Nonswelling Macroporous Synbeads for Improved Efficiency of Solid-Phase Biotransformations

Alessandra Basso,[a] Paolo Braiuca,[a] Luigi De Martin,[a] Cynthia Ebert,[a] Lucia Gardossi,*[a] Paolo Linda,[a] Silvia Verdelli,[a] and Andrea Tam[b]

Abstract: An application of novel, highly porous nonswelling resins (Synbeads) for enzymatic catalysis on solid supports is reported. These new resins combine easy handling of the beads, chemical stability, improved accessibility of proteins and higher productivity relative to swelling polymers. The present study demonstrates that the resin porosity greatly affects the efficiency in solid-phase biotransformations and that Synbead resins are valuable alternatives to swelling polymers for solid-phase chemistry and biocatalysis. The present study investigates the influence of key parameters, such as porosity and reactive functional-group density, on the reaction efficiency.

Keywords: biotransformations · combinatorial chemistry · enzyme catalysis · penicillin G acylase · polymer resins · solid-phase synthesis

Introduction

Organic synthesis on solid supports is a rapidly developing methodology that offers several advantages over traditional synthesis in solution. This could be exploited on an industrial scale by providing a simple route for product purification, enabling the polymers to be reused and the use of large amounts of reactants to force the equilibrium of unfavourable reactions, in addition to possibly recycling unreacted reagents.

Recently, polymeric-functionalised matrices have become increasingly popular and have been applied with success to fine chemical and foodstuff production. Combinatorial chemistry and parallel synthesis of compound libraries on polymeric supports are efficient methods for the generation of new substances with a known profile of properties.[1]

The application of enzymes in combinatorial chemistry has attracted significant attention over the last decade.[2] Enzymatic methods have opened up advantageous alternatives to classical chemical techniques, since enzyme-catalysed transformations often proceed under very mild conditions and are highly selective. In solid-phase chemistry, enzymatic catalysis has been exploited in the preparation of glycopeptides,[3] synthesis and hydrolysis of peptides[4] and deprotection of oligonucleotides.[5] Many hydrolytic enzymes (proteases, esterases, glycosidases and amidases) have also been investigated for their ability to selectively cleave enzyme-scissile linker groups.[6–8]

In general, the support resin plays a pivotal role and novel polymers having specific advantages over existing resins are continually being developed.[9–13] An optimal polymer for solid-phase-chemistry applicability must be stable to mechanical stress, acid–base and chemical treatments and also allow a good diffusion of chemical reactants. In the case of biocatalysis, the substrate must be accessible to enzymes.

The accessibility of reactants and enzymes to the substrate is usually associated with the swelling properties of a polymer.[14] However, a number of solid supports that do not swell by a measurable extent have been reported to be applicable for solid-phase chemistry.[5,15–16]

It has been recently demonstrated that the design of a specific support for solid-phase biocatalysis can increase the efficiency of reactions catalysed by large enzymes, such as penicillin G acylase (PGA $M_r = 88$ KDa).[17] Due to its strict selectivity towards phenylacetic derivatives,[8] PGA is very attractive for the design of enzyme-labile *exo*-linkers in combinatorial biocatalysis.

Here we report on the design and development of nonswelling Synbeads, which, thanks to their porosity, can be efficiently applied to solid-phase chemistry and biocatalysis.

The present study investigates the influence of key parameters, such as porosity and reactive functional-group density,

[a] Dr. A. Basso, P. Braiuca, Dr. L. De Martin, Prof. C. Ebert, Dr. L. Gardossi, Prof. P. Linda, S. Verdelli
Dipartimento di Scienze Farmaceutiche
Università degli Studi, Piazzale Europa 1
34127 Trieste (Italy)
Fax: (+39) 040-52572
E-mail: gardossi@units.it

[b] A. Tam
Resindion SrL, Mitsubishi Chemical Corporation
Via Roma 55, 20082 Binasco, Milan (Italy)

 DOI: 10.1002/chem.200305243 © 2004 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

on the process efficiency. This information will be helpful for the selection, on a rational basis, of optimal commercial Synbead resins for solid-phase chemistry and biocatalysis, also on an industrial scale and eventually for the design of novel nonswelling resins.

Results and Discussion

A new class of nonswelling polymers (Synbeads) were specifically designed and prepared for efficient use in solid-phase chemistry. They belong to the well-known family of Sepabeads®, a class of polymers used for the immobilisation of enzymes in pharmaceutical and food applications.[18] Synbeads show chemical resistance in a wide range of reaction conditions and, similarly to Sepabeads®, have good mechanical stability. They are methacrylic copolymers formed by a monomer with the functional group and a cross-linker.[19–20] Synbeads have terminal aminoethyl (C2A) or aminohexamethyl (C6A) residues, which have a length of 2.5 Å and 7.5 Å, respectively, and contain more than 1 mmol of amino groups per gram of dry resin.

Synbeads were designed to obtain an internal geometry with large, flat surfaces to improve mass transfer. They are prepared through a suspension polymerisation technique, namely by mixing the functionalised monomer, a cross-linker and an adequate porogenic agent. This process leads to the formation of a matrix that combines a *nonconventional high porosity*, generated by the removal of the porogenic agent after the polymerisation, with an *internal microporosity*, due to cross-linking.

The macroporosity enhancement was obtained by increasing the concentration of the porogenic agent and decreasing the concentration of the cross-linker in the composition of the polymerisation phase. As reported in Figure 1 and Table 1, within the family of C6A Synbeads, four polymers (C2A, C6A, C6A2 and C6A3) each with a different porosity were prepared and compared. They have the same particle size (150–300 m), but a pore diameter varying from 380 Å to 5400 Å.

The beads' morphology was investigated by using electron microscopy. There is an evident decrease of macroporosity on the surface of the polymers from C6A3 (Figure 2a) to C6A2 (Figure 2b) and C6A (Figure 2c), whereas the microscopic aspect of C6A and C2A (Figure 2d) is similar. The effect of the porogenic agent used in the polymerisation process on the macroporosity of the C6A beads is shown in Figure 2e.

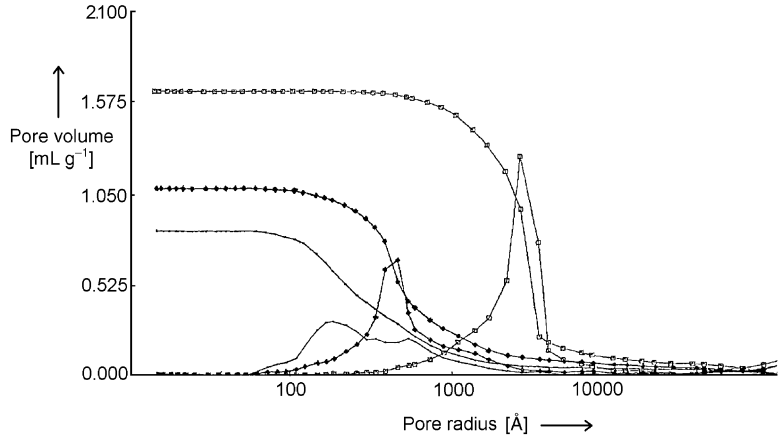


Figure 1. Pore determination by the Mercury injection method using an Autopore 9220 (□ = C6A3, ■ = C6A2; • = C6A).

Table 1. Physical properties of Synbeads.

Resin	Solvent retention [%]	Mean particle diameter [μm]	Pore diameter [Å][a]	Surface area [m^{2}g^{-1}][b]
C2A	59	320	380	130
C6A	56	240	380	130
C6A2	63	210	950	40
C6A3	69	200	5400	15

Determined by using the: [a] Mercury injection method with Autopore 9220; [b] BET single-point method with Flowsorb 2300.

Unlike conventional swelling resins (e.g., $PEGA_{1900}$) for solid-phase organic chemistry, Synbeads are rigid because of the high degree of cross-linking in the resin (>30%); this means they do not modify their morphology when suspended in water or organic solvents (either hydrophobic or hydrophilic), as demonstrated in Figure 3.

The amount of amino groups on the resin, obtained after the polymerisation process, represents the total exchange capacity of the resins; this was determined by acid–base titration (Table 2). The chemical accessibility and reactivity of these amino groups was assayed by exploiting Fmoc chemistry. Five acylation cycles of the resin with Fmoc-Phe were performed and the released Fmoc (cleaved with piperidine) was quantified after each cycle.[17] Table 2 shows the maximum acylation values obtained for all the resins. Complete acylation was reached within three cycles for all the resins; in the case of C6A3, it was already observed after the first cycle, probably due to its larger porosity.

Chain length does not significantly affect the chemical reactivity of the terminal amino groups, as is evident by comparing the results obtained with the C2A and C6A polymers.

The apparently poor value of chemical accessibility for both C6A2 and C6A3 resins actually corresponds to the maximum acylation achievable when NH_2 groups are acylated by Fmoc-Phe. This comes from the calculation of the number of Fmoc-Phe groups per 100 Å^2, which is comparable in all the resins. From the values of total exchange capacity and surface area it results that Synbeads have more than ten NH_2 groups per 100 Å^2. Due to the steric hindrance of the Fmoc-Phe group (Fmoc-Phe occupies about 25 Å^2), the maximum acylation achievable in the highest packed situation for all the polymers considered corresponds, in theory, to no more

© 2004 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

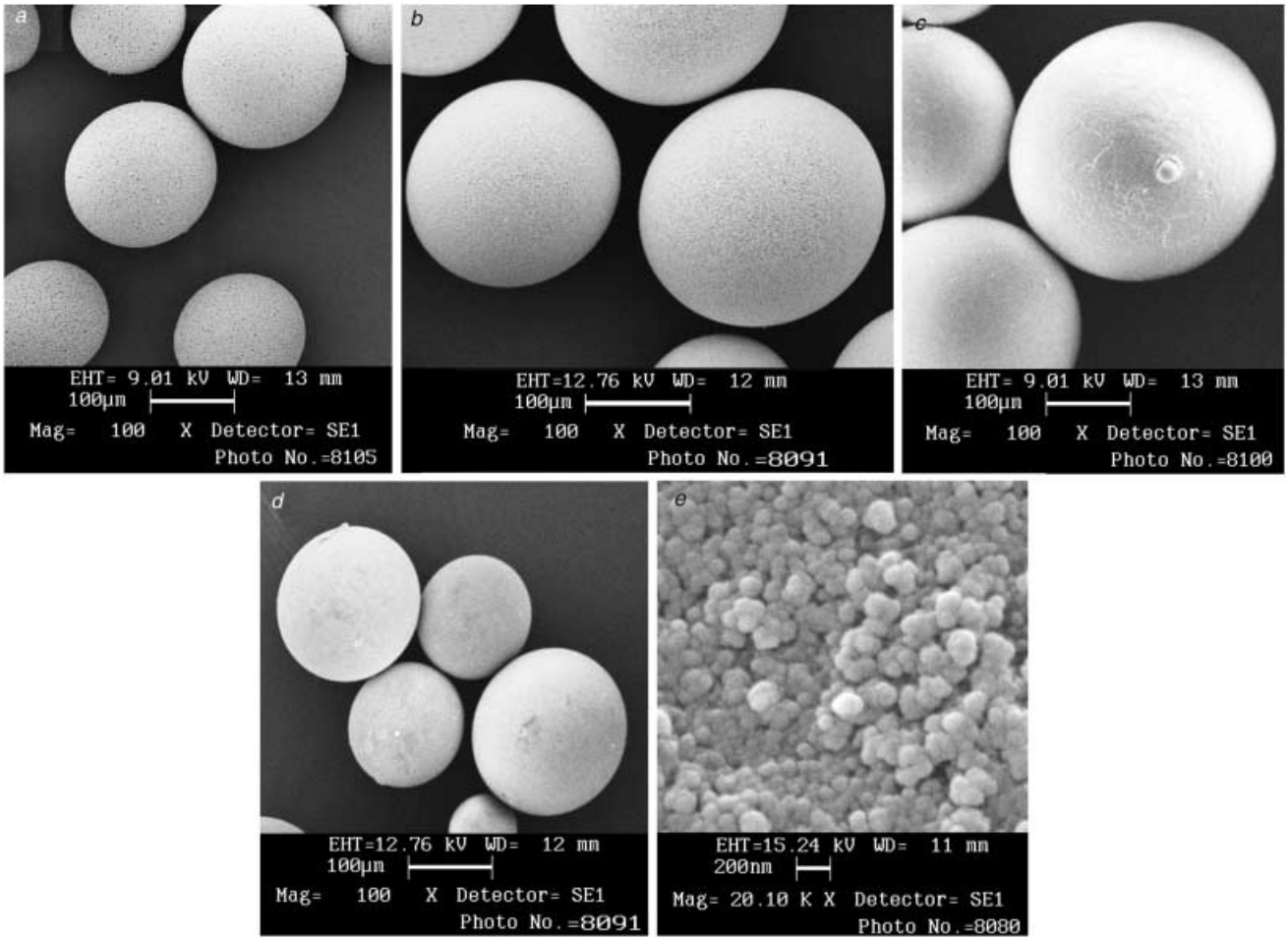


Figure 2. Electron micrograph of the Synbeads showing the beads of a) C6A3, b) C6A2, c) C6A, d) C2A and e) the surface of the C6A beads.

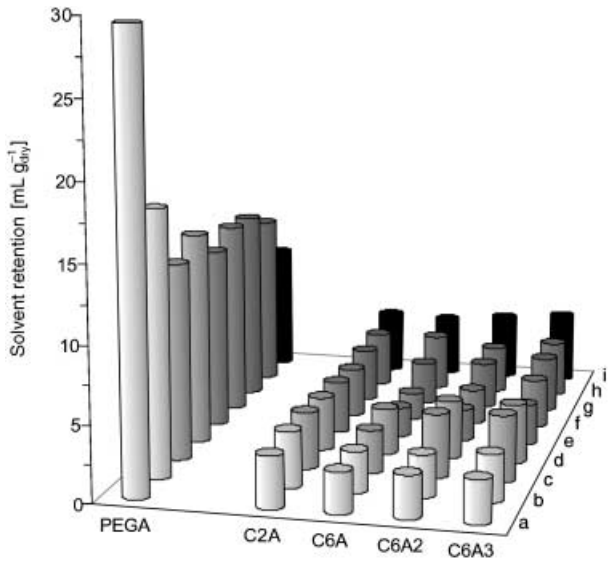


Figure 3. Solvent retention of the Synbeads in different solvents. Data are compared to the behaviour of $PEGA_{1900}$ in the same solvents. a) Water; b) methanol; c) 2-propanol; d) *N,N*-dimethylformamide; e) 1,4-dioxane; f) acetonitrile; g) dichloromethane; h) toluene; i) hexane.

Table 2. Chemical properties of Synbeads.

Resin	Total exchange capacity[a]	Chemically accessible amino groups[b] [mmol g_{dry}^{-1}]	Fmoc-acylated amino groups per 100 Å^2
C2A	2.20	0.53	2.5
C6A	2.24	0.46	2.1
C6A2	1.77	0.09	1.4
C6A3	1.58	0.06	2.4

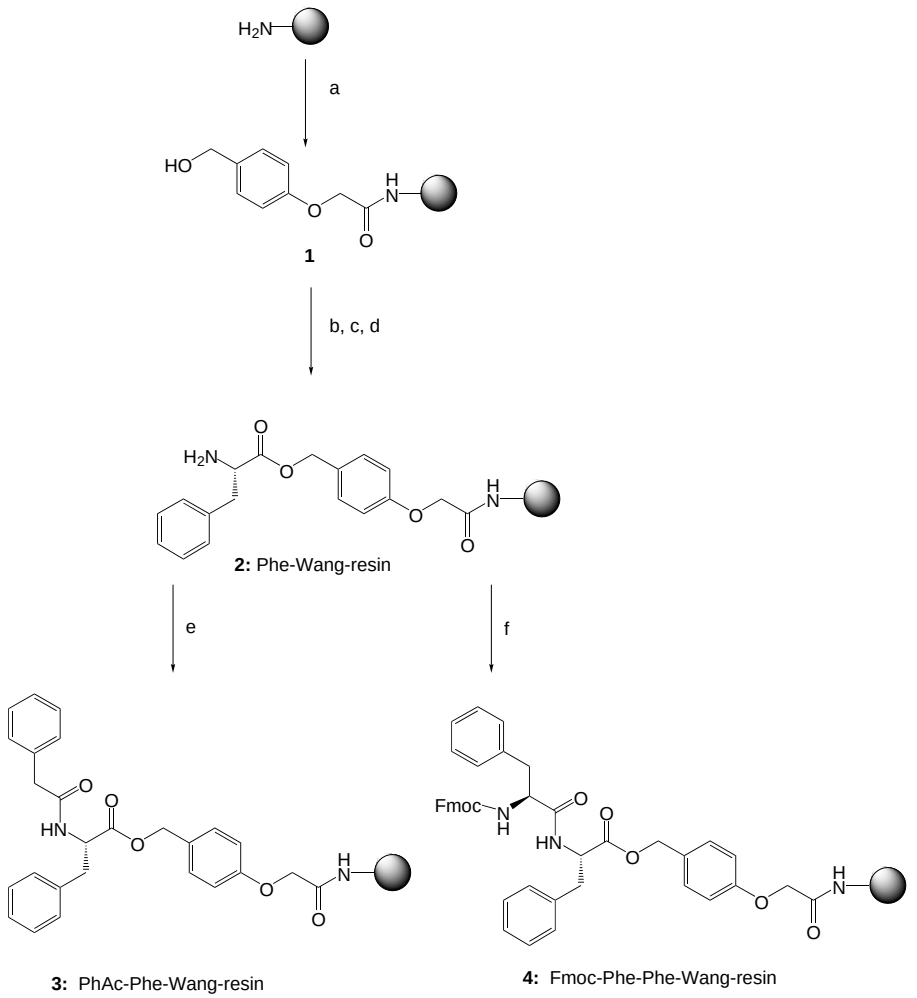
Determined by: [a] Acid–base titration; [b] Chemical coupling of Fmoc-Phe on the resins and subsequent deprotection with piperidine. Quantitative determination of the Fmoc group by UV analysis.

than four amino groups per 100 Å^2. As a consequence, the yields reported in Table 2 correspond to the maximum acylation achievable for all the polymers; although with less sterically hindered substrates higher acylation yields can be achieved. In principle, during the polymer-preparation process the amino-group density of the nonswelling polymers could be chosen on a rational basis. Therefore, further novel types of Synbeads with improved chemical accessibility could be specifically designed for different solid-phase chemistry purposes.

 © 2004 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

The applicability of Synbeads to solid-phase biocatalysis was then verified experimentally by studying the biocatalysed hydrolysis and synthesis of amide/peptide bonds on substrates **3** and **2**, respectively. PGA-catalysed hydrolysis of the phenylacetic acid derivative was investigated by using substrate **3** (PhAc-Phe-Wang-resin), which had been previously used as a model substrate for enzyme accessibility studies.[17] Substrate **3** was chemically synthesised according to Scheme 1 by using widely reported procedures of solid-phase peptide synthesis. After the Fmoc-Phe coupling, all the unreacted amino groups were capped with acetic anhydride in a one-step reaction.

It was also verified during the chemical synthesis of the substrate, that the achieved acylations (expressed in terms of mmol per g_{dry} resin) corresponded to the chemical accessibility reported in Table 2. These values were obtained by determining the release of the Fmoc group (step d) from the intermediate substrate Fmoc-Phe-Wang-resin. In addition, the final yield of the chemical synthesis of **3** was quantified by means of LC–MS analysis through cleavage of PhAc-Phe from the Wang linker, obtaining data in agreement with the Fmoc quantification reported in Table 2. This indicates that the resins are suitable for solid-phase chemistry and for multistep chemical treatments.



Scheme 1. Synthesis of substrates **3** and **4**: a) HMPA coupling (DIC/HOBT in DMF); b) Fmoc-Phe coupling (DIC/DMAP in DMF); c) capping with acetic anhydride in DMF; d) Fmoc deprotection by piperidine (20% piperidine); e) PhAcOH coupling (HOBT/HBTU/DIPEA in DMF); f) Fmoc-Phe chemical coupling (DIC/DMAP in DMF) or enzymatic coupling (thermolysin).

One of the major drawbacks of solid-phase biocatalysis is the diffusion of the enzymes inside the beads; this is even more evident when dealing with large enzymes, such as PGA.[15] The effect of the modified porosity was evaluated by measuring the release of phenylacetic acid (PhAcOH) (see Table 3) from substrate **3** on C6A, C6A2 and C6A3 after enzymatic hydrolysis by PGA. Experimental results reported in Table 3 clearly indicate that the highest enzymatic release of PhAcOH by PGA from substrate **3** (35%) was obtained using C6A3 Synbeads, which have the largest porosity (5400 Å). With C2A or C6A no significant difference in the yields was observed and the low conversions recorded suggest that the enzyme is not diffusing inside the pores of these resins. These low yields are comparable to those already reported for other swelling and nonswelling supports, such as Tentagel (Table 3), xerogels[16] or controlled-pore glass supports (CPG).[7,15]

Table 3. Results obtained in the hydrolysis of substrates **3** (PhAc-Phe-Wang-resin) and **5** (PhAc-Gly-Gly-Wang-resin) by PGA, expressed as percentage of conversion [%] and μmol of released PhAcOH per gram of dry resin.[a]

Resin	Substrate	PhAcOH release	
		[μmol g_{dry}^{-1}]	[%][b]
$PEGA_{1900}$	**3**	18	12
Tentagel	**3**	4	<1
C2A	**3**	12	2
C6A	**3**	10	2
C6A2	**3**	10	11
C6A3	**3**	21	35
C6A3	**5**	39	64

[a] Conditions: PGA (5 mg) (lyophilised from Fluka), Kpi buffer (6 mL, 0.1 M, pH 8.0) and substrate (0.01 mmol) at RT, 40 r.p.m. (blood rotator). [b] At 24 h when the reaction equilibrium was reached. Calculated on the basis of chemically accessible amino groups (Table 2).

The best performing polymer, Synbead C6A3, was then tested by chemically synthesising a different substrate PhAc-Gly-Gly-Wang-resin (**5**). Relative to **3**, substrate **5** exhibits a peptidic portion that is less sterically hindered; this is then expected to have a higher conformational adaptabili-

© 2004 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

ty inside the enzyme active site. Substrate **5** was prepared by using a synthetic procedure similar to those reported in Scheme 1 for substrates **3** and **4** (see Experimental Section).

It is worth noting that by using substrate **5** on C6A3 the enzymatic release of phenylacetic acid almost doubled (64%); this is an even higher yield than reported when using PGA on insoluble supports. The significant increase in the yield is ascribable to improved enzyme–substrate recognition.

The efficiency and versatility of Synbeads is even more remarkable when our results are compared with conversions previously reported. Phenylacetic acid is removed by PGA with quite low yields compared with commercial swelling polymers, such as $PEGA_{1900}$ (12%, Table 3, 13% ref. [7]) or with rigid supports, such as CPG (10%)[7] or xerogels (<5%).[16]

It must also be underlined that when the reaction volume is taken into account, 100 mg of wet $PEGA_{1900}$ occupy tenfold the volume of 100 mg of wet C6A3 (in both cases the diameter of the wet beads is 150–300 μm); this difference is of major importance, especially for the implementation of large-scale processes. When comparing the productivity of the two systems, expressed per reaction volume, a 15-fold improvement is achievable with the C6A3 polymer (11 $\mu mol\,cm^{-3}$ for C6A3 compared with 0.7 $\mu mol\,cm^{-3}$ for $PEGA_{1900}$).

After investigating the efficiency of Synbeads in hydrolyses catalysed by the large PGA, a smaller hydrolase, thermolysin from *Bacillus thermoproteolyticus rokko* (M_r= 35 KDa) was used for peptide synthesis. This protease is highly efficient in peptide synthesis and amino acid resolution on a solid phase, as demonstrated by Ulijn et al.[4] We attempted the enzymatic synthesis of the dipeptide Fmoc-Phe-Phe-Wang-resin[4a] starting from the substrate **2** (Phe-Wang-resin, see Scheme 1) and using Fmoc-Phe as the reactant.

The aim was to verify whether the porosity of the support affects the efficiency of this small enzyme as well, so the enzymatic synthesis was performed on the three polymers C6A, C6A2 and C6A3. Reactions were monitored by quantifying the unreacted Phe and the product of the enzymatic reaction Fmoc-Phe-Phe, both of which are released from the Wang linker under acidic conditions.

Yields varying from 1% (C6A) to 45% (C6A2) and 88% (C6A3) were obtained after an 8 h reaction (Figure 4a). Complete conversion was achieved with C6A3 (97%) after 12 h and C6A2 (98%) after 24 h, whereas only 30% was obtained with C6A in 24 h. This result is related to the diffusion limits of the thermolysin into the polymers and confirms that, for efficient catalysis on these rigid polymers, porosity is of major importance.

Data obtained in thermolysin-catalysed peptide synthesis demonstrate that, firstly, the enzymatic synthesis of peptides on C6A3 is very efficient and can be a valuable alternative to the chemical synthesis and, secondly, that these rigid supports (in particular C6A3) are a valid alternative to swelling polymers in enzyme applications on a solid phase. In addition, the product Fmoc-Phe-Phe was recovered very cleanly after cleavage with TFA on C6A3, as shown by Figure 4b and c.

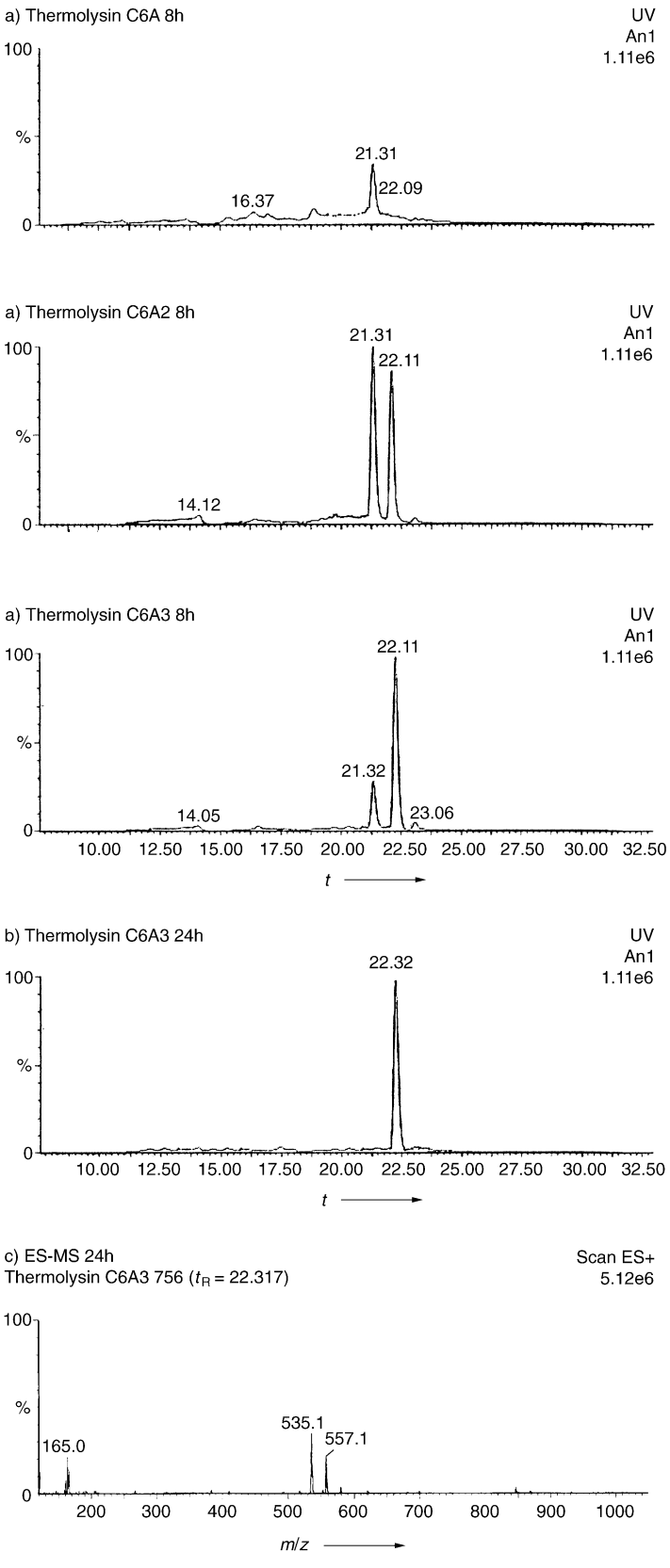


Figure 4. LC–MS analysis of Phe and Fmoc-Phe-Phe after cleavage by TFA from Synbeads, relative to the thermolysin-catalysed synthesis. t_R: Phe 21.31 min, Fmoc-Phe-Phe 22.11 min. Reactions after a) 8 h incubation for all the resins and b) 24 h for C6A3; c) ES spectrum of the product Fmoc-Phe-Phe after 24 h reaction cleaved from C6A3 (535 $[M+1]^+$, 557 $[M+1+Na]^+$).

 © 2004 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

In conclusion, Synbeads can be efficiently applied to chemical and biocatalysed processes. In both cases these supports offer the remarkable advantage of easy recovery of pure products. The porosity of C6A3 polymers allows efficient diffusion of enzymes, even as large as PGA. Finally, Synbeads are also very good for solid-phase enzymatic peptide synthesis.

We are currently involved in the optimisation of the C6A3 polymer to increase the chemical accessibility of its amino groups with the aim of producing a new class of efficient, highly porous and highly loaded polymers.

Experimental Section

Synbeads were prepared by Resindion on the basis of the procedures described in refs. [19] and [20]. The different resins were prepared from mixtures of monomers containing 10–50% of cross-linking agent. Resindion will provide free samples of the resins for research purposes.

General procedure for solid-phase chemistry: All reactions were performed in reactor syringes (StepBio) and mixed in a blood rotator (40 rpm).

Solvent retention: The solvent retention of Synbeads ($PEGA_{1900}$) was quantified on the basis of the weight difference between wet and dried resin (heated overnight in an oven at 110 °C).

Chemical syntheses: Before chemical synthesis the resins were washed three times with MeOH/DMF, MeOH, DCM and DMF (3 mL of each). The resins (1 g of wet resin) were weighed in reactor syringes and suspended in DMF.

Chemical accessibility: The chemical accessibility of amino groups was determined by acylation with Fmoc-PheOH (3 equiv) in the presence of *N,N′*-diisopropylcarbodiimide (DIC) (4 equiv) and 4-dimethylaminopyridine (DMAP) (0.1 equiv) in DMF. After each step of acylation the Fmoc group was released in piperidine (20% in DMF) and quantified by UV detection (290 nm). Loading $(\mathrm{mmol\,g^{-1}}) = [\mathrm{Abs}(290\,\mathrm{nm}) \times V(\mathrm{mL})]/[4950(\varepsilon) \times \mathrm{amount}(g_{dry})]$.

Wang-linker coupling: The Wang linker was attached to the resin by reacting 4-(hydroxymethyl)phenoxyacetic acid (HMPA) (3 equiv) in the presence of DIC (4 equiv) and 1-hydroxybenzotriazole (HOBT) (6 equiv). The mixtures were then mixed overnight. The resins were filtered next and washed with MeOH/DMF, MeOH, DCM and DMF. The synthesis step was repeated twice (2 h).

*Synthesis of **3***: The OH-group of the Wang linker was treated with PhAc-Phe (3 equiv) in the presence of DIC (4 equiv) and DMAP (0.1 equiv) in DMF. The reaction was performed in two cycles, the first of 2 h and the second overnight. After each cycle the resin was filtered and then washed with MeOH/DMF, MeOH, DCM and DMF.

*Synthesis of **4** and **5***: After the Wang linker was attached, Fmoc-GlyOH (3 equiv) was coupled in the presence of DIC (4 equiv) and HOBT (6 equiv). The unreacted OH-groups of the Wang linker and the unreacted amino groups of the polymer were capped with acetic anhydride (10 equiv) in DMF overnight. The Fmoc group was deprotected with a solution of piperidine (20%). A second Fmoc-GlyOH (10 equiv) was coupled in the presence of HOBT (12 equiv), *O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU) (12 equiv) and *N,N′*-diisopropylethylamine (DIPEA) (7.5 equiv). Deprotection of the Fmoc group was carried out in piperidine (20% in DMF, 2 h, RT). PhAcOH (3 equiv) was coupled in the presence of DIC (4 equiv) and HOBT (6 equiv). A similar synthetic procedure was applied to the synthesis of **4** by using Fmoc-Phe as the reagent.

PGA-catalysed hydrolyses: Enzymatic hydrolysis of substrates **3** and **5** from the resins were performed by washing about 50 mg of the wet functionalised resin with Kpi buffer (0.1 M, pH 8.0). The resin was then suspended in the same buffer (6 mL) and in the presence of lyophilised PGA (5 mg, Fluka, 14 $\mathrm{U\,mg^{-1}}$). The reactions were allowed to mix for 24 h at RT.

Thermolysin-catalysed peptide synthesis: The enzymatic synthesis was performed by using Fmoc-Phe (10 equiv) and thermolysin (5 mg) in the buffer (1 mL) on a weighed amount of the resins (corresponding to 0.002 mmol of amino groups).

Analyses: At the end of the reaction the mixtures were filtered and the resins were washed with CH_3CN/H_2O (1:1) (12×3 mL). The liquid phase was recovered in a flask and dried under vacuum. It was then redissolved in CH_3CN/H_2O (1:1) (1 ml) and centrifuged, before being passed through membrane filters (0.45 μm). The samples were analysed with a reversed-phase HPLC system. Product amounts were calculated by using calibration curves of PhAcOH. The peptide structures were then cleaved through the Wang linker with a solution of trifluoroacetic acid/H_2O (95:5) to confirm the conversion results.

LC–MS analyses were performed at the University of Edinburgh using a Waters 2790 Separation Module, a Waters 486 Tuneable Absorbance Detector at 254 nm and a Luna 5μ C18 column (250×2 mm) coupled to a Micromass Platform II Spectrometer. Gradient conditions: from 100% A to 15% A in 20 min, then isocratic for 5 min. Solution compostitions: A) H_2O, 0.1% TFA; B) CH_3CN, 0.1% TFA. Flow rate: 0.2 $\mathrm{mL\,min^{-1}}$.

Theoretical measurements: Atomic distances, molecule sizes and surfaces were calculated by means of a Molecular Operating Environment (MOE) running on a SGI Octane workstation.

Acknowledgement

The authors would like to acknowledge MIUR (Rome, Italy) and Region Friuli Venezia Giulia for funding to L.G. and P.L. The authors express their gratitude to Prof. Sabine L. Flitsch for the LC–MS analyses and to Dr. Rein V. Ulijn for helpful discussion. Thanks are due to the anonymous referees for useful suggestions.

[1] a) C. T. Walsh, *ChemBioChem* **2002**, *3*, 124–134; b) F. Balkenhohl, C. von dem Busche Hünnefeld, A. Lansky, C. Zechel, *Angew. Chem.* **1996**, *108*, 2436–2488; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2288–2337; c) J. S. Fruchtel, G. Jung, *Angew. Chem.* **1996**, *108*, 19–46; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 17–42; d) L. A. Thompson, J. A. Ellman, *Chem. Rev.* **1996**, *96*, 555–600; d) M. T. Reetz, *Angew. Chem.* **2001**, *113*, 292–320; *Angew. Chem. Int. Ed.* **2001**, *40*, 284–310; e) S.-I. Nishimura, *Curr. Opin. Chem. Biol.* **2001**, *5*, 325–335.

[2] a) P. C. Michels, Y. L. Khmelnitsky, J. S. Dordick, D. S. Clark, *Trends Biotechnol.* **1998**, *16*, 210–215; b) V. V. Mozhaev, C. L. Budde, J. O. Rich, A. Y. Usyatinsky, P. C. Michels, Y. L. Khmelnitsky, D. S. Clark, J. S. Dordick, *Tetrahedron* **1998**, *54*, 3971–3982; c) D. H. Altreuter, D. S. Clark, *Curr. Opin. Biotechnol.* **1999**, *10*, 130–136; d) J. L. Krstenansky, Y. L. Khmelnitsky, *Bioorg. Med. Chem.* **1999**, *7*, 2157–2162.

[3] a) M. Meldal, F.-I. Auzanneau, O. Hindsgaul, M. M. Palcic, *J. Chem. Soc. Chem. Commun.* **1994**, 1849–1850; b) M. Schuster, P. Wang, J. C. Paulson, C.-H. Wong, *J. Am. Chem. Soc.* **1994**, *116*, 1135–1136.

[4] a) R. V. Ulijn, B. Baragaña, P. J. Halling, S. L. Flitsch, *J. Am. Chem. Soc.* **2002**, *124*, 10988–10989; b) R. V. Ulijn, N. Bisek and S. L. Flitsch, *Org. Biomol. Chem.* **2003**, *1*, 621–622; c) R. V. Ulijn, N. Bisek, P. J. Halling, S. L. Flitsch, *Org. Biomol. Chem.* **2003**, *1*, 1277–1281.

[5] H. Waldmann, A. Reidel, *Angew. Chem.* **1997**, *109*, 642–644; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 647–649.

[6] R. Reents, D. A. Jeyaraj and H. Waldmann, *Adv. Synth. Catal.* **2001**, *343*, 501–513.

[7] U. Grether, H. Waldmann, *Chem. Eur. J.* **2001**, *7*, 959–971.

[8] R. Reents, D. A. Jeyaraj, H. Waldmann, *Drug Discovery Today* **2002**, *7*, 71–76.

[9] W. Li, B. Yan, *J. Org. Chem.* **1998**, *63*, 4092–4097.

[10] J. Rademann, M. Grøtli, M. Meldal, K. Bock, *J. Am. Chem. Soc.* **1999**, *121*, 5459–5466.

[11] D. E. Bergbreiter, *Med. Res. Rev.* **1999**, *19*, 439–450.

[12] F. Rasoul, F. Ercole, Y. Pham, C. T. Bui, Z. Wu, S. N. James, R. W. Trainor, G. Wickham, N. J. Maeji, *Biopolymers* **2000**, *55*, 207–216.

© 2004 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

[13] A. J. Mendonca, X.-Y- Xiao, *Med. Res. Rev.* **1999**, *19*, 451 – 462.
[14] R. Quarrel, T. D. W. Claridge, G. W. Weaver, G. Lowe, *Mol. Diversity* **1995**, *1*, 223 – 232.
[15] J. Kress, R. Zanaletti, A. Amour, M. Ladlow, J. G. Frey, M. Bradley, *Chem. Eur. J.* **2002**, *8*, 3769 – 3772.
[16] A. Basso, L. De Martin, C. Ebert, P. Linda, L. Gardossi, R. V. Ulijn, S. L. Flitsch, *Tetrahedron Lett.* **2003**, *44*, 6083 – 6085.
[17] A. Basso, L. De Martin, L. Gardossi, G. Margetts, I. Brazendale, A. Y. Bosma, R. V. Ulijn, S. L. Flitsch, *Chem. Commun.* **2003**, 1296 – 1297.
[18] C. Mateo, O. Abian, G. Fernandez-Lorente, J. Pedroche, R. Fernandez-Lafuente, J. Guisan, A. Tam, M. Daminati, *Biotechnol. Prog.* **2002**, *18*, 629 – 634.
[19] K. Itagaki, H. Kusano, E. Miyata, K. Nakajima, (Mitsubishi Chem. Ind. Ltd.) JP 62074904, **1987** [Chem. Abstr. **1988**, *108*, 18846].
[20] H. Kusano, E. Miyata, Y. Kubo, (Mitsubishi Chem. Ind. Ltd.) JP 63143942, **1988** [Chem. Abstr. **1989**, *110*, 20794].

Received: June 17, 2003
Revised: September 1, 2003 [F5243]

 © 2004 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim