A poly(acryloylmorpholine)-based bead matrix of improved versatility for solid (gel) phase peptide synthesis

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We have described recently a novel, bead-form, phenolic poly(acryloylmorpholine)-based matrix for application in the solid (gel) phase method of peptide assembly[1]. Preparation of this support, a de-*O*-acetylated copolymer of acryloyl morpholine, *N,N'*-diacryloyl piperazine and *N*-[2-(4-acetoxyphenyl)ethyl]-acrylamide was facilitated by a specially-developed suspension polymerization procedure. During subsequent peptide synthesis, the carboxylic acid terminals of the growing peptide chains were anchored throughout the bead copolymer by phenolic ester linkages. Thus, as prepared, the matrix is applicable only in schemes of peptide synthesis employing *N*-terminal protecting groups which may be cleaved under non-basic conditions. This precludes the use of, for example, the currently-popular fluorenylmethoxycarbonyl (Fmoc) amino acids.

To be adaptable for use in schemes of peptide synthesis involving both acid-catalysed and base-catalysed deprotection strategies, a solid (gel) phase peptide support must be amenable to simple functional modification to introduce appropriate, selectively-labile, peptide anchorage points. There have been some reports of poly(*N,N*-dimethylacrylamide)-based copolymers of this type. A communication by Atherton *et al.*[2] reports the suspension polymerization of *N,N*-dimethylacrylamide, *N,N'*-ethylenediacrylamide and *N*-[6-(*N'*-t-butoxy-carbonyl-β-alanylamino)hexyl]-acrylamide. The copolymer so produced was deprotected by mild acid hydrolysis to expose pendant amino groups. The latter were reacted further to incorporate different residues, effective as functional points of attachment for peptide synthesis. Arshady *et al.*[3] have communicated an alternative approach to this type of amine-functionalized copolymer. Bead copolymerization of *N,N*-dimethylacrylamide, *N,N'*-ethylenediacrylamide and *N*-acryloyl sarcosine methyl ester was followed by amination of the carboxymethyl residues of the copolymer by treatment with ethylene diamine.

More recently, Stahl *et al.*[4] have reported the successful bead copolymerization of *N,N*-dimethylacrylamide, *N,N'*-ethylenediacrylamide and the hydrochloride of *N*-(6-aminohexyl)-acrylamide to give materials of similar type.

All three procedures involved a suspension polymerization procedure in which at least the major monomer, *N,N*-dimethylacrylamide, was dissolved in an *aqueous* phase dispersed in a continuous organic phase. Inevitably, less polar monomers in the polymerization mixture will tend to partition between the two phases. The use of the hydrochloride of *N*-(6-aminohexyl)-acrylamide by Stahl *et al.* was adopted to obviate this possibility. For poly(acryloylmorpholine)-based networks, we have found that introduction of monomeric amine salts in the copolymerization mixture results in mechanically unstable materials of low crosslink density[5].

We are prompted to relate here the application of our successful alternative strategy[1] for suspension polymerization, in which the various monomers are maintained 'salted-in' an *organic* phase dispersed in saturated brine, to the synthesis of a new, poly(acryloylmorpholine)-based copolymer of similar versatility to those described above.

The procedure involves copolymerization of acryloyl morpholine, *N,N'*-diacryloyl piperazine and a new, purpose-synthesized, monomer, *N*-[3-(*N'*-benzyloxycarbonylaminomethyl)benzyl]-acrylamide. A two stage synthesis was employed to prepare this monomer. An equimolar quantity of 3-aminomethylbenzylamine in ethanol was added to benzyloxycarbonyl chloride in petrol (b.p. 60°–80°C). The precipitated material was collected and extracted with chloroform to leave the crude hydrochloride of 3-(*N*-benzyloxycarbonylaminomethyl)benzylamine which was purified by recrystallization from water. The desired monomer was obtained by acryloylation in chloroform solution layered with aqueous sodium hydroxide.

The experimental procedure for the preparation of the new bead copolymer (*Figure 1*) was as follows. Freshly distilled acryloyl morpholine (84.3 g, 0.60 mol), *N*-[3-(*N'*-benzyloxycarbonylaminomethyl)benzyl]-acrylamide (38.8 g, 0.12 mol), *N,N'*-diacryloylpiperazine (5.83 g, 0.03 mol) and α,α'-azobisisobutyronitrile (9.0 g) were dissolved in 1,1,2,2-tetrachloroethane (685 cm^3). After displacement of dissolved oxygen by bubbling with nitrogen, the solution was dispersed with mechanical stirring in an oxygen-free continuous aqueous phase, consisting of a sodium chloride-saturated mixture of aqueous 0.20% xanthan gum solution (920 cm^3) and aqueous 0.16% hydroxypropylmethyl cellulose solution (183 cm^3). Stirring was carefully regulated to give a droplet size distribution of 50–250 μm.

Polymerization was initiated by raising the temperature to 70°C. After 4 h, the temperature was allowed to fall to 55°C and heating continued overnight. After syphoning off most of the brine, the copolymer gel was washed re-

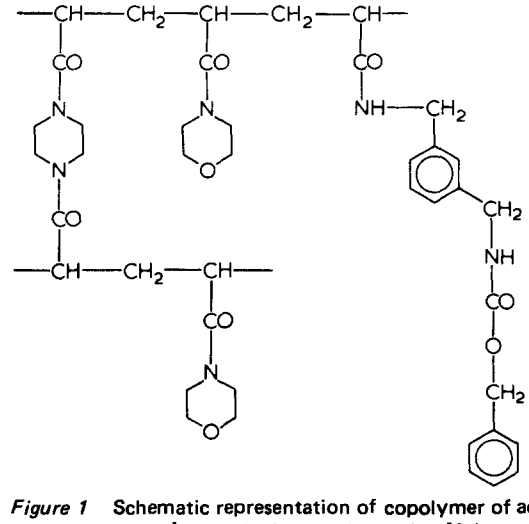

Figure 1 Schematic representation of copolymer of acryloyl morpholine, *N,N'*-diacryloylpiperazine and *N*-[3-(*N*-benzyloxycarbonylaminomethyl)benzyl]-acrylamide

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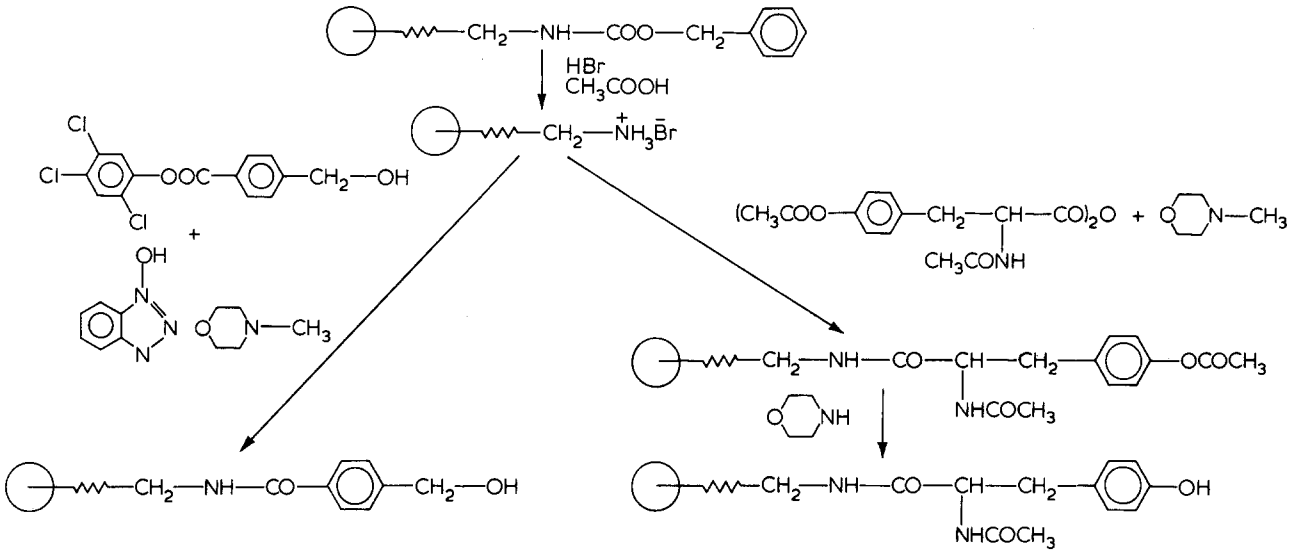

Figure 2 Conversion of benzylamino-substituted copolymer to functional derivatives suitable for use in solid (gel) phase peptide synthesis

peatedly with distilled water and then with ethanol. Equilibration with ether and drying *in vacuo* gave the copolymer in the form of discrete beads (118.7 g, 92%). Beads of 45–211 μm (107.4 g, 84%) were obtained by dry sieving.

Treatment of the copolymer beads with HBr in acetic acid effected quantitative deprotection of the benzylamino residues pendant on the hydrocarbon backbone. The hydrobromide salt of the deprotected matrix could then be adopted readily (*Figure 2*) for solid (gel) phase peptide synthesis involving either repetitive acid-catalysed or repetitive base-catalysed deprotection cycles.

For example, reaction with 4-hydroxymethylbenzoic acid 2,4,5-trichlorophenyl ester[3] in the presence of 1-hydroxybenzotriazole and *N*-methylmorpholine leads to quantitative conversion of the benzylamino residues to 4-hydroxymethylbenzoyl groups. This material is suitable for use in all strategies of solid (gel) phase peptide synthesis.

Alternatively, reaction of the hydrobromide salt of the deprotected matrix with *N,O*-diacetyl-L-tyrosine anhydride, prepared by treatment of *N,O*-diacetyl-L-tyrosine[6] with dicyclohexylcarbodiimide in the presence of *N*-methylmorpholine, led to quantitative acylation by *N,O*-diacetyl-L-tyrosyl groups; subsequently these groups were de-*O*-acetylated selectively to provide points of attachement for protected amino acids and peptides via base-labile phenyl ester linkages.

The advantages and disadvantages of the phenyl ester mode of peptide attachment in solid (gel) phase peptide synthesis have been discussed elsewhere[7,8]. In our own work, we found that utilization of the phenolic residue of L-tyrosine as a point for peptide attachement also provides a useful reference amino acid for analytical control in subsequent amino analysis.

Irrespective of the pendant functional groups used ultimately for attachment of the peptide under synthesis, coupling of the first protected amino acid of the desired sequence was achieved by reaction of the appropriate protected amino acid in dimethylformamide with dicyclohexylcarbodiimide in the presence of a catalytic quantity of 4-dimethylaminopyridine at 25°C. Thereafter, standard, appropriate, chain elongation strategies were employed. Details of the application of the new support matrix to the synthesis of oligopeptides will be reported in due course.

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