COUPLING CAPACITY OF SOLID PHASE SEQUENCING SUPPORTS

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1. Introduction

The first step of the solid phase peptide sequencing method [1] is the immobilisation of the peptide on an insoluble matrix. A variety of solid supports containing free amino groups have been employed. These include aminopolystyrene [1], aminopropylglass [2], triethylenetetraminepolystyrene [3], aminoethyl-aminopropylglass [4] and a modified polyacrylamide [5]. Claims have been made for the relative merits of these supports. Attachment yield is influenced by a variety of factors including the nature and size of the peptide and the type of solid matrix employed and a wide range of yields has been reported [6]. The coupling yield clearly depends upon the number of amino groups per unit weight of resin available under the conditions of coupling for which we have coined the term 'coupling capacity'. This factor itself is related to the degree to which matrix swells in the coupling solvent. Coupling capacity will vary from one batch of the same resin to another and in some cases will depend upon the age of the resin and the conditions under which the resin has been stored. We describe here a simple quantitative method for the measurement of coupling capacity. It can be applied to any of the amino-matrices hitherto employed for solid phase peptide sequencing and in the presence of any of the solvents normally used in the peptide coupling step. The method is based upon a procedure described earlier for monitoring free amino groups during solid phase peptide synthesis [7].

2. Principle of the method

The basis of the method is shown in fig.1. In the first step an immobilised Schiff's base (II) is formed between the resin and the aldehyde (I). Excess reagent is removed by washing and a soluble Schiff's base (IV) formed by reaction with benzylamine (III). The spectrophotometric determination of the concentration of (IV) is a measure of the coupling capacity of the resin, expressed in mmol/g resin.

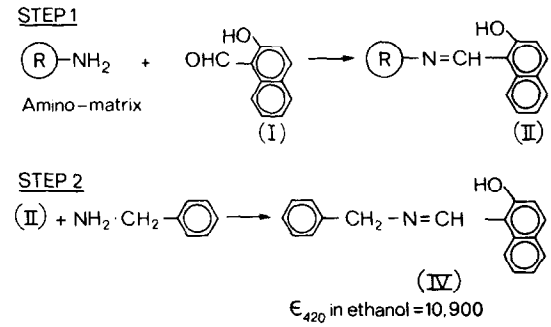

Fig.1. The principle of the method.

3. Materials and methods

3.1. *Reagents*

2-Hydroxy-1-naphthaldehyde was purchased from Fluka and benzylamine from BDH Chemicals. Dimethyl formamide was redistilled in vacuo.

3.2. *Resins*

Aminopolystyrene [1], aminopropylglass [2] and triethylenetetraminepolystyrene [3] were synthesised as described earlier. *t*-Butyloxycarbonyl-β-alanylhexamethylenediamine polydimethylacrylamide [5] was a generous gift from Drs E. Atherton and R. C. Sheppard.

Table 1
A comparison of the coupling capacity of solid phase sequencing matrices

Sequencing matrix	Coupling capacity (amino groups mmol/g)
Aminopolystyrene	2.48–2.57
Triethylenetetramine sequencing resin	0.25–0.33
β-Aminopropylglass	0.20–0.22
β-*N*-(2-aminoethyl)-3-amino-propylglass	0.11–0.13
β-Alanyl-hexamethylenediamine-polydimethylacrylamide	0.26–0.33

The amino groups were deprotected with methanolic HCl immediately before use.

3.3. *Measurement of coupling capacity*

The resin (1–5 mg) was mixed with 0.2 M 2-hydroxy-1-naphthaldehyde (I) in dimethylformamide (2 ml) and allowed to react 15 h at room temperature, then the resin was washed four times with dimethylformamide (3 ml) and then with ethanol until the ultraviolet absorption at 270 nm of the washes was zero (A_{270} of compound I 3600 in ethanol) then 0.4 M benzylamine in ethanol (2 ml) was added to the resin. The ultraviolet absorption of the supernatant was measured at 420 nm after a further 45 min (A_{420} of compound IV in ethanol 10 900). The contribution of the excess benzylamine to the absorption can be neglected.

4. Results

A comparison of the coupling capacities of five supports commonly used in solid phase sequencing (table 1) shows that aminopolystyrene has a coupling capacity measured in dimethylformamide ten times greater than that of the other supports. The figure obtained for the modified polyacrylamide resin which is maximally swollen in dimethylformamide agrees well with the β-alanine content 0.24–0.33 mmol/g, based upon the amount of the β-alanine employed in the synthesis of the resin [1,8]. When the measurement was performed in ethanol the availability of amino groups in aminopolystyrene was reduced by a factor of six.

5. Discussion

The method described above permits a direct comparison to be made between the number of available amino groups of different solid phase peptide sequencing supports under the conditions employed for coupling of the peptide to the support. It allows comparison of one batch of the same resin to be made with another and should allow the stability of these supports to be measured over a period of time. Thus, it should remove some uncertainties from the attachment procedure.

Other methods have been employed for estimating amino groups on solid phase sequencing supports. The method described here is clearly superior to a widely used version of the ninhydrin reaction which is a qualitative test for the presence of amino groups on supports [9]. Another method based on trinitrobenzylsulphonic acid [10] suffers from the disadvantage that the reagent must be extremely pure and is susceptible to the presence of trace metals. Further, measurements are made in borate buffer and not under coupling conditions. A qualitative procedure employing trinitrobenzylsulphonic acid has also been described [11]. The measurements described here suggest that aminopropylglass, triethylenetetraminepolystyrene and a modified polyacrylamide support all have approximately the same coupling capacities in dimethylformamide. Other criteria such as the size and nature of the peptide and the type of coupling reaction to be employed would have to be applied in deciding which support to use in a particular case.

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