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Role of Capping in Peptide Synthesis

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ABSTRACT

Protecting groups like Fmoc and coupling both steps are essential to monitoring the Fmoc SPPS (Solid Phase Peptide Synthesis) reaction completion. Reliable methods are used to detect the unreacted number of amino groups for monitoring these two essential reaction steps of coupling and cleavage. The ability to detect the complete coupling, incomplete coupling or failure of coupling we use many colour tests in the laboratory and based on this the Fmoc peptide chemistry allows the control of the completion of the Fmoc cleavage. The most important test used is the Kaiser test and highly recommended to monitor the coupling and cleavage steps. If the result of colour tests is positive after coupling, then the second coupling should be performed. Then again use the colour test to detect the level of coupling. If the result is still slightly positive, repeat coupling with the smaller modification of reagents such as used PyBOP instead of HOBT AND HOAT. These colour tests help in revealing the presence of unreacted amino-functional groups. Thus, we need to block these free N-terminal of amino-acids which help in avoiding the making of deletion of sequence.

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INTRODUCTION

Solid-phase peptide synthesis and classical solution synthesis are the methods used to synthesize the different types of laboratory peptides which have many biomedicine properties. The significant synthesizing steps of these two methods are coupling and deprotection. (Angeletti *et al.*, 1997). In coupling action, we join the N-terminal of one amino-acid to the carboxyl group of another amino acids through a covalent bond. In contrast, in deprotection step, we used the cleavage of protecting group (Fmoc) from N-terminal of amino acids which help to make the covalent bond or linkage with the activated carboxyl group of another amino acid. (Fields and Noble, 1990). To ensure the completion of these steps, we have many colour tests such as Kaiser's test, Isatin test, chloranil test, bromophenol blue test. We proceed with the synthesis based on the results of these tests. (Wellings and Atherton, 1997). SPPS and classical solution methods also include one of the most important steps, and it is capping. (Isidro-Llobet *et al.*, 2009).

In the capping step, we block the end of free or unreacted N-terminal of amino acids. It is necessary to prevent the reaction of this free-N-terminal with the side chain of another or itself residues of the peptide chain. Capping is following by the coupling reaction, preformed to block any unreacted amino groups permanently or to acetylene the N-terminal of unreacted amino acids. Capping is very useful during the synthesis of difficult or long peptide chain to minimize deletion products. The capping solution ratio 1:1:3 acetic anhydride, base dimethylformamide and pyridine and

DIPEA may be used the base. So, capping after coupling can improve the results during solid-phase peptide synthesis by blocking uncoupled ends of free amino groups. (Doig and Baldwin, 1995). For example, if we create the sequence CHEMISTRY and one specific spot on the resin, we are failed to couple the serine residue; We have H2N-TRY-CO- resin at one place. Suppose we don't use capping; we might generate the sequence used capping, we essentially generate Ac-NH-TRY-CO-resin which would not disturb anymore, and we would end up with sequence CHEMISTRY, HEMISTRY, CHMISTRY, CHEISTRY, CHEMSTRY, CHEMISTRY, CHEMISY, CHEMISTY and maybe even CHEMISTR. These are all of a similar length to our desired product and hard to purify away via HPLC. But if we used capping step, we can only get HEMISTRY, EMISTRY, MISTRY, ISTRY, STRY, TRY, RY, Y- most of these are significantly shorter and easier to purify away (Fields and Fields, 1994).

MATERIALS AND METHODS

Monitoring of Peptide Coupling and Capping

Several qualitative tests have been developed to check the coupling and deprotection of peptide synthesis. The well-known test is the Kaiser test. In this test, we confirm the completion of coupling of primary amines which shows an intense blue colour. In contrast, secondary amines such as terminal proline, produce a less intense red-brown colour after reaction with ninhydrin reagent. (Chan and White, 2000). The alternative tests recommended for N-terminal proline are chloranil test and isatin test—these tests outcomes a blue colour after the reaction of ninhydrin reagent with unprotected N-terminal proline. The protocols for testing should be followed very carefully. Fmoc protecting group loss from excess heating when reacted with pyridine (Contained in the testing reagents). The results are positive, and the colour is blue. There are some additional coupling testes, and these are bromophenol blue test and 2,4,6 trinitrobenzene sulfonic acid test. These tests are based on the acid-base reaction. All tests mentioned above are used for detection of amines such as proline or N-methyl amino acids as well as primary amines. The resin should wash before performing the analysis to remove basic and acidic impurities. (Warrass and Lippens, 2000).

If Coupling is Not Complete

If N-terminal amine was unreacted, then the monitoring tests indicate that the coupling should be done. The various colour shows that a significant amount of unreacted amines is present. The peptide is likely to be aggregated to changing coupling conditions such as switching to a different solvent (NMP, DMSO or DCM/DMF instead of DMF) of different coupling reagents (HOAT or PYBOP instead of HOBT) may also be beneficial (Hancock and Battersby, 1976; Wellings and Atherton, 1997). Unreacted amines are still present after the second coupling; $(CH_3CO)_2O$ should be used to cap. Blocking prevents the removed of peptide impurities by capping at unreacted sites. The desired peptide sequence has different HPLC retention characteristic than the capped impurities making it easier to isolate. Capping can also from a purge of the crude peptide. (Kaiser *et al.*, 1970).

Test for Monitoring Solid-phase reactions

The Kaiser test is very important to detect 1^0 amines. It is generally utilized in SPPS to detect the coupling reactions are either completed. Ninhydrin molecules react with the deprotected N-terminal of amino acids and produce an intense blue colour. This test is not reliable to determine the secondary amines. (Boas and Mirsharghi, 2014). The N-terminal amino acid such as proline, pipecolic acid or tetrahydroisoquinoline-3-carboxylic acid, we used tests such as chloranil and isatin. The Fmoc protecting group is not entirely stable in the conditions utilized in the Kaiser test, and false-positive tests can be produced. Alternative tests for monitoring solid-phase peptide coupling reactions have been reported, which including bromophenol blue test and 2,4,6 trinitrobenzene-sulphonic acid test. (Sarin *et al.*, 1981).

Kaiser Test (Ninhydrin Test)

Kaiser Test Solutions

Reagent A: Dissolve 16.5 metric capacity unit of 25ml of distilled water. Dilute 1.0 ml of this solution with 49 ml of pyridine (freshly distilled from ninhydrin) (Wellings and Atherton, 1997).

Reagent B: Dissolve 1.0 g of ninhydrin in 20 ml of n-butanol. Reagent C: Dissolve 40 g of phenol in 20 ml n-butanol. Kaiser check Procedure: Take 10-15 beads of an organic compound in a test tube and label its S. Take this S test tube and another empty test tube label as R (reference) and added two-three drops of chemical reagents that are mentioned above A, B, and C. Heat each the tubes at 110°C for five minutes. Compare the colour with reference.

A) Colourless or faint blue colour: complete coupling, proceed with synthesis.

B) Dark blue solution but beads are colourless: nearly complete coupling, extend coupling or unreacted cap chains

C) The solution is light blue, but the beads are dark blue: coupling incomplete, recouple

The solution is intensely blue, and each beads colour is also blue, which shows the unsuccessful coupling, check organic compound, reagents, then recouple.

Isatin test for Unprotected amino acids

Isatin Solution: Add two grams of isatin to 60ml of alcohol. Stir it at room temperature for two hours. Filter it to get rid of any unmelted isatin. Dissolve 2.5 g of Boc-Phe-OH within the filtrate. (Yan, 1998). Isatin Procedure: Place a tiny quantity of the sample (4-5 mg of resin-peptide) in a small test tube. Add two to three drops of the isatin and warmth at a 100 °C for five minutes. If the colour of the beads is blue, the coupling reaction is incomplete. (Wellings and Atherton, 1997)

Chloranil test for Secondary Amines

Chloranil Solutions Reagent A: Mix one milligram of an organic compound with forty-nine millilitre of N, N-dimethylformamide (DMF). Reagent B: Dissolve one gram of p-chloranil in 49 ml of DMF. Chloranil Procedure: Place one to five mg of rosin in an exceedingly small test tube. Add two drops of each chemical reagents A and B. Let the mixture stand at room temperature for five minutes. Observe the colour of the beads. If the colour of the beads is blue, the secondary alkane is present. (Boas and Mirsharghi, 2014)

Bromophenol Blue test

Bromophenol Blue Solution: Dissolve three mg of tetrabromo-phenol sulfonephthalein in one hundred millilitres of DMF.

Tetrabromo-phenol sulfonephthalein Procedure: Transfer 10-15 beads to a test tube. Fastidiously rinse the beads with DMF solution. (Milton and Milton, 1990). With a pipette, withdraw the rinse liquid without removing the beads. Add two-three drops of the Bromophenol Blue solution. Observe the colour of the beads. A) If the beads are blue to blue-green, the coupling reaction is not complete and a second coupling could be needed.B) If the colour of the beads is chromatic, then the coupling reaction is almost complete. If the beads are yellow with a trace of inexperienced, the coupling reaction is complete.

2,4,6-Trinitrobenzenesulphonic assessment

2,4,6-Trinitrobenzene-sulphonic assessment Solutions Reagent A: 1 Chronicles a pair of,4,6 trinitrobenzene sulphonic acid in DMF Reagent B: 100% DIPEA in DMF 2,4,6-Trinitrobenzene-sulphonic assessment Procedure: Take away a number of resin beads and wash them totally with DMF. Suspend the beads in reagent DMF. Add one drop of chemical reagent A and one drop of chemical reagent B. Enable the sample to stand at temperature for five minutes. Wash the beads with DMF and observe the colour. Red beads indicate that the coupling is not complete. (Hancock and Battersby, 1976).

RESULTS AND DISCUSSION

Standard Capping Procedure

Scheme 1: General Capping for all style of resins 1. Filter and wash the resin many times with DMF. 2. Suspend the resin in the DMF solution containing anhydride and a base. DIPEA is also substituted for the base. 3. Gently shake the above solution at room temperature for half an hour. 4. Filter and wash the resin with DMF. 5. Perform a Kaiser test. If the Kaiser test isn't negative, repeat the capping procedure. Scheme 2. Capping 2-chlorotrityl chloride resin 1. Wash the loaded resin with 3xCH_2Cl_2. 2. Make the capping solution. The capping solution in CH2Cl2: MeOH: DIPEA in the ratio of (17:2:1). 3. Dump the capping solution on the loaded resin and rock for one hour at room temperature. 4. After one hour, force the capping solution with an element and wash the resin with 2 x CH2Cl2 and 1x DMF.

Colour tests for capping

Suppose the colour tests are still positive when the planned coupling time is completed. The resin is filtered off, washed with DMF and IPA, and a second coupling is typically performed. If the test is slightly positive, then recoupling is generally performed with a lower quantity of reagents. Some significant changes are: • modification of solvent, use DMF/DCM 1:1 rather than DMF, • modification of coupling chemical reagent, use tatou rather than TBTU, • modification of additive, use TBTU+HOAT rather than TBTU+HOBT.

CONCLUSION

There are many colour tests such as Kaiser's test, isatin test, chloranil test, bromophenol blue test plays an important role to reveal the completion of capping takes place or not. If the result of tests is negative, it means incomplete coupling, and they need to be capped to stop the formation of the deletion peptide sequence. Capping can acylate the unreacted N-terminal of amino acids and stop the formation of deletion sequences of peptides. It also helps to improve the yield of synthesized peptides.

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