ZINC CHLORIDE-CATALYZED CHLOROMETHYLATION OF RESINS FOR SOLID PHASE PEPTIDE SYNTHESIS

R. S. FEINBERG* and R. B. MERRIFIELD

Rockefeller University, New York, New York 10021

Abstract—Zinc chloride has been found to catalyze chloromethylation of resins for solid phase peptide synthesis, producing low levels of chloromethylation more accurately and under more convenient conditions than does SnCl₄. The preparation of a stable solution of the ZnCl₂ catalyst in THF is described, and data are presented detailing the effects of varying temperature, time, and the concentrations of catalyst and chloromethyl methyl ether on the chloromethylation reaction.

The initial step in the solid phase synthesis of peptides[1]—functionalization of the solid support—is usually accomplished by Friedel-Crafts chloromethylation of a styrene-divinylbenzene copolymer. If supports of any desired substitution are to be conveniently prepared, this alkylation must be an easily controlled reaction leading to resins with predictable levels of chloromethyl substitution. Original chloromethylation procedures[1-3] employed chloromethyl methyl ether and stannic chloride as the catalyst, but because tin halides are among the stronger catalysts of Friedel-Crafts alkylations, the reaction is very fast and must be carefully controlled by cooling and efficient mixing. In our laboratory, efforts to change the scale of these procedures have given variable results, and it has proved especially difficult to obtain resins with the low chloromethyl substitutions (0·03–0·3 millimoles/g) desired for the synthesis of larger peptides.

A more easily controlled chloromethylation especially well suited to the production of resins of low substitution can be achieved by using zinc chloride as the catalyst. Zinc chloride has previously been used in the alkylation of more highly crosslinked polymers leading to ion-exchange resins,[4,5] and for soluble polystyrenes,[6-8] and we have found that it induces a smooth, controllable chloromethylation of the 1% divinylbenzene-styrene copolymer beads most often used in solid phase peptide synthesis. Though it has the disadvantage of being a hygroscopic solid, zinc chloride is very soluble in tetrahydrofuran and a 1 M solution in THF is easily prepared from ZnCl₂ that has been dissolved in hydrochloric acid and then fused to remove water and Zn(OH)₂. Provided the THF is dry and peroxide-free, the catalyst solution is quite stable over a period of weeks.

The chloromethylation study was performed on individual 0·5 g samples of 1% divinylbenzene-styrene copolymer beads in sufficient liquid (6 ml) to form a mobile suspension. The samples were removed from agitation in a heating bath at appropriate intervals and analyzed for chlorine content after washing, but without drying or re-weighing the resin. Table 1 shows the effects of varying the amount of catalyst employed. The speed of

Table 1. Chloromethylation with varying ZnCl₂ concentrations

ZnCl₂ (mmol/g resin)	ClCH₂-Substitution (mmol/g resin)
0·02	0·11
0·05	0·17
0·10	0·19
0·20	0·20
0·40	0·20

0·5 g resin, 4·0 ml ClCH₂OCH₃, 2·0 ml CH₂Cl₂, 2 h at 40°.

chloromethylation reached a limiting value at a concentration of 0·2 millimoles of ZnCl₂/gram of resin, and this catalyst concentration was therefore chosen for all succeeding experiments to maximize substitution and minimize the effects of minor pipetting errors when adding the zinc chloride solution.

As Table 2 shows, the extent of chloromethylation varied almost linearly with the concentration of chloromethyl, methyl ether, other factors being held constant, so that the chloromethylation reaction could be conveniently moderated by dilution with an inert solvent. Solvents that complex Friedel-Crafts catalysts such as tetrahydrofuran and dioxane

Table 2. Chloromethylation with varying $ClCH_2OCH_3$ concentrations

Chloromethylating solution (ml/g resin)	$ClCH_2$-Substitution (mmol/g resin)
4·0 $ClCH_2OCH_3$ 7·8 CH_2Cl_2	0·07
6·0 $ClCH_2OCH_3$ 5·8 CH_2Cl_2	0·13
8·0 $ClCH_2OCH_3$ 3·8 $CHCl_2$	0·18
10·0 $ClCH_2OCH_3$ 1·8 CH_2Cl_2	0·20
11·8 $ClCH_2OCH_3$ 0·0 CH_2Cl_2	0·22

0·5 g resin, 0·1 ml 1·0 M $ZnCl_2$ in THF, 2 h at 50°.

compete for the $ZnCl_2$ with chloromethyl methyl ether and are known to inhibit chloromethylation.[7] Methylene chloride, however, does not dissolve zinc chloride and appears to be unreactive as an alkylating agent under the conditions employed. There was no measurable alteration in the physical properties of chloromethyl resins produced by zinc chloride catalysis without or in the presence of methylene chloride, though descreased concentrations of chloromethyl methyl ether are thought to favor formation of cross-links within the beads.[6] Resin beads that had undergone chloromethylation swelled in organic solvents to the same extent as nonchloromethylated controls, indicating that little or no cross-linking occurred between polymer chains.[6]

The results of chloromethylations moderated by varying both the temperature and the chloromethyl methyl ether concentration are shown in Fig 1 and Table 3. The most versatile set of conditions was obtained at 40° (Fig 1, Curves 2–4) where variation of the chloromethyl methyl ether concentration produced resins containing from 0·05 to 0·5 millimoles of chlorine per gram over a period of 4 h. This range of substitution includes those levels most often employed in solid phase peptide syntheses, but where resins of unusually low chloromethyl content are desired, accurate substitution can be achieved at 30° (Fig 1, Curves 5 and 6). At this temperature, samples taken over a time span of 4 h gave
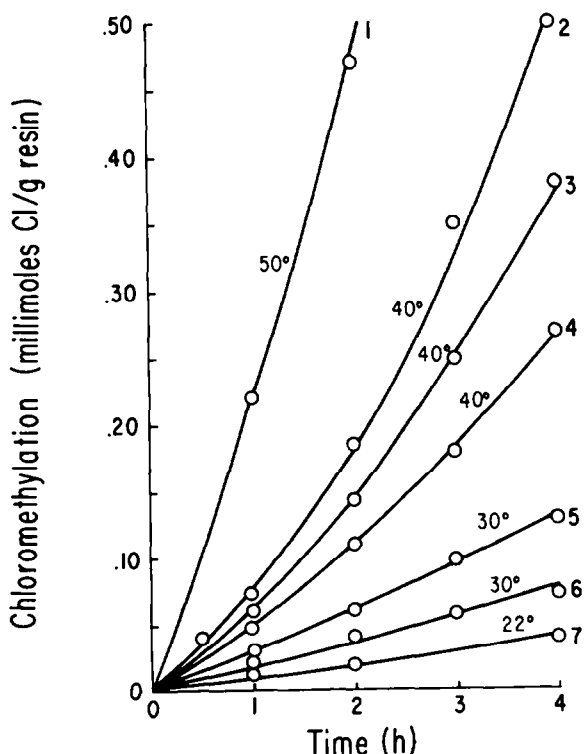

Fig 1.   Chloromethylation of 1% divinylbenzene-stryrene coplymer beads.

0·5 g samples containing 0·1 ml 1·0 M $ZnCl_2$ in THF, and (4·0 ml $ClCH_2OCH_3$ + 1·9 ml CH_2Cl_2) Curves 2, 5 and 7; (3·0 ml $ClCH_2OCH_3$ + 2·9 ml CH_2Cl_2) Curves 1 and 3; (2·0 ml $ClCH_2OCH_3$ + 3·9 ml CH_2Cl_2) Curves 4 and 6.

Table 3. Extended chloromethylation

Hours	Temp	ClCH₂-Substitution (mmol/g resin)
1	40°	0·07
2	40°	0·14
3	40°	0·23
4	40°	0·42
5	40°	0·50
6	40°	0·84
7·5	40°	1·27
24	40°	2·74
1	50°	0·22
2	50°	0·47
3	50°	1·01
4	50°	1·27

0·5 g resin, 0·1 ml 1·0 M ZnCl₂ in THF, 3·0 ml ClCH₂OCH₃, 2·9 ml CH₂Cl₂.

values ranging from 0·03–0·13 millimoles chlorine/g, allowing a desired resin in this substitution range to be prepared with great certainty. Increasing the temperature to 50° produced very high levels of substitution, and the curve (Fig 1, Curve 1) is too steep to permit accurate control for low levels of alkylation.

On a single sample size of resin, repeated experiments gave reproducible results. However, as procedures were scaled up from half-gram to 10 g or 25 g samples, the chloromethylations produced curves parallel to, but 20–30% lower than, those given in Fig 1. This lower reactivity for larger batches of resin probably results from factors such as less efficient stirring and temperature control. Therefore, to extrapolate values from Fig 1 to larger-scale chloromethylations, at least one point on the parallel curve generated under the specific physical conditions employed should be determined. The curves have a definite sigmoid shape, though attemts to link this phenomen to the generation of methanol during the reaction were equivocal.

Zinc chloride-catalyzed chloromethylation, when used in conjunction with the cesium salt method of esterification,[9] provides a route to the controlled formation of resins bearing a predetermined number of peptide chains, with no potentially reactive chloromethyl sites remaining. All of the chlorine in chloromethyl resins prepared as described above was displaceable by the cesium salts[9] of protected amino acids such as Boc-glycine and Boc-valine, and one such quantitatively substituted resin (containing 0·19 mmole of Boc-valine per gram) was shown to compare favorably with resins prepared by the less readily controlled SnCl₄-catalyzed chloromethylation. The effectiveness of the new preparation was demonstrated by the synthesis of

the test peptide Leu-Ala-Gly-Val, using Bpoc-amino acids[10] and standard DCC coupling procedures. The resulting tetrapeptide, before purification, contained less than 0·05% of any of the deletion sequences possible in the solid phase synthesis of this peptide.

EXPERIMENTAL

1. *Preparation of* 1·0 M ZnCl₂ *solution in THF.* ZnCl₂ (1·5 g) was placed in a 25 ml Erlenmeyer flask, ca 3 drops of conc HCl and 5 drops H₂O were added and the flask was swirled and heated until all solids dissolved. Heating was gradually increased to evaporate the water, leaving a crust of solids which was then melted* by stronger heating. When the ZnCl₂ became a clear mobile liquid with no further evolution of bubbles, the flask was placed in a dessicator and allowed to cool, and the resulting glass was dissolved in THF (10 ml) freshly distilled from LiAlH₄. The exact concentration of the resulting soln was determined by pipetting a sample into water containing several drops of HNO₃ and titrating with 0·1 M AgNO₃.

2. *Chloromethylation:* 0·5 g *samples.* To the resin (0·5 g Bio-Beads, SX1, 200–400 mesh, Bio-Rad Laboratories) in a 13 × 2 cm Teflon-stoppered test tube was added 3·9 ml CH₂Cl₂. The 1·0 M ZnCl₂/THF soln (0·1 ml) was pipetted onto the test tube wall and washed in with 2·0 ml ClCH₂OCH₃. The test tube was then agitated at 40° in a Buchler Rotary Evapo-Mix shaker bath. After 2 h, the tube was removed, cooled, the chloromethylating soln was withdrawn by inserting a filter stick. The resin was washed 2 × 6 ml THF-CH₂Cl₂ (1:1), 4 × 6 ml THF, and 3 × 10 ml CH₃OH. Measurement of the chlorine content of this sample gave 0·11 millimoles Cl/g.

For the parameters of temp, time and chloromethyl methyl ether concentrations used in other half-gram samples, see Fig 1 and Table 3.

3. *Determination of extent of chloromethylation.* Pyridine (4 ml) was added to the resin sample (0·5 g) in its test tube which was then stoppered and heated to 100° for 1·5 h. The tube was then cooled, DMF (6 ml) and conc HNO₃ (6 ml) were added, and the tube was swirled on an ice bath until it cooled to room temp. Contents of the tube were transferred to a small beaker using additional DMF (up to 10 ml) to transfer resin and liquids quantitatively. The chloride was then titrated potentiometrically with 0·1 M AgNO₃, using a silver billet and a K₂SO₄/PbSO₄ reference electrode on a Radiometer Type TTT1c pH meter.

4. *Chloromethylation:* 10 g *samples.* The resin (10 g Bio-Beads, SX1), ClCH₂OCH₃ (60 ml) and CH₂Cl₂ (58 ml) were stirred 5 min in a 200 ml round-bottomed flask, 1·0 M ZnCl₂ in THF (2·0 ml) was then added and the suspension was stirred at 40° for 3 h. The suspension was then poured onto a fritted glass filter funnel and the resin was washed with 4 × 100 ml THF, 3 × 100 ml THF/H₂O (3:1), 3 × 100 ml THF/3N HCl (3:1), 3 × 100 ml THF/H₂O (3:1), 5 × 100 ml THF, and 3 × 100 ml CH₃OH. The resin was dried overnight under high vacuum at room temperature and the sample was analyzed potentiometrically, giving Cl = 0·18 millimoles Cl/g resin.

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