Overcoming Peptide Problems by Design

Introduction

Peptides are complex molecules and each sequence is unique with regard to its chemical and physical properties. While some peptides are difficult to synthesize, many peptides are relatively straightforward to synthesize but may still be difficult to purify after synthesis. A common problem with many peptides is insolubility in aqueous solution. For purification, these hydrophobic peptides often have to be dissolved in nonaqueous solvents, or in particular buffers. These solvents or buffers may be unsuitable for use in biological and other assay systems, and the customer may therefore be unable to use the peptide for the intended purpose.

Consequently, when we receive an order for a peptide or set of peptides, the sequences are analysed using predictive algorithms to determine if there is likely to be any problem with peptide assembly or solubility [1,2]. The sequences are also examined for sequence-specific problems by our peptide chemists, who make the final assessment. In instances where peptides are identified as being very difficult to synthesize or insoluble in aqueous solvents, rather than reject the order, there are a number of options we can suggest to improve the outcome of the synthesis. These all involve modifying the peptide sequence(s) or ending(s), which may or may not be feasible, given the intended application for the peptide(s). See the Appendix for a brief guide to the properties of the common amino acids. The following guide to the types of problems and how they can be reduced or overcome by designing a modified peptide is intended as a general guide only and the design of particular peptides should be arrived at by a process of consultation with Mimotopes staff.

Topics in this article:

Options for Peptides that are Difficult to Synthesize

1. Shorten sequence

As peptide length increases generally the purity of the crude product becomes lower. Most peptides of 15 residues or less can be obtained without major difficulty. However, as peptide length increases above 20 residues, yield of correct peptide becomes a major consideration. In many cases, decreasing the length below 20 can give more favourable results.

2. Decrease the number of hydrophobic residues

Peptides with a predominance of hydrophobic residues, especially in the region 7-12 amino acids from the C terminus, can often have assembly problems. This is thought to be due to beta-sheet formation between peptide chains during synthesis, which produces incomplete coupling. In these cases, replacing one or more residues with more polar residues, or adding a Gly or Pro residue to help break up regular peptide structure may help.

3. Minimise "difficult" residues

Peptides with multiple cysteine, methionine, arginine and tryptophan can often be difficult to synthesize. Serine can often be used as a non-oxidising replacement for cysteine, and norleucine can be used as a methionine replacement. Lysine can be used in place of arginine while tyrosine or phenylalanine or other hydrophobic residues such as leucine are sometimes adequate replacements for tryptophan.

Options to Improve Solubility

1. Change N or C terminus.

For acidic peptides (i.e. peptide has an overall negative charge at pH 7), we recommend a peptide format of

Acetyl-peptide-COOH

(acetyl group at amino terminus and free acid at carboxy terminus) to maximise the negative charge. For basic peptides (i.e. peptide has an overall positive charge at pH 7), we recommend a peptide format of

H-peptide-amide

(free N-terminal amino group, and amide C-terminus) to maximise the positive charge.

2. Shorten or lengthen sequence

Some sequences contain a large number of hydrophobic residues such as Trp, Phe, Val, Ile, Leu, Met, Tyr and Ala;

generally we see solubility problems in peptides where >50% of the residues are these hydrophobic amino acids. In order to increase the polarity of the peptide, it may be useful to lengthen the sequence, provided the added amino acids increase peptide polarity. Alternatively, the sequence may be shortened to eliminate hydrophobic residues and hence increase peptide polarity. The more polar the peptide, the more likely it is to be soluble in aqueous buffers.

3. Add solubilising residues

For some peptides, it is possible to arbitrarily add a set of polar residues to improve solubility. We recommend for acidic peptides to add Glu-Glu to the N or C terminus and for basic peptides to add Lys-Lys to the N or C terminus. If a charged group cannot be tolerated, we recommend the addition of Ser- Gly-Ser to the N or C terminus. Obviously there are cases where the N and C termini cannot be altered, and this approach would not be applicable.

4. Alter sequence by substituting one or more residues

Peptide solubility may be improved by changing some residues within the sequence. Often, a single replacement can dramatically improve solubility and that change may be relatively conservative; for example replacing Alanine with Glycine.

5. Alter sequence by selecting a different "frame" for a set of overlapping peptides

If a number of sequential or overlapping peptides of set length from a sequence are to be made, a change in the starting point of each peptide may make a difference by creating a better balance between hydrophobic and hydrophilic residues in individual peptides, or by separating "difficult" residues into different peptides (e.g. two cysteines into separate peptides instead of together in one peptide).

Appendix

General Notes on the Effects of Particular Amino Acid Residues on Peptide Properties

The twenty genetically-coded amino acids, and the other common amino acids occurring in proteins, can be classified in various ways according to the properties they share. The following is a list of the most common ones, their three-letter code and single-letter code, and different ways of classifying them.

1. The Genetically-coded Set:

Alanine Ala A	Glutamine Gln Q	Isoleucine Ile I
Methionine Met M	Phenylalanine Phe F	Valine Val V
Cysteine Cys C	Arginine Arg R	Lysine Lys K
Asparagine Asn N	Glycine Gly G	Tryptophan Trp W
Aspartic acid Asp D	Serine Ser S	Leucine Leu L
Proline Pro P	Histidine His H	Tyrosine Tyr Y
Glutamic acid Glu E	Threonine Thr T	

2. Other Common Amino Acids in Proteins:

Hydroxyproline (hydroxylated proline - two isomers) Cystine (oxidised cysteines) Pyroglutamic acid (cyclised N-terminal glutamic acid)

3. Other Amino Acids Commonly used in Peptide Design:

Alpha-amino butyric acid (cysteine replacement) Beta-amino alanine (straight chain isomer of alanine) Norleucine (linear sidechain isomer of leucine)

4. The amino acids can be grouped by hydrophilicity/hydrophobicity as:

HYDROPHILIC: D, E, H, K, N, Q, R, S, T, Hydroxyproline, pyroglutamic acid

HYDROPHOBIC: A, F, I, L, M, P, V, W, Y, alpha-amino butyric acid, beta-amino alanine, norleucine

INDETERMINATE: C, G

5. Notes on Other Groupings of Amino Acids

Amino acids subject to oxidation under relatively mild conditions: cysteine, methionine

Amino acids subject to deamidation or dehydration: asparagine, glutamine, C-terminal amides

Amino acids subject to degradation during peptide preparation: methionine, tryptophan

Amino acids which can carry a positive charge: lysine, arginine, N-terminal end of peptide, histidine

Amino acids which can carry a negative charge: aspartic acid, glutamic acid, C-terminal end of peptide, tyrosine

References

1. Milton, R.C. de L., Milton, S.C.F. and Adams, P.A. (1990) Prediction of difficult sequences in solid phase peptide synthesis. J. Am. Chem. Soc. 112; 6039-6046.

2. Fauchere, J.L. and Pliska, V. (1983) Hydrophobic parameters of amino acid side chains from the partitioning of N-acetyl-amino-acid amides. Eur. J. Med. Chem. 18; 369-375.

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