Solid Phase Peptide Synthesis of Full-Length Modified Histones

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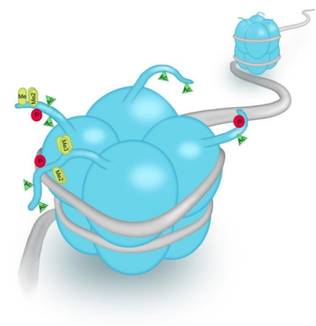
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Introduction

Histones are globular proteins that are subject to a wide variety of posttranslational modifications. These histone modifications, which occur predominantly on the unstructured N-terminal tails, form an epigenetic code central in the regulation of regular and disease-specific cellular processes, in particular DNA replication, repair and transcription.



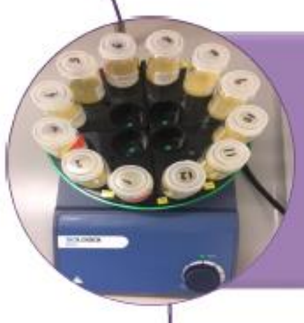
The growth of epigenetics research has necessitated improved access to purified histones with specific post translational modifications. We have utilized our extensive knowledge in solid phase peptide synthesis to develop procedures for the automated synthesis of full length histones in a linear fashion from C to N termini. Any post translational mark can be site specifically incorporated during the synthesis, without a requirement for the separate generation of cysteine or thioester containing fragments typical in native chemical ligation approaches.

Synthetic Process



Solid Phase Peptide Synthesis

- HMPB-Chemmatrix resin (PCAS BioMatrix Inc) loaded with the C-terminal amino acid (0.30-0.35 mmol/g)
- Automated Fmoc-SPPS performed on a PreludeX synthesiser (Gyros Protein Technologies)
- Residues double coupled using DIC/OXYMA chemistry. HATU/DIEA used for challenging couplings



Cleavage

- Assembled histones are cleaved with a TFA:TIS:EDT:H_2O cocktail for 4 h at RT
- NH_4I/DMS added to prevent Met oxidation
- Crude histones isolated by precipitation from diethyl ether



Purification

- First pass purification using RP-HPLC with a Luna C18 column
- Second pass purification using cation exchange with a Resource S column
- Fractions pooled based on SDS-PAGE/MS analysis
- On-column desalting and lyophilisation



Analysis

- SDS-PAGE
- ESI-MS
- Analytical RP-HPLC
- Quantification based on UV absorbance at λ = 280 nm

Histone H3.1

ARTKQTARKS TGGKAPRKQL ATKAARKSAP ATGGVKKPHR YRPGTVALRE IRRYQKSTEL LIRKLPFQRL VREIAQDFKT DLRFQSSAVM ALQEACEAYL VGLFEDTNLC AIHAKRVTIM PKDIQLARRI RGERA

Histone H4

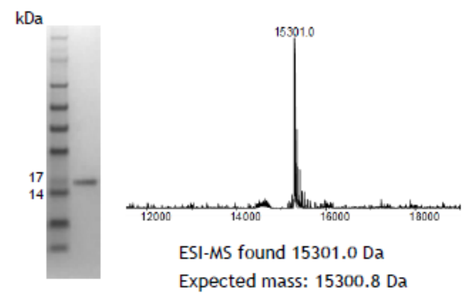
SGRGKGGKGL GKGGAKRHRK VLRDNIQGIT KPAIRRLARR GGVKRISGLI YEETRGVLKV FLENVIRDAV TYTEHAKRKT VTAMDVVYAL KRQGRTLYGF GG

- Highlighted residues indicate known sites of post-translational modification
- Lys = acetylation or methylation; Arg = methylation; Ser = phosphorylation

H3.1K79me2

ARTKQTARKS TGGKAPRKQL ATKAARKSAP ATGGVKKPHR YRPGTVALRE IRRYQKSTEL LIRKLPFQRL VREIAQDFK(me2)T DLRFQSSAVM ALQEACEAYL VGLFEDTNLC AIHAKRVTIM PKDIQLARRI RGERA

- 0.10-0.15 mmol scale with resin split at strategic points (Ser^{57} & Ala^{29})
- Ala^{21}-Thr^{22} pseudoproline
- Arginine residues in N-terminal tail coupled with HATU/DIEA
- Fmoc-$Lys^{79}(Me)_2$-OH.HCl site specifically incorporated
- 2 mg (net) of purified lyophilised histone isolated after two-step purification

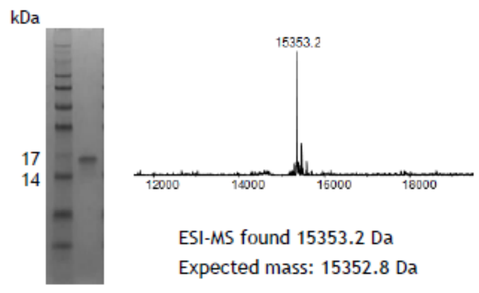


H3.1S28phos

ARTKQTARKS TGGKAPRKQL ATKAARKS(phos)AP ATGGVKKPHR YRPGTVALRE IRRYQKSTEL LIRKLPFQRL VREIAQDFKT DLRFQSSAVM ALQEACEAYL VGLFEDTNLC AIHAKRVTIM PKDIQLARRI RGERA

Synthesis protocol as for H3.1K79me2 with following amendments:

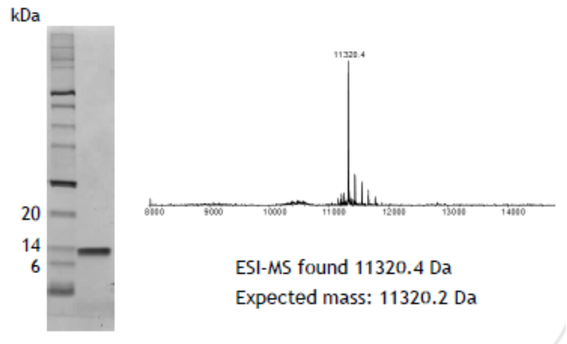
- Fmoc-Ser^{28}(PO(OBzl)OH)-OH site specifically incorporated using HATU/DIEA chemistry
- Fmoc deprotection using 5% DBU/DMF for residues 1-28 to prevent dehydroalanine formation at pSer
- 2 mg (net) of purified lyophilised histone isolated after two-step purification



Ac-H4K12ac

Ac-SGRGKGGKGL GK(ac)GGAKRHRK VLRDNIQGIT KPAIRRLARR GGVKRISGLI YEETRGVLKV FLENVIRDAV TYTEHAKRKT VTAMDVVYAL KRQGRTLYGF GG

- 0.10 mmol synthesis scale
- Glu^{53}-Thr^{54} pseudoproline
- Fmoc-Asp^{24}(OMpe)-OH used to limit aspartimide formation
- Fmoc-Lys^{12}(Ac)-OH site specifically
- Incorporated
- 3 mg (net) of purified lyophilised histone isolated after two-step purification



Conclusions

- The synthesis of full-length modified histones has been accomplished by linear Fmoc solid phase peptide synthesis, without the need to resort to a native chemical ligation fragment-based approach.
- Methylation, acetylation and phosphorylation modifications have been successfully incorporated into H3 and H4 histones at specific sites and any modification can be accommodated by the inclusion of the appropriate commercial building block.
- Histones are isolated as lyophilised solids following a two-step RP-HPLC/IEX purification procedure with purity and identify confirmed by SDS-PAGE/ESI-MS.