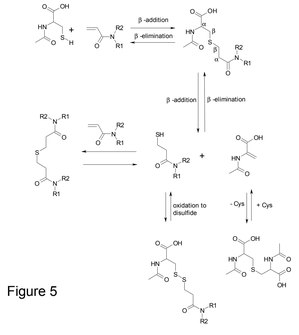

Figure 5

2.2. Catalytic effect

We have not experienced the outstanding catalytic effect. Maybe the electron structure is different for the acryl amides (more polarized than simple unsaturated ketones), so less sensitive to the usual catalytic effect. Looking through the usually active PTC catalysts they found active we suspect that part of the usual catalytic effect was simply solubilization effect.

2.3. Effect of Molar Ratio

We also studied the effect of molar ratio of 1 and Ac-Cys on yield of the Michael adduct. The molar ratio of 1:1 looks to be ideal for the Michael addition. His also contradicts the 1:2 Michael acceptor/thiol ratio usually detected. The reason for decreasing yield most probably caused by the complicated reversible reaction equation systems related to the rearrangement of the S-alkylated acetyl cystein (figure 5).

2.4. pH

We also studied the catalytic effect of the medium on the Michael addition, but in the range of 6.4 and 8.4 pH we did not detected any remarkable effect.

3. Covalent Protein Mapping Fragments as Self-Assembly Library Building Blocks

The self-assembly theory is based on the phenomenon that an active site determines the size of the expected product, which is formed in a reaction that is compatible with the proteins. It could be seen as an extension of the Covalent Protein Mapping concept if we take out the cystein SH and substitute it for instance a thiol fragment which could fill the free space of the enzyme site properly
There are many types of concepts how build self assembly systems suitable for lead generation:
• **The Sunesis technologies** use the usual the disulfide linkers to connect disulfide type of leads on the hot spot in many ways[1]. Usually the anchor point is a Cys SH, to which the first fragments is linked in a reversible reaction and subsequently the second fragment of appropriate size is attached to it. (see figure 1)
• **Sharpless at Scripps developed click reaction**[5] which generates tetrazole ring with the reactions of terminal azides and acetylenes in presence of copper(I) type catalysts.
• **Manetsch at Florida University**[6] uses the Target Guided Synthesis term for selective reaction between sulfonazides and thioamides.
The supposed mechanism of self assembly reaction is not clear. Erlanson emphasize the importance of the equilibrium networks in the self-assembly reaction, in Sharpless and Manetsch approach the reversible reaction is reduced to a reversible step and the emphasis is on the high expected yield and the selectivity of the reaction.

As we experienced the selectivity of the Michael addition to acryl amides and possibility for increase the yield by using catalyst could be suitable for library formation. The spatial needs of this reaction are considerably less, than the above listed reaction types. We also believed that based upon our “normal” wet chemistry experience, the scope of the reaction most probably could be extended to other electron donating groups.
The fragment pools are soluble in water and as shown in the above lines the Michael addition comfortably happens in aqueous conditions.
Thus we tried reaction of acryl amides with the following fragment types:

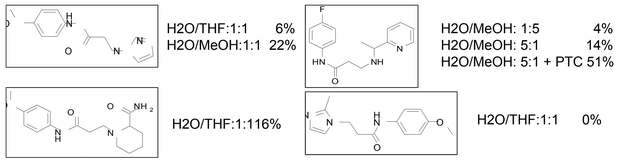

Figure 6

As could be seen from the above data the initially poor yield of 2,2-pyridylethylamine reaction the suitable reaction conditions and catalyst might elevate the effectiveness of the reaction considerably.
We also believe that in case of protein we can take the advantage the spatial selection of the fragments to be assembled, and thus the synthesis could take place this way effectively and selectively. We look for partner for this protein work.

Conclusions

InFarmatik designed synthesized and evaluated an acryl amide type Covalent Protein Mapping Fragment Library. The selectivity and reactivity of the acryl amides in model reaction proved to be sufficient for use the library in protein mapping.
The extension of Michael addition of SH to other relevant functional groups gave the opportunity to design synthetically divers and good physicochemical property libraries. Due to the ability to fine tune the various Michael reactions with reaction conditions there is an opportunity to use this technique for creation of self-assembly structures.

References:

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