A Special Cellulose Membrane Support for the Combinatorial and Parallel Synthesis of Peptide Libraries Suitable for the SC²-type Manufacturing of High Density Multi-purpose Chemical Micro-arrays

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Abstract

Starting from a commercially available cellulose paper, we have designed a membrane support which is particularly suited for the SC² process. This AC-D membrane provides a non-hydrolizable primary amino functionality, it is resistant to all chemically and mechanically operations required for peptide assembly, but it readily dissolves in the side-chain deprotection mixture containing 95 % trifluoroacetic acid.

The new support can be utilized as planar synthesis support for the solid-phase assembly of chemical compounds by several types of combinatorial and parallel synthesis techniques such as the Filter Disc method [2, 3], the SPOT-synthesis [4] or the Cut & Combine method [5]. This material will open the further miniaturization, automation and integration of high throughput synthesis & biological screening processes for immunological, functional genomics, proteomics and drug discovery studies.

Introduction

We have recently developed a new process for the manufacturing of multiple copies of high density multi-purpose chemical micro-arrays which we call SC2 (**S**potting **C**ompound **S**upport **C**onjugates). The concept is described in Fig. 1. It involves the stepwise solid phase assembly of peptides or peptide mixtures permanently anchored to a particular type of support material which can be dissolved post synthesis to yield soluble peptide-support conjugates. These conjugates are suitable for the direct spotting on planar surfaces such as a glass microscope slide by respective picoliter pipetting devices, thus generating multiple copies of a synthesized array [1].

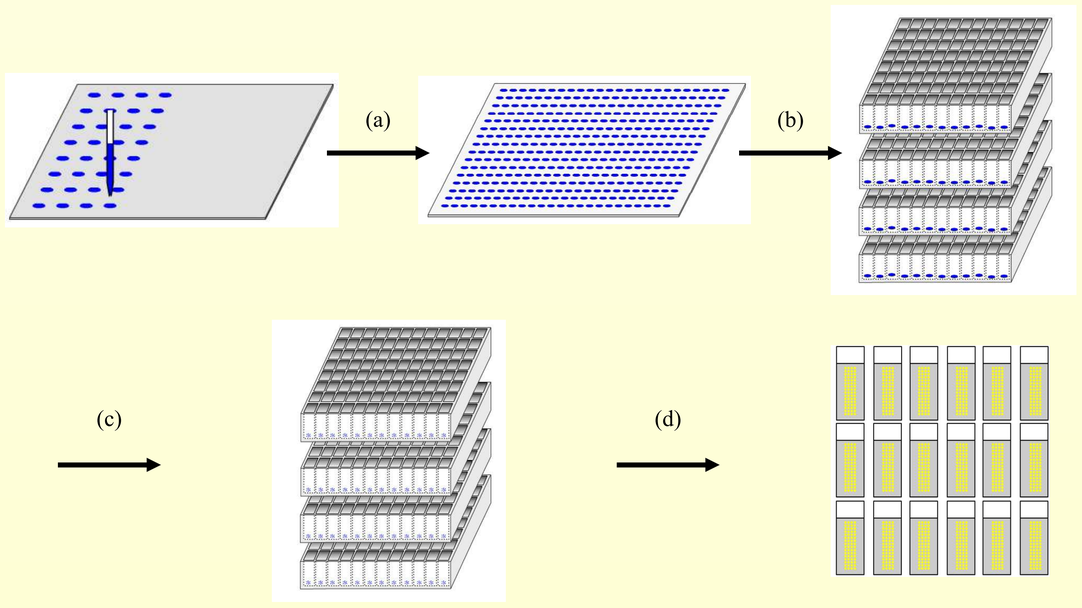


Fig. 1: The steps of the SC² - process: a) Synthesis of compounds on a cellulose solid support e.g. by the SPOT-method; b) Distribution of the synthesized solid supported compounds into microtiter plates; c) Dissolving of the solid support in TFA and precipitation of the cellulose-compound conjugates in ether; d) Dissolving the cellulose-compound conjugates in DMSO and printing of multiple copies on glass slides

Results and Discussion

The properties of the solid support employed in the SC2 is crucial for the success of the process. While the support must be stable during the synthesis procedure, it should readily dissolve after synthesis to provide soluble compound-support conjugates suitable for the printing of micro-arrays. Additionally, the dried compound-support conjugates have to stay attached to the surface of the glass-slides during the assay of the micro-arrays. Cellulose turned out to be the material of choice.

We have developed a new aminated AC-D cellulose membrane for the SC2 process which combines the high stability to hydrolysis of the compound-support linkage of our standard AC-S synthesis membrane [6] with an improved solubility of the membrane in TFA. No hydrolysis was observed for both AC-D and AC-S membranes even in conc. Ammonia as shown in Fig. 2 while most of the amino-functionalities of the esterified membrane with Fmoc-ß-Ala were hydrolysed off after 2 h even at pH 9.

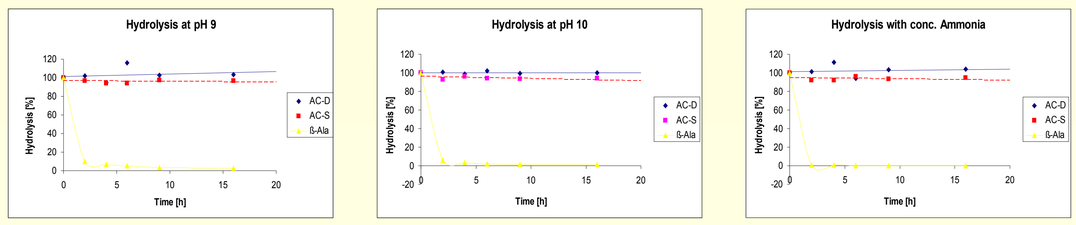


Fig. 2: Stability of the new AC-D membrane in comparison to the standard acid-stable AC-S and the traditional membrane, esterified with Fmoc-ß-Alanine, to hydrolysis in aqueous buffers at pH 9, pH 10 and to conc. Ammonia at room temperature. The amino group content was measured with bromophenol blue before and after the treatment as described earlier [6].

The new AC-D membrane is completely soluble after 4 h in 95% TFA as shown in Fig. 3. The mass increase of the AC-S and ß-alanine membrane after thoroughly drying *in vacuo* over night is attributed to swelling of the cellulose matrix.

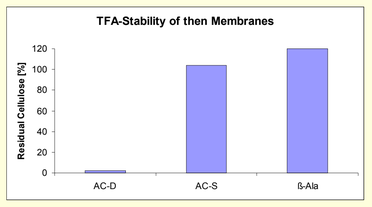
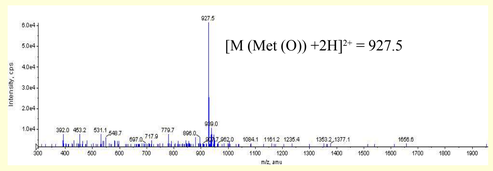


Fig. 3: Membrane pieces were added to a mixture of 95% TFA, 3% TIBS and 2% water and treated in an ultrasonic bath for 4 h. Undissolved cellulose was collected by filtration and dried over night *in vacuo*.

To evaluate the performance of the new AC-D membrane during synthesis, we assembled a 15-mer peptide. The ESI-MS of the crude product is shown in Fig. 4.

Fig. 4: ESI-MS of Ac-VLMEWLKTRPILSPL-NH2, synthesized with the automated INTAVIS MultiPep synthesizer, employing Fmoc-chemistry. MS analysis showed methionine oxidation as the main product: Calculated [M+H]+= 1837; Found [M (Met (O)) +2H]²⁺ = 927.5.



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