polymer reports

Catalytic transfer hydrogenation of poly(acryloylmorpholine)-based peptide-resin assemblies and of derived peptide segments

R. Epton, S. J. Hocart, G. Marr and P. W. Small
Department of Physical Sciences, Wolverhampton Polytechnic, Wulfruna Street, Wolverhampton WV1 1LY, UK
(Received 22 April 1982)

Catalytic transfer hydrogenation using *in situ* generated Pd black has been used to cleave benzyl ester linked peptides from poly(acryloylmorpholine)–peptide solid (gel) phase assemblies with concurrent removal of peptide *N*-terminal and side-chain protecting groups. Generation of Pd black *in situ* also facilitates peptide deprotection by catalytic transfer hydrogenation in free solution.

Keywords Catalytic transfer hydrogenation, solid (gel) phase, poly(acryloylmorpholine), peptide synthesis

INTRODUCTION

We have reported previously the utilization of bead-form, crosslinked, poly(acryloylmorpholine) derivatives as support matrices in an efficient, low-cost, reaction strategy for solid (gel) phase peptide synthesis[1,2]. A feature of the strategy is that mild reaction conditions are used throughout. When assembly of a target peptide is complete, the peptide is detached from the resin, in protected form, usually by autocatalysed transesterification with 2-dimethylaminoethanol[3,4]. The resulting labile acrylpeptide ester is then allowed to undergo autocatalysed hydrolysis to yield a protected acylpeptide acid which is purified by conventional procedures. Subsequently, *N*-terminal and side-chain deprotection may be effected cleanly and in high yield by catalytic transfer hydrogenation[5–8].

In the event that a final deprotected peptide is required rather than a protected acylpeptide segment, the three-stage protocol is obviously time consuming. However, the alternative, traditional tactic of concurrent detachment and deprotection by treatment with anhydrous hydrogen fluoride is an aggressive one which can exacerbate purification difficulties. Recently, Colombo[9] has reported a solution to this problem. Simultaneous detachment and deprotection was achieved, in the case of polystyrene resins, by catalytic transfer hydrogenation with 1,4-cyclohexadiene and *in situ* generated palladium black. In order to secure good cleavage yields, the peptide was assembled while anchored, *via* a *C*-terminal benzyl ester linkage, on a long 'spacer arm' pendant on the polystyrene backbone[10]. The excellent yields reported encouraged us to try a similar approach for the detachment of peptides which had been assembled while attached, *via* a *C* terminal benzyl ester linkage, on a poly(acryloylmorpholine)-based resin[11]. Such supports undergo gelation better than polystyrene resins in all common peptide solvents. It was our hypothesis that the greater freedom of movement of the polymer chains comprising the poly(acryloylmorpholine)-based gel network would be sufficient to allow good contact between the benzyl ester linkages and the catalyst surface. Under these circumstances, a long 'spacer arm' could be unnecessary.

EXPERIMENTAL

Materials and methods

A bead copolymer of acryloyl morpholine *N*-[3-(*N*'-benzyloxycarbonylaminomethyl)benzyl] – acrylamide and *N*,*N*'-diacryloylpiperazine (molar ratio 20:4:1) (Copolymer 1) was used as the support matrix. Reactions involving solid (gel) supports were carried out with the aid of modified Corley–Sach–Anfinsen reactors[12,1].

De-O-benzyloxycarbonylation (deprotection) of Copolymer 1

Copolymer 1 (6.0 g) was treated with 45% HBr in glacial acetic acid (100 cm^3) over 16 h at 25°C. Excess reagent was removed and the gel was washed with acetic acid (3 × 80 cm^3) and ether (2 × 80 cm^3). The resin was subjected to alternate washes with *N*,*N*-dimethylacetamide (5 × 80 cm^3) and ether (5 × 80 cm^3) and dried *in vacuo* to give deprotected Copolymer 1 (5.6 g, ~98%). Infra-red analysis (KBr disc) confirmed the loss of the benzyloxycarbonyl groups (C=O, 1710 cm^{-1} absent).

p-*Hydroxymethylbenzoylation of deprotected Copolymer 1*

Deprotected Copolymer 1 (1.0 g) was treated with 4-hydroxymethylbenzoic acid 2,4,5-trichlorophenyl ester[13] (0.83 g, 2.5 mmol) and 1-hydroxybenzotriazole (0.135 g, 1

0032-386/82/111685-03$03.00

mmol) in *N,N*-dimethylformamide (25 cm^3). After 5 min, *N*-methylmorpholine (0.2 g, 2 mmol) was added and the swollen gel agitated for 4 h. The excess reagent was removed and the resin subjected to alternate washes with dichloromethane (5 × 20 cm^3) and ether (5 × 20 cm^3) to give the *p*-hydroxymethylbenzoyl derivative of deprotected Copolymer 1 (Copolymer 2) (1.0 g, ~95%). The resin gave a negative fluorescamine test[14].

Boc–Tyr(Bzl)–Gly–Gly–Phe–Leu–O–[Copolymer 2] Copolymer 2 (5.0 g, hydroxymethylbenzoyl content 1.0 mmol g^{-1}) was used as the starting point for the preparation of this assembly. The reaction and washing conditions employed for each stage in the synthesis were as described previously by us for the preparation of the corresponding phenolic poly(acryloylmorpholine)-based assembly[1]. The peptide resin assembly gave the following amino acid analysis $Tyr_{1.07}$ $Gly_{2.01}$ $Phe_{1.07}$ $Leu_{1.00}$.

H–Tyr–Gly–Gly–Phe–Leu–OH.HCO_2H. Boc–Tyr(Bzl)–Gly–Gly–Phe–Leu–O–[Copolymer 2] (0.250 g, 0.154 mmol attached peptide) 25 cm^3, Corley–Sachs–Anfinsen reactor) was allowed to swell in 98% HCO_2H (10 cm^3) and reaction allowed to proceed, with nitrogen stirring, over 3 h at 25°C. The excess reagent was then drawn off and the gel washed with CH_2Cl_2 (2 × 5 cm^3), $MeCONMe_2$ (2 × 5 cm^3), 10% Et_3N in $MeCONMe_2$ (3 × 5 cm^3), Et_2O (2 × 5 cm^3) and finally $MeCONME_2$ (2 × 5 cm^3). The excess solvent was then removed and the resin was redispersed in $MeCONMe_2$ (2 cm^3) containing $Pd(OAc)_2$ (0.16 g, 0.72 mmol). After 10 min, 98% HCO_2H (0.5 cm^3) was added and the mixture gently nitrogen stirred for 16 h at 25°C. The reaction liquor was then drawn off and the gel subjected to alternate washes with 98% HCO_2H (5 × 5 cm^3) and Et_2O (5 × 5 cm^3). The combined reaction liquor and washings were evaporated under reduced pressure to give an oil, which solidified on trituration with Et_2O. Precipitation from $MeOH/Et_2O$ gave crude H–Tyr–Gly–Gly–Phe–Leu.HCO_2H (0.086 g, 93%); HPLC on Spherisorb 5 ODS with $MeCN/H_2O/HCO_2H$ (60/38/2) gave one major peak (integration >92%) with minor impurities. Crystallization from MeOH gave pure H–Tyr–Gly–Gly–Phe–Leu–OH.HCO_2H (0.034 g, 37%), m.p. 162°–164°C d. HPLC (system as above) gave one peak (integration 100%); $Tyr_{1.01}$ $Gly_{2.01}$ $Phe_{1.07}$ $Leu_{1.00}$, (Found C, 57.60; H, 6.57; N, 12.25; O, 23.42%. $C_{29}H_{39}H_5O_9$ requires C, 57.89; H, 6.53; N, 11.64; O, 23.93%).

H – Gly – Gly – Pro – Arg – Gly – OH.2HCO_2H. Boc–Gly–Gly–Pro–Arg(NO_2)–Gly–OH[2] (0.200 g, 0.440 mmol) was dissolved in 98% HCO_2H (5 cm^3) and reaction allowed to proceed over 3 h at 25°C. $Pd(OAc)_2$ (0.40 g, 1.8 mmol) was then added and the resulting suspension was stirred magnetically for 16 h at 25°C. The suspension of palladium black was then removed by filtration, and the reaction solvent removed by evaporation under reduced pressure to give an oil which, on trituration with ether gave crystalline H–Gly–Gly–Pro–Arg–Gly–OH.2HCO_2H (0.150 g, 82%); m.p. 137°–140°C d; HPLC (system as above) gave one major peak (integration >97%); $Gly_{2.88}$, $Arg_{1.05}$, $Pro_{1.00}$, (Found: C, 42.60; H, 6.40; N, 21.17. $C_{19}H_{34}N_8O_{10}$ requires C, 42.69; H, 6.41; N, 20.97).

RESULTS AND DISCUSSION

The synthesis of the primary poly(acryloylmorpholine)-based bead copolymer (Copolymer 1) (*Figure 1a*) utilized in this work has been described previously by us[11]. To prepare Copolymer 1 for solid (gel) phase peptide synthesis, the pendant benzyloxycarbonyl groups were replaced by *p*-hydroxymethylbenzoyl groups (Copolymer 2) (*Figure 1b*). This was effected in two stages. First, the benzyloxycarbonyl groups were removed by treatment with hydrogen bromide in acetic acid. Second, the aminomethyl groups so exposed were subjected to acylation with 4-hydroxymethylbenzoic acid 2,4,5-trichlorophenyl exter, thereby effecting conversion to *p*-hydroxymethylbenzoylaminomethyl residues[13].

For the study of catalytic transfer hydrogenolysis and simultaneous deprotection within the poly(acryloylmorpholine) support matrix, a model peptide assembly (*Figure 1c*) was elaborated. The model, polymer-bound peptide was a fully protected derivative of the opioid peptide, [Leu] enkephalin. The solid (gel) phase strategy employed was that previously used by us to prepare the same protected peptide using a corresponding phenolic poly(acryloylmorpholine)-based support[1]. Formic acid was selected as the hydrogen donor for the catalytic transfer process in preference to the 1,4-cyclohexadiene used by Colombo[9]. Formic acid is an excellent solvent for the poly(acryloylmorpholine) matrix and, moreover, can be used to effect acidolytic cleavage of *t*-butoxycarbonyl groups immediately prior to hydrogenolysis.

To effect initial removal of the *t*-butoxycarbonyl groups from the model peptide assembly, it was allowed to swell in neat 98% formic acid and the resulting gel was left to stand until the infra-red carbonyl absorption at 1710 cm^{-1}, due to the *t*-butoxycarbonyl group, was no longer evident. The gel was then thoroughly washed, neutralized with triethylamine and equilibrated with dimethylacetamide. The catalyst precursor, palladium (II) acetate was then added. After allowing the latter to diffuse into the gel, 98% formic acid was again introduced. This resulted in the *in situ* generation of palladium black and, subsequently, catalytic transfer hydrogenation. After 16 h, work-up of the reaction mixture gave a 93% yield of crude [Leu]-enkephalin formate, purity >92% as estimated by h.p.l.c. Recrystallization from methanol gave a product which, on h.p.l.c., was found to have a purity of 100%. This result

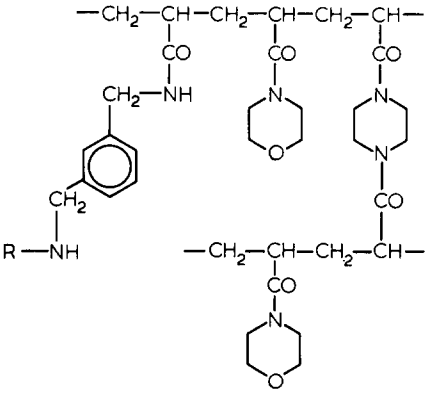

Figure 1 Schematic representation of (a) copolymer 1 (R = $C_6H_5CH_2OCO$–), (b) copolymer 2 (R = *p*-$HOCH_2C_6H_4CO$–) and (c) peptide-resin assembly prior to hydrogenolysis [R = Boc–Tyr(Bzl)–Gly–Gly–Phe–Leu–O–$CH_2C_6H_4CO$–]

clearly confirms our hypothesis that an elaborate 'spacer arm' is unlikely to be necessary for catalytic transfer hydrogenolysis using *in situ* generated palladium black, provided that the solid (gel) phase support matrix is sufficiently well swollen. In this context, it is interesting to note that Colombo[15] has recently utilized catalytic transfer hydrogenolysis to liberate a peptide, thymosin α_1, which has been assembled while attached to a purpose-synthesized, polystyrene-based, nitrobenzhydrylamine resin *via* the side-chain amide group of a C terminal asparagine residue. Provided that two or three successive hydrogenation cycles were used, peptide recovery was 84%.

We have found that the *in situ* method for the generation of palladium black both simplifies and expedites the deprotection of protected peptides by catalytic transfer hydrogenation in free solution. In previous solution work, palladium on charcoal[5] or palladium black[6,7], which had been freshly prepared by the standard method[16], have been employed. Palladium on charcoal is a catalyst of inferior activity. The standard method for the preparation of palladium black is time-consuming and, moreover, results in a product which rapidly deteriorates. This leads often to irreproducibility in hydrogenation conditions. An example of the *in situ* method for the generation of palladium black and subsequent catalytic transfer hydrogenation in solution is provided by the reductive deprotection of the protected peptide Boc-Gly-Gly-Pro-Arg(NO_2)-Gly-OH[2]. The protected peptide was dissolved in 98% formic acid and allowed to react for 3 h to effect removal of the *t*-butoxycarbonyl groups. Catalytic transfer hydrogenation was then initiated by addition of palladium (II) acetate, which was immediately reduced to palladium black. After 16 h, work of the reaction mixture gave an 82% yield of H-Gly-Gly-Pro-Arg-Gly-OH.2HCO_2H, purity >97% as estimated by HPLC.

CONCLUSIONS

Catalytic transfer hydrogenation, using formic acid and *in situ* generated palladium black in dimethylacetamide as gelation solvent, provides an effective method for the detachment of peptides, originally attached *via* benzyl ester linkages, from poly(acryloylmorpholine)-based matrices. *N*-terminal and side chain deprotection is effected simultaneously. This approach is preferable to the traditional, but more aggressive, tactic of using anhydrous hydrogen fluoride to achieve the same ends. Catalytic transfer hydrogenation, using formic acid as solvent and *in situ* generated palladium black, provides a simple and reliable method for *N*-terminal and side chain deprotection of peptides in free solution. We are now using both procedures routinely in our laboratory. Perhaps, the ultimate strategy for catalytic transfer hydrogenation, useful when forcing reaction conditions are required, would be to partially neutralize the formic acid by addition of a tertiary amine after generation of the palladium black. This would increase dramatically the concentration of the formate anion, which is believed to be the active hydrogenating agent in these reactions[8].

REFERENCES

1 Epton, R., Goddard, P., Hocart, S. J., Marr, G. and Hudson, D. *Int. J. Biol. Macromolecules* 1982, **4**, 233
2 Buckle, M., Epton, R., Marr, G., Small, P. W. and Hudson, D. *Int. J. Biol. Macromolecules* 1982, **4**, 275
3 Hudson, D. and Kenner, G. W. *Int. J. Biol. Macromolecules* 1980, **2**, 63
4 Barton, M. A., Lemieux, R. V. and Savoie, J. Y. *J. Am. Chem. Soc.* 1972, **95**, 4501
5 Felix, A. M., Heimer, E. P., Lambros, T. J., Tzougraki, C. and Meienhofer, J. *J. Org. Chem.* 1978, **43**, 4194
6 Sivanandaiah, K. M. and Gurusiddappa, S. *J. Chem. Research (S)* 1979, 108
7 El Amin, B., Anantharamaiah, G. M., Royer, G. P. and Means, G. E. *J. Org. Chem.* 1979, **41**, 3442
8 Anwer, M. K. and Spatola, A. F. *Synthesis* 1980, **11**, 929
9 Colombo, R. *Chem. Lett.* 1980, 1119
10 Sparrow, J. T. *J. Org. Chem.* 1976, **41**, 1350
11 Epton, R., Hocart, S. J. and Marr, G. *Polymer* 1980, **21**, 481
12 Corley, L., Sachs, D. H. and Anfinsen, C. B. *Biochem. Biophys. Res. Commun.* 1972, **47**, 1353
13 Arshady, R., Atherton, E., Gait, M. J., Lee, K. and Sheppard, R. C. *J.C.S. Chem. Comm.* 1979, 423
14 Felix, A. M. and Jimenez, M. H. *Anal. Biochem.* 1973, **52**, 377
15 Colombo, R. *J. Chem. Soc. Comm.* 1981, 1012
16 Greenstein, J. P. and Winitz, M. in 'The Chemistry of the Amino Acids', Vol. 2, Wiley, New York, 1961, p 1233