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A total solid-phase synthesis of DILP8

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Summary

We have developed a cysteine anchoring method for the synthesis of DILP8 and its analogues. The first is to synthesis of DILP8A SS13-18, C14-MeOBzl, C24-Acm and activate it as DILP8A S13-18, C14-SSPyr C24-Acm. A next step is to synthesize the DILP8BC16-Acm. The desired peptide, DILP8 with Cys(Acm) at A-24 and B-16, was then dissolved in 75% HOAc by addition of Iodine in MeOH and 4M HCl in dioxane. The reaction mixture was monitored by HPLC and the excess iodine was reduced with ascorbic acid. Purification of the peptide was achieved by HPLC. Pure synthetic DILP8 showed a single peak on analytical HPLC with corrected molecular ion. By using the above methods, enough peptide and highly homogenous pure DILP8 were generated.

Introduction

Dilp8 shares some sequence homology with the human INSL4 (Figure 1). Since Dilp8 has been found to coordinate Drosophila tissue growth with developmental timing[1], a similar mechanisms has been speculated in disease states in humans where alteration in growth or tissue inflammation can delay puberty. However, there is no report about the chemical synthesis of this DILP8 yet.

Methods, Results and Discussion

A solid-phase syntheses of DILP8 has been performed by using the modified method as described before[2].

The first in the method is to make DILP8, A chain, S-S 13-18, Cys(S-S-Pyr) 14 and Cys (Acm) 24 DHSSRSYNNIPYCC(SSPyr)LNQCEEEFFC(Acm) and the B chain, SFCSLERMKKFAMEAC(Acm)EHLFQADEGARRD were dissolved in 50% acetic acid. The reaction was carried out at 50ºC during 4 hours. After adding 6M urea, the reaction mixture was evaporated to 8ml and was loaded onto a gel filtration column (P-6) eluted by 1 M HOAc. The desired peptide, DILP8 with Cys(Acm) at A-24 and B-16, was then dissolved in 75% HOAc by addition of Iodine in MeOH and 4M HCl in dioxane.

Synthesis of A Chain: DILP8A SS13-18 , C14-MeOBzl , C24-Acm (Mw 3093): Fmoc-Cys(Acm)-O-TrtCl polymer (Nova) 700 mg (~ 1mmol/g) was used as solid support. Cleavage condition: 25ml TFA, 1.5ml m-Cresol, 1.5ml TIPS, 0.5ml Trifluoroacetic acid 1 hr, precipitated with ether. Solid material was filtered off, and dissolved in ~50% MeCN (~400ml), oxidized with Iodine. Crude peptide was purified by HPLC 25x250mm, 0-100% CH3CN in 0.1%TFA linear gradient, desired fraction combined and lyophilized.

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Activation of A Chain : DILP8A SS13-18 , C14-SSPyr , C24-Acm (Mw 3081): When DILP8A SS13-18 , C14-MeOBzl , C24-Acm peptide in 25ml TFA, aldrithiol-2 (~150mg) and 1ml thioanisole were added. The reaction mixture was kept 45min at room temperature and then diluted with ether. The solids was collected by filtration. The crude peptide was purified by HPLC 25x250mm, 0-100% CH3CN in 0.1%TFA linear gradient, desired fraction combined and lyophilized.

Synthesis of B Chain : DILP8B C16-Acm (Mw 3478):

Fmoc-Asp(tBu)-O-TrtCl polymer (Nova) 700 mg (~ 1mmol/g) was used as solid support. Cleavage conditions: 25ml TFA, 1.5ml m-Cresol, 1.5ml TIPS, 0.5ml Trifluoroacetic acid 1 hr. The reaction mixture was precipitated with ether. The crude peptide was purified by HPLC 25x250mm, 0-100% CH3CN in 0.1%TFA linear gradient, desired fraction combined and lyophilized.

Coupling of A and B Chain : DILP8AB SSA13-A18 , SSA14-B3 , CysA24-Acm , CysB16-Acm :

Activated A-chain (DILP8A SS13-18 , C14-SSPyr , C24-Acm) and B-chain (DILP8B C16-Acm) were dissolved in 50% AcOH (25ml) and kept at 50 °C. The completion of reaction was controlled by HPLC during 4 hours. After addition of 6ml 6M urea, the reaction volume has been reduced to 7-8ml by evaporation. The peptide was isolated by gel-filtration (P-6, 1M AcOH), and desired fractions were combined and lyophilized

Formation of DILP8 : SSA13-A18 , SSA14-B3 , SSA24-B16:

DILP8AB SSA13-A18 , SSA14-B3 , CA24-Acm , CB16-Acm (~25mg) was dissolved in 20 ml 75% AcOH (25ml) and followed by addition of 1ml 4M HCl and Iodine (MeOH) in dioxane into mixture. The reaction was monitored by HPLC. Excess of iodine was reduced with ascorbic acid. In the next step, the reaction mixture was diluted with water to ~100ml. The peptide was purified by HPLC 25x250mm, 0-100% CH3CN in 0.1%TFA 150min linear gradient, and desired fractions were combined and lyophilized.

The reaction mixture was monitored by HPLC and the excess iodine was reduced with ascorbic acid. Purification of the peptide was achieved by HPLC (Figure 2). Pure synthetic DILP8 showed a single peak on analytical LC/MS with corrected molecular ion. This synthesized DILP8 provides us the opportunity to explore the novel biological activity of DILP8.

References

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