A Literature and Experiment Based Evaluation of Synthesis Techniques for Prothymosin-α Derived Peptides

General Protein Information

Prothymosin-α (ProTα) is a protein made up of 110 amino acids and located primarily in the nucleus of cells. The precise function of this protein is unclear, however evidence strongly suggests it is associated with several cellular activities and, in particular, cell proliferation. Prior research indicates that ProTα has the following attributes and qualities:

- Chromatin Remodeling Activity
- Immunomodulatory Activity
- Growth-Promoting Effect
- Zinc-Binding Property
- Enhancement of Estrogen Receptor Transcriptional Activity
- Protection of Cellular Apoptosis
- Oxidative Stress Defense Gene Regulation
- Enhancement of T Lymphocyte Function

Because of ProTα’s high level of expression in a wide variety of cell types and the fact that it is relatively conserved (especially in vertebrates), as can be seen in the alignments of Figure 1 to the right, it is expected to play an essential role in organisms. Recent research has also revealed that this protein possesses significant anti-HIV activity and efforts are underway to understand how it suppresses viral replication. The usual synthetic peptides derived from the central region of ProTα has also been shown to be effective at inhibiting HIV replication. These small ProTα derived peptides can also be used as a means to test the effects of variations in sequence identity in order to increase the desired functional response in cells (anti-HIV activity for example). There is further evidence that ProTα may be phosphorylated in vivo, and we hypothesize that this phosphorylation may be significant with regards to its anti-HIV activity. Since the phosphorylation state of a protein can be used to control its activity (i.e. functionally turn it on or off), we would also like to know if phosphorylation increases or decreases the protein’s anti-HIV properties. The protein itself can undergo many known post-translational modifications and these too are shown in Figure 1 to the right and include the following:

- Initiator Methionine Removal
- N-Acetylation
- Phosphorylation of Serine Residues
- Phosphorylation of Threonine Residues
- Caspase Cleavage at Three Binding Sites

In addition to its wide range of functions and post-translational modifications, ProTα has also been found to exist at multiple sites in the human genome. Interestingly, the duplication events did not produce repeated genes ordered sequentially, but rather created copies of the gene throughout the genome as can be seen in Figure 2 to the right. Similar patterns exist in other species which indicates a temporally spaced series of duplication events, especially in vertebrates. For example, Homo sapiens (humans) have 19 copies[3], Pan troglodytes (chimpanzee) 22[5], Mus musculus (mouse) 26[7], Bos taurus (cow) 13[6], Danio rerio (zebra fish) 21[6], and Gallus gallus (chicken) 12[7]. As can be seen by comparing the human karyogram in Figure 2 to the chimpanzee’s in Figure 3, a broad level of evolutionary conservation exists with regard to ProTα gene location. This is most apparent when viewing chromosomes 6, 7, 11, 13, 14, 17 and 20, which have gene placements in identical locations. The pattern of placement throughout the genome indicates that the gene may be part of a transposon and/or that the various duplicates may be expressed in response to varied signals which correspond to the different functions that ProTα possesses. Further research would be necessary to evaluate these hypotheses.

Evaluation of Synthesis Techniques

The goal of the experimental portion of this project was to develop useful techniques for synthesizing small ProTα derived peptides to be used to evaluate the protein’s anti-HIV activity. Specifically, three such peptides were created which addressed the issues associated with the creation of phosphorylated peptides in vitro as well as glutamimide formation as an unwanted side reaction during synthesis.

The first two peptides dealt with the difficulty of creating peptides with bulky phosphorylated residues. A ten residue amino acid chain corresponding to residues 81-90 in the human ProTα sequence (EAES/ATGKRA) was first created with a phosphoserine at a 0.1 mmol scale. All reagents were in 0.4 mmol excess except for the phosphoserine which was used in 0.3 mmol excess to reduce wasted reagent. The difficulty of the phosphoserine coupling was circumvented by allowing the coupling times of the residue and the subsequent residue to be increased to 2 hours (from the usual 1 hour). The resultant peptide was purified via HPLC and then analyzed by mass spectrometry. It was found to be highly successful with only a small percentage of the product containing deletions. What’s more, the vast majority of the deletions that did occur were in residues other than the phosphoserine meaning that the conditions and extended coupling time used for that residue were adequate.

The second phosphorylated peptide synthesized utilized the same sequence and conditions except that a phosphothreonine was used and not a phosphoserine. In the case of this peptide, both the conditions used and results obtained were the same meaning the extended coupling times again produced proper incorporation of the residue.

The final peptide synthesized was derived from residues 66-75 of human ProTα (EEEG/DEEED) and was created to model techniques-to evade cyclization and glutamimide formation viz-siz synthesis of larger peptide sequences. It utilized a benzyl ester protecting group (Obz) on the glutamate residues to prevent both synthetic as well as analytical problems attributable to negative charges. Additionally, this peptide used a nonconventional phosphorylated residue in the form of a benzyl protected glycine at position 71 as a way to prevent the previously mentioned glutamimide formation. As before, a 0.4 mmol surplus of reagents was used for all residues except for the Fmoc-(Dmb)Gly-OH where a 0.3 mmol excess was used. The products were then purified and analyzed and found to contain a much smaller percentage of cyclized peptides compared to the non-Dmb protected product.

Conclusion

The experimental goals of this project centered on developing high-fidelity synthesis techniques for both the highly charged phosphorylated as well as other glutamimide prone ProTα derived peptides. These were both elucidated with a high success rate attributable to altered coupling times and conditions during the synthesis process. The literature evaluation focused on mapping the points of post-translational modifications, comparing the human sequence to various phylogenetic lineages, assessing and describing the various functions attributed to the peptide as well as where these functions are expressed, and assessing the expression of the protein throughout the cell cycle. As research continues, elucidation of the evolution of the protein and the ramifications this has towards the overall functions will certainly be explored. Additional testing of various ProTα derived peptides for anti-HIV activity will also undoubtedly continue.

References