Proceedings of the 36th European Peptide Symposium
Michal Lebl (Editor)
European Peptide Society, 2022 *https://doi.org/10.17952/36EPS.2022.269*

Chemical Synthesis of Palmitoylated Histone Protein

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Introduction

Histone proteins H2A, H2B, H3 and H4 form a histone core, which is composed of two copies of each of them. The core is wrapped around by DNA to form a nucleosome. The *N*-terminus of each histone protein in nucleosome receives many post-translational modifications, such as acetylation, methylation and phosphorylation. These modifications are dependent on the changes in the living environments, diseases and foods, which results in the epigenetic regulation of genes. Recently, an unusual modification of histone was found [1]: an *O*-palmitoylation of histone H4 of Ser^{47}. Due to the hydrophobicity of the palmitoyl (Pal) group, this modification will lead to the local increment of the hydrophobicity on highly basic histone, and then donate novel function(s) by possible change of nucleosome structure and/or its interactor(s). Therefore, the detailed analysis of this modification will lead to the discovery of the novel function of histone modification. Here, we report the total chemical synthesis of Ser(Pal)^{47}-histone H4.

Results and Discussion

The structure of Ser(Pal)^{47}-H4 is shown in Figure 1. In this synthesis, the thioester method, which uses the direct aminolysis of the amino-protected peptide thioester by the other segment [2], was used. As the middle segment having the Pal group is expected to be highly hydrophobic, we postulated that the thioester method, which uses an organic solvent for coupling, is advantageous to condense segments including hydrophobic ones. The segment coupling was designed to be performed at the *C*-terminus of Gly^{28,56}, in order to avoid the potential danger of racemization of the *C*-terminal amino acids during the condensation reaction. Three segments, which correspond to H4(1-28) **1**, H4(29-56) **2**, H4(57-102) **3**, were prepared by the solid-phase peptide synthesis (SPPS).

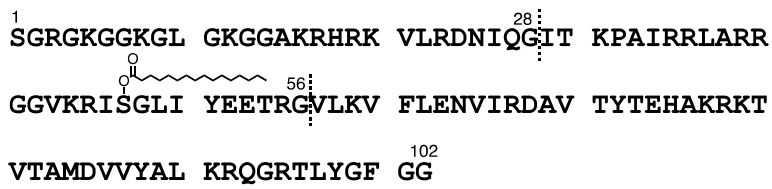

Fig. 1. Structure of Ser(Pal)^{47}-histone H4. The broken lines indicate the sites of segment coupling.

To efficiently assemble the entire sequence, three segments were ligated in one-pot using the reactivity difference between the aryl and alkyl thioesters, following the previously developed method [3]. Therefore, segment **1** was prepared as a reactive aryl thioester, whereas segment **2** as an alkyl thioester, which is only activated by the addition of silver ions. The synthesis of these thioesters was realized by the post-synthetic thioesterification method using the *N*-alkylcysteine as the *N*-to-*S* acyl shift device [4]. The side chain amino protection was achieved by the hydrophilic and acid/base stable 4-pyridylmethoxycarbonyl (*i*Noc) group [5]. This group can be easily removed by Zn in acetic acid treatment after the ligation.

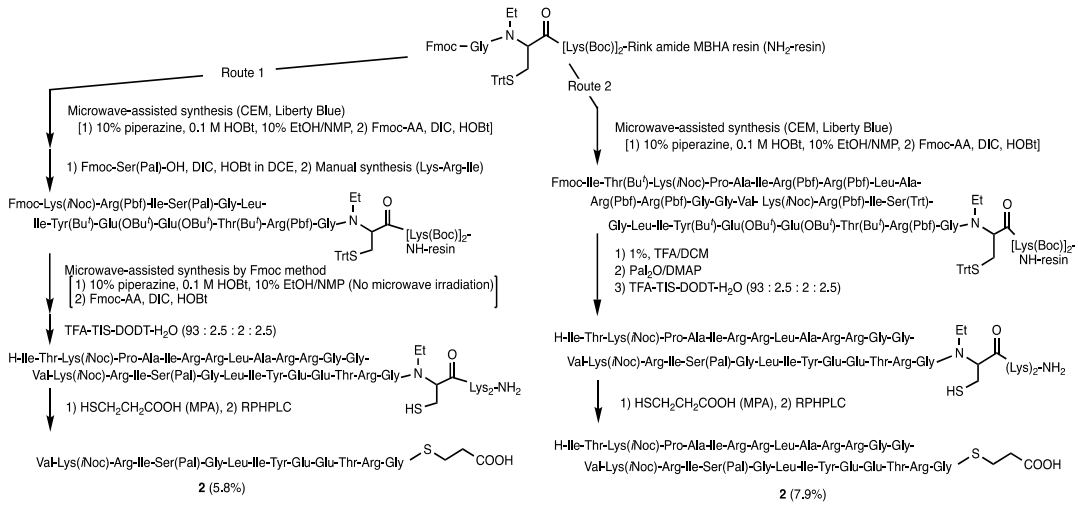

Fig. 2. Two synthetic routes of segment 2. Fmoc-Ser(Pal)-OH and Fmoc-Lys(iNoc)-OH were introduced manually.

For the introduction of the palmitoyl group to the side chain hydroxy group of Ser[47], two synthetic routes were examined: 1) introduction of the preformed Fmoc-Ser(Pal)-OH during SPPS of segment **2**, 2) introduction of Fmoc-Ser(Trt)-OH for Ser[47] during SPPS, followed by on-resin selective removal of Trt group and palmitoylation. In the first method, when standard microwave synthesis (CEM, Liberty Blue) was applied, the purity of the product was low, which might be due to the partial decomposition of the *O*-palmitoyl group during the piperidine treatment. Therefore, the microwave irradiation was switched off during the piperidine treatment and the desired product was successfully obtained after thioesterification reaction in 5.8% isolated yield (Fig. 2, Route 1). In Route 2, the standard microwave synthesis could be applied and segment **2** was obtained in 7.9% yield. Route 2 is better than Route 1 in point of the yield and the applicability of the standard microwave protocol.

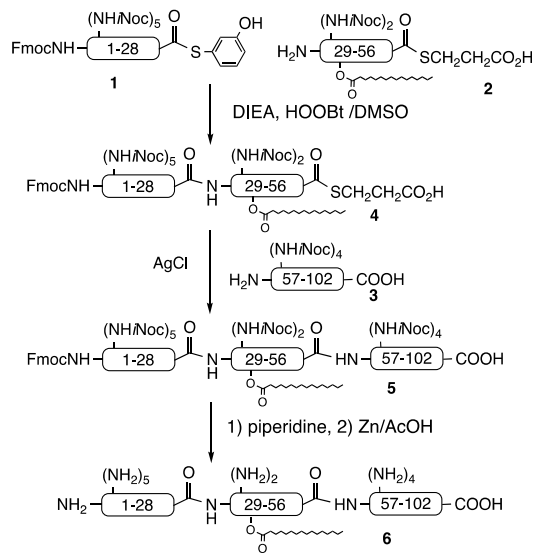

Fig. 3. Ligation to obtain Ser(Pal)[47]-histone H4.

Three segments were then condensed by the one-pot thioester method as shown in Figure 3. The peptide aryl thioester **1** and palmitoylated peptide alkyl thioester **2** were dissolved in DMSO containing HOOBt, and DIEA was then added to initiate the reaction. The reaction efficiently proceeded within 12 h to give the intermediate alkyl thioester **4**. Without purification, peptide **3** and silver ions were added to the mixture to perform the second ligation. This reaction also proceeded efficiently to give the polypeptide **5**. The Fmoc group removal by piperidine followed by the *i*Noc group removal by Zn in acetic acid treatment efficiently proceeded to give the desired product **6** in 20% overall yield [6]. From the SPPS of the segment to the ligation reaction, no major solubility problem was observed.

The introduction of the palmitoylated H4 into the nucleus as well as the nucleosome formation using palmitoylated H4 are being undertaken.

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