Process Control in the Solid Phase Peptide Synthesis by Titration of Free Amino Groups

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The development of the solid phase synthesis by Merrifield [1,2] and the automation of the procedure [3–7] have greatly expanded the field of peptide synthesis. The solid phase procedure as a stepwise procedure does not allow purification of intermediate products; it is therefore of the utmost importance to obtain quantitative reactions in each step. Several authors have described methods for controlling the degree of reaction.[8–11] All of these methods except one,[10] however, necessitate that a sample be taken out for analysis, a procedure which will cause difficulties during automatic peptide synthesis.

The important reactions to control during the solid phase synthesis are the deblocking of the amino group of the *N*-terminal amino acid and the coupling with the next amino acid. As a direct non destructive measurement of the amount of the free amino groups is preferable to an indirect procedure we have tried to obtain this by titration of the entire batch with perchloric acid. The titration has been carried out with 0.1 N perchloric acid in glacial acetic acid/methylene chloride in the ratio 1/1 (v/v). So after deblocking and treatment with triethylamine the titration shows the amount of free amino groups available for coupling and after the coupling it shows whether any amino groups have not reacted. Typical titration curves for the two different titrations are shown in Fig. 1. The titration values during the synthesis and the amino acid analysis of the synthesized tetra peptide resins Ala-Leu-Phe-Gly and Phe-Gly-Leu-Ala are shown in Table 1.

The process control here described is performed by adding a few steps in the normal cycle, thus titration and treatment with triethylamine for neutralizing the perchloric acid followed by successive washings before coupling, and titration before treatment with HCl in acetic acid. The method allows repetition of deblocking and coupling if the titrations should disclose insufficient yield of these reactions. In the latter case, however, a treatment with triethylamine is necessary to liberate

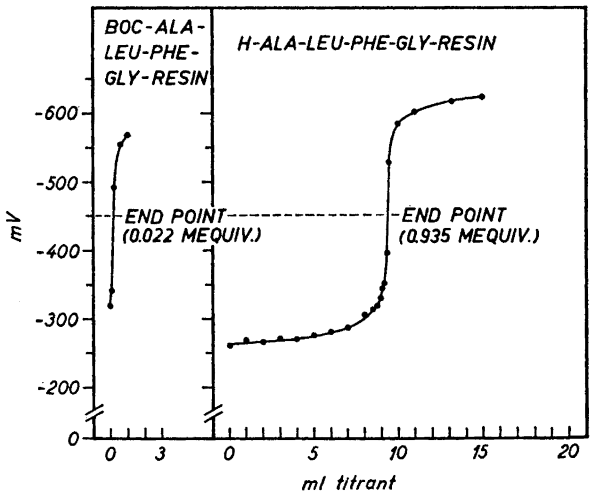

Fig. 1. Titration curves. To the left after coupling with BOC-alanine, and to the right after cleavage of the BOC-group.

Table 1. Amount of free amino groups after coupling and after deblocking determined by perchloric acid titrations and the amino acid analysis after hydrolysis of the resin bound tetra peptides. The peptide Ala-Leu-Phe-Gly-resin was titrated manually whereas Phe-Gly-Leu-Ala-resin was titrated automatically.

Peptide	Ala	Leu	Phe	Gly
Titration values after coupling, mequiv.	0.02	0.02	0.01	—
Titration values after deblocking, mequiv.	0.94	0.97	1.03	1.04
Amino acid analysis relative to Gly	0.88	0.89	0.95	1.00
Peptide	Phe	Gly	Leu	Ala
Titration values after coupling, mequiv.	0.11	0.05	0.03	—
Titration values after deblocking mequiv.	0.95	0.97	1.04	1.03
Amino acid analysis relative to Ala	0.89	0.93	0.95	1.00

the titrated amino groups before a repeated coupling.

The titrations were carried out manually as well as automatically, but manually initiated. Glass and calomel electrodes were used, however, in the second example a calomel electrode with a secondary salt bridge consisting of 10 % of saturated aqueous LiCl in glacial acetic acid was investigated. The possibility of developing a system allowing automatic titration initiated from the control unit of the punched tape controlled automatic peptide synthesizer,[4,5] is under investigation in order to allow automatic repetition of reactions with insufficient yield.

Experiments are being carried out to investigate whether destruction of labile amino acids occurs during the titrations. Titrations of methionine containing peptides have till now, however, not resulted in detectable amounts of methionine sulfone determined by amino acid analysis after hydrolysis of the resin bound peptide in 6 N hydrochloric acid/acetic acid 1/1 (v/v).

Experiments are also being carried out in order to investigate whether the titration may decrease the yield in the synthesis. By a 9 times repeated titration of 1.32 mequiv. of resin bound alanine, an average decrease of 0.023 mequiv. per titration was found. The standard error of estimate *i.e.* the accuracy of the titration was 0.008 mequiv. This result, however, may not be valid for the synthetic procedure as the experiment only comprises titration and removal of perchloric acid. The linear decrease in absolute values seems to show the presence of an amino group inactivating impurity present in any of the solvents or reagents used.

The approximate duration of the procedures involved in the process control are:

A) Titration after deblocking, 1 h.
B) Titration after coupling, 0.5 h.
C) Triethylamine treatment and washings with methylene chloride acetic acid, 0.5 h.

Consequently, one synthesis cycle is extended by about 2 h which, however, is justified by the importance of obtaining an analytical process control.

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