Novabiochem®
innovations 3/05

Synthesis of highly amyloidogenic human IAPP using pseudoproline dipeptides

Human islet amyloid polypeptide (IAPP), hAmylin, is a mono-cyclic, C-terminally amidated, 37-residue peptide hormone (Figure 1) [1]. It is produced together with insulin by pancreatic islet β-cells [2] and is involved in the regulation of carbohydrate metabolism [3]. Amylin is also thought to play a role in the pathology of type-II diabetes mellitus, as it is the major constituent of plaque deposits found in the islets of type-II diabetic patients [4], and Amylin fibrils are toxic to insulin-producing cells [5].

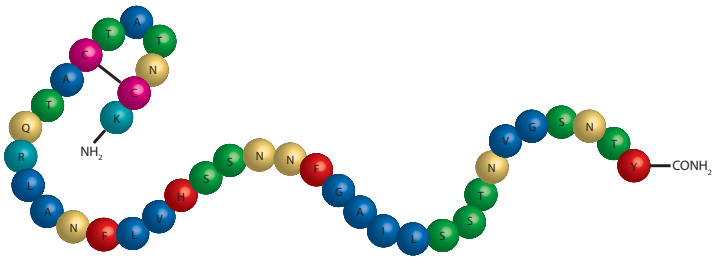

Fig. 1: Schematic representation of hAmylin$_{1-37}$.

The Fmoc SPPS of hAmylin$_{1-37}$ has been recently described by Abedini & Raleigh [6]. As expected, the inherent propensity of amylins to aggregate was found to make the synthesis of this peptide extremely problematic. Highly heterogeneous products were obtained even when double couplings of standard Fmoc-amino acid derivatives were employed at every cycle. However, the authors found that by simply making three pseudoproline dipeptide substitutions, hAmylin$_{1-37}$ could be obtained in excellent purity. This work is reproduced here by kind permission of Prof. Raleigh and the American Chemical Society.

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"In our hands, pseudoproline derivatives have proven very effective - particularly in the synthesis of peptides with difficult and long sequences. Using pseudoprolines, we saved time and money for repeat synthesis of failed sequences. We now routinely use pseudoproline derivatives for our peptide synthesis. I would highly recommend using them for peptide synthesis in the manufacturing industry as well. I am glad Novabiochem took the lead in manufacturing pseudoprolines in bulk".

Ved Srivastava, Amylin Pharmaceuticals Inc, San Diego, CA

"Pseudoproline dipeptides have greatly increased our success rate for synthesizing both long and difficult peptides. If we are able to integrate pseudoprolines into our syntheses, we can easily machine-synthesize peptides up to 80 amino acids in length. Routine use of pseudoprolines in our peptide syntheses has considerably increased the yield and purity, as well as decreased the number of failed syntheses. They are wonderful products!"

Yingwei He, Protein Chemistry Dept., Abgent, San Diego, CA.

"Biomol started incorporating pseudoproline derivatives into its everyday schedules for routine peptide synthesis some eight years ago. Over the intervening years, the use of these reagents on a routine basis has led to a dramatic reduction in the necessity for repeat synthesis. When coupled with an undoubted improvement in the yield and purity of crude peptides obtained, this has meant considerable financial savings in terms of both synthesis and purification costs. We are firmly of the opinion that the benefits of incorporation of pseudoproline analogs into peptide synthesis protocols is fully justifiable on both scientific and commercial grounds and is to be recommended on a routine basis."

Paul Sheppard, Biomol International Lp, Exeter, UK.

General guidelines for the use of pseudoproline dipeptides

- Optimal results are obtained if the pseudoproline dipeptides are spaced 5-6 residues apart throughout the sequence.
- The optimum separation between a pseudoproline dipeptide and a Pro residue is 5-6 amino acid residues.
- The minimum separation between a pseudoproline dipeptide and another pseudoproline dipeptide or Pro residue is 2 residues.
- Aim to insert a pseudoproline dipeptide before regions of hydrophobic residues.

Fig. 2: Primary sequence of 1) hAmylin$_{1-37}$, showing location of disulfide bond; 2) Ac-hAmylin$_{8-37}$. Residues highlighted with color indicate sites of pseudoproline dipeptide substitution.

Optimization of the synthesis of hAmylin

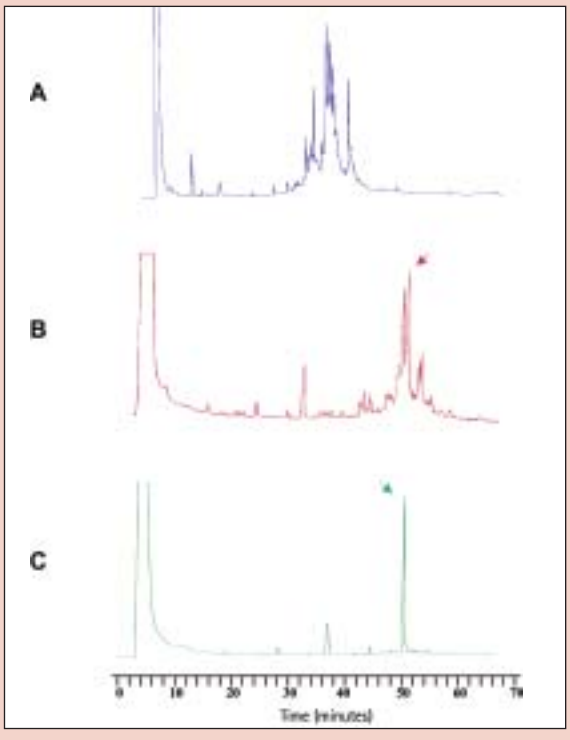
The synthesis of amylin was optimized using the highly aggregation prone 8-37 fragment as a model system. Three different strategies were investigated:

1) Double couple all β-branched amino acid residues and those that immediately follow;
2) Double couple all amino-acid residues;
3) Substitute dipeptides A^{8}T, S^{19}S, L^{27}S for the corresponding pseudoproline dipeptides. Double couple all β-branched residues, pseudoproline dipeptides and residues following either of these.

All the syntheses were carried out on an ABi 433A peptide synthesizer using HBTU activation. For strategy 3, the dipeptides indicated in Figure 2 were substituted with pseudoproline dipeptides, in accordance with the above guidelines.

Strategy 1 totally failed to produce any of the target peptide, instead producing a mixture of truncated peptides indicative of extensive aggregation during peptide assembly (Figure 3A). The material obtained from strategy 2 was also highly heterogeneous (Figure 3B). Some of the desired peptide was produced but this co-eluted with two deletion products and could not be easily isolated. The synthesis using pseudoproline dipeptides on the other hand afforded the desired peptides in a purity of greater than 90% (Figure 3C).

Fig. 3: HPLC profiles of crude Ac-hAmylin$_{8-37}$ obtained using A) double coupling of all β-branched residues and those residues which directly follow; B) double coupling of all residues; C) double coupling of all pseudoproline, β-branched residues and those which directly follow either of these [7].



H-Lys-Cys-Asn-Thr-Ala-Thr-Cys-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-Asn-Asn-Phe-Gly-Ala-Ile-Leu-Ser-Ser-Thr-Asn-Val-Gly-Ser-Asn-Thr-Tyr-NH_2 **1**

Ac-Ala-Thr-Gln-Arg-Leu-Ala-Asn-Phe-Leu-Val-His-Ser-Ser-Asn-Asn-Phe-Gly-Ala-Ile-Leu-Ser-Ser-Thr-Asn-Val-Gly-Ser-Asn-Thr-Tyr-NH_2 **2**

Synthesis of linear hAmylin$_{1-37}$

The synthesis of full-length linear hAmylin$_{1-37}$ was carried out using the optimized conditions of strategy 3. The Cys residues were introduced using Fmoc-Cys(Trt)-OH. UV monitoring of Fmoc deprotection reactions indicated efficient coupling and no aggregation. Treatment of the peptidyl resin with TFA/anisole/thioanisole/ethanedithiol (90:3.33:3.33:3.33) afforded crude material of excellent quality as shown in Figure 4. Characterization of this material by ES-MS confirmed the major component to be the desired peptide.

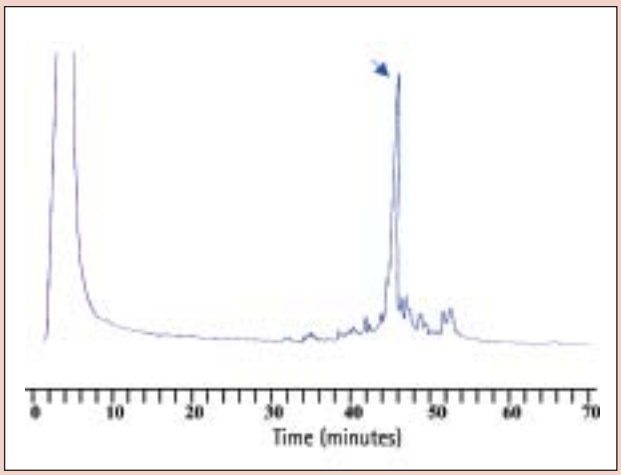


Fig. 4: HPLC profile of crude linear hAmylin1-37 obtained using the pseudoproline dipeptide strategy 3. Arrow indicates elution position of the desired product [7].

Air oxidation of linear hAmylin$_{1-37}$

The high purity of the crude linear hAmylin$_{1-37}$ enabled disulfide-bond formation to be carried out directly by air oxidation without the need for prior purification. Crude peptide was first dissolved in 6M GuHCl (5.7 mg/ml) and then diluted with 50 mM Tris buffer (pH 8.5). The reaction was monitored by HPLC as shown in Figure 5.

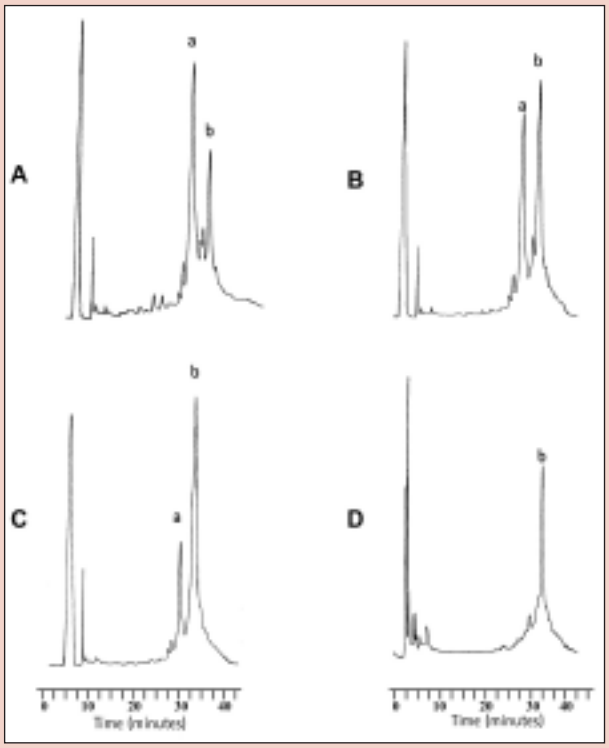


Fig. 5: HPLC profiles of crude hAmylin1-37 as a function of time in 43mM Tris-0.86 M GuHCl after (A) 2 min, (B) 1 h, (C) 3 h, and (D) 24 h. (a) Crude linear peptide; (b) cyclic peptide [7].

Ordering information

05-20-1000	Fmoc-Ala-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1005	Fmoc-Ala-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1010	Fmoc-Asn(Trt)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1008	Fmoc-Asn(Trt)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1011	Fmoc-Asp(OtBu)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1126	Fmoc-Asp(OtBu)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1115	Fmoc-Gln(Trt)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1125	Fmoc-Gln(Trt)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1002	Fmoc-Glu(OtBu)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1122	Fmoc-Glu(OtBu)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1127	Fmoc-Gly-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1124	Fmoc-Gly-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1119	Fmoc-Ile-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1118	Fmoc-Ile-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1004	Fmoc-Leu-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1009	Fmoc-Leu-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1003	Fmoc-Lys(Boc)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1116	Fmoc-Lys(Boc)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1121	Fmoc-Phe-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1128	Fmoc-Phe-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1012	Fmoc-Ser(tBu)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1117	Fmoc-Ser(tBu)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1130	Fmoc-Trp(Boc)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1013	Fmoc-Trp(Boc)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1014	Fmoc-Tyr(tBu)-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1007	Fmoc-Tyr(tBu)-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1001	Fmoc-Val-Ser($\Psi^{Me,Me}$pro)-OH	1 g 5 g
05-20-1006	Fmoc-Val-Thr($\Psi^{Me,Me}$pro)-OH	1 g 5 g

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