Characterization of a Side Reaction Using Stepwise Detection in Peptide Synthesis with Fmoc Chemistr

Yan Yang[1], William V. Sweeney[1], Susanna Thörnqvist[2], Klaus Schneider[2], Brian T. Chait[2] and James P. Tam[3]

[1] Department of Chemistry, Hunter College of CUNY, New York, NY 10021

[2] Laboratory for Mass Spectrometry and Gaseous Ion Chemistry, The Rockefeller University, New York, NY 10021

and

[3] Department of Microbiology and Immunology, Vanderbilt University, A5119 MCN, Nashville, TN 37232

I. Introduction

In solid phase peptide synthesis, it is important that the repetitive steps proceed rapidly, in high yield, and with minimal side reaction to prevent the accumulation of by-products (1). Solid phase peptide synthesis almost always employs either Boc or Fmoc chemistry. Boc chemistry requires acidic conditions for deblocking, and the potential side reactions have been extensively studied. However, in Fmoc chemistry (2) repetitive basic conditions are required for deblocking, and only a few of the base-catalyzed side reactions have been characterized. We present here a method used to demonstrate that a side reaction well known in Boc chemistry (3) but thought not to occur under the conditions of Fmoc, in fact occurs with both approaches: aspartimide formation.

In this method the progress of synthesis was monitored by stepwise micro-scale TFA cleavage in conjunction with reversed-phase HPLC and mass spectrometry (MS) for identification of products. The resins were sampled after each coupling step. Then the peptide fragments generated by TFA cleavages were examined by HPLC and MS. By comparing each peptide fragment, the side reaction due to aspartimide formation was detected and eventually defined. Peptide ladder mass spectrometric analysis was used for a mixture of the collected peptide fragments to provide further corroborative evidence for the aspartimide formation.

TECHNIQUES IN PROTEIN CHEMISTRY VI