A GUIDE TO HANDLING AND STORING PEPTIDES

Scenario: You have received a custom synthesized peptide from Mimotopes. What do you do next?

Topics in this article:

Storage of Dry Peptide

When peptides are received, ensure they are kept in a cool, dark place. For best preservation, store them under refrigeration at 4°C or colder, away from bright light. Dry peptides are stable at room temperature for days to weeks, but for long term storage, -20°C is preferred.

Contamination with moisture will greatly decrease long term stability of solid peptides. Each time you use some of the peptide, remove the container from cold storage and allow it to equilibrate to room temperature or slightly warmer before opening it. This will reduce the uptake of moisture from the air onto the cold surface of the solid peptide or the inside of the container. After removing the required quantity, re-seal the container, preferably under an atmosphere of dry nitrogen. This can be achieved by blowing a gentle stream of dry nitrogen into a plastic bag housing the container, taking great care to avoid blowing the peptide powder right out of the container. After the air is displaced, quickly cap the container, and then return it to cold storage. This procedure will minimize the oxidation of air-sensitive peptides as discussed later.

Redissolving Peptides

Peptides are not very useful if they are insoluble in the aqueous buffers required for testing in bioassay systems. Mimotopes always advises the feasibility of peptides prior to accepting orders for synthesis, but in some cases the researcher has little choice and may have to deal with difficult peptides. To some degree, solubility difficulties can be predicted, and by careful design of the peptide these difficulties can be minimised. Even apparently minor changes to peptide polarity can sometimes significantly improve solubility.

Choice of Container

An ideal container for peptide manipulation would be scrupulously clean, chemically resistant, optically clear, strong, and available sterile in a size suitable for the amount of peptide you have. Glass containers are generally satisfactory for this purpose. If a plastic is used, the choice is generally between plastics which are crystal clear but not solvent resistant (e.g. polystyrene) and those which are chemically resistant but translucent (e.g. polypropylene).

We ship peptides in polypropylene tubes, mainly because of their resistance to breakage. Being solvent-resistant, they can be used as a vessel for redissolving peptides, but if best visibility is required the peptide should be transferred to a clear (preferably glass) container.

A Strategy for Dissolving Single Peptides

There is no "ideal" solvent that will solubilize all peptides, maintain their integrity and be compatible with biological assays. Thus, a series of increasingly powerful solvents may have to be tried until the peptide dissolves. For peptides with a solubility problem, the following may be of help.

Step 1. In general, try to dissolve the peptide in sterile distilled water or sterile dilute acetic acid (0.1%) solution to give a stock solution at a higher concentration than required for the assay. The peptide can be diluted later with the assay buffer, but if the assay buffer is used initially and the peptide fails to dissolve, recovery of the pure peptide free of nonvolatile salts and/or organic solvents can be difficult. Use of water or dilute acetic acid allows the peptide to be dried down without any nonvolatile residues, enabling another attempt at dissolving the peptide using more powerful solvents.

If the peptide persists as visible particles, sonication can be tried. Sonication breaks down lumps of solid peptide to smaller particles and vigorously stirs the solution,

improving the rate of dissolution, but does not alter the inherent solubility of the peptide in the particular medium being tried. If, after sonication, the "solution" has gelled, has a persistent haziness, or has a scum (not bubbles) floating on the surface, the peptide has probably not dissolved but is simply finely suspended.

Step 2. If the peptide was insoluble, look at the peptide sequence before proceeding. What proportion of the amino acid residues are hydrophobic (A,F,I,L,M,P,V,W,Y,C)? How many positive (K,R,H and N-terminus) and how many negative (D, E and C-terminus) charges are there? What is the overall (net) charge at neutral pH?

If the sequence has little or no net charge at any pH, move to step 3., below. If the sequence has a net charge at neutral pH, addition of dilute acetic acid as suggested above (for basic, positively charged peptides) or dilute aqueous ammonia or ammonium bicarbonate (for acidic, negatively charged peptides) with further sonication should improve solubility. The final concentration of acetic acid or ammonia/ ammonium bicarbonate you use will depend on what your assay system can tolerate. If the peptide still refuses to dissolve, you can at least remove the volatile buffer solution by lyophilisation and try alternative solvents on the same peptide sample.

Step 3. If the sequence has little or no net charge at any pH, and if the number of hydrophobic residues approaches 50% or more, the above procedures will probably be inadequate. Addition of high purity acetonitrile, dimethylsulfoxide (DMSO), dimethylformamide (DMF) or the use of chaotropic salts such as guanidine hydrochloride or urea will dissolve most peptides. Again, the choice from this list, and the concentration to use will depend on compatibility with your assay system.

If it is known that the peptide is slightly soluble in aqueous solution, it is better to dissolve it completely in a small amount of neat acetic acid, acetonitrile, DMSO or DMF and slowly dilute with water rather than progressively adding such solvents to a suspension of the peptide in water. This is because the rate of dissolution of the peptide into a water/solvent mixture may be slow, by comparison with its rate of dissolution in neat solvent, and therefore if the water/solvent mixture is used first, much more nonaqueous solvent than necessary may eventually be added to get the peptide to dissolve.

A Strategy for Dissolving Several Peptides

The kind of individual treatment described above starts to become impractical when handling larger numbers of peptides, say 10 or more. Although exceptions can be found to the success of any generalised procedure, a recommended strategy for redissolving greater numbers of peptides with varied properties is outlined below. Under this procedure, the final working stock solution of each peptide may consist of different levels of solvent, buffer etc. For users of Mimotopes' PepSetsTM peptide libraries, where uniformity may be more of an issue, please refer to the Peptide Technical Note accompanying the PepSet.

i. Add 0.1% acetic acid/water to give a target peptide concentration of 1-5mg/mL, and sonicate.

ii. To any insoluble peptides add pure acetic acid to bring the concentration of acetic acid to 10%(v/v), and sonicate.

iii. To any peptides still insoluble add acetonitrile to 20%(v/v), and sonicate.

iv. Lyophilise any remaining insoluble peptides to remove the water, acetic acid, and acetonitrile. To the solid, add neat DMF dropwise until the peptide dissolves. Dilute this solution slowly with water to give approximately 10%(v/v) DMF. If the peptide precipitates at any stage during this step, stop adding water and add a little more DMF until the peptide redissolves. Such peptides may be too insoluble in water to be used at concentrations equal to the others in the set.

v. Dilute each solubilized peptide with the solvent found to be effective for it, to bring the stock solutions to the same peptide concentration. This simplifies calculations and subsequent handling. Further dilutions, as needed for the bioassay, can be made in the assay buffer e.g. as a dilution series (titration). Dilution of a relatively insoluble peptide with buffer at this step may successfully avoid precipitation because it is now at a low concentration (below its solubility limit).

vi. Except after addition of DMF, all solutions can be easily lyophilised to return the peptide to a form suitable for long term storage, if required.

This is only one of a large number of possible procedures. The one chosen depends on the assay system, and the need for a particular buffer or peptide concentration. Contact Mimotopes for free technical advice if you wish to use a particular buffer not mentioned here.

Storage of peptide solutions

The shelf life of peptide solutions is limited, especially for peptides containing C, M, W, N and Q. To prolong the storage life of peptides in solution, use sterile buffers around pH5 to 6, and freeze aliquots for storage at -20°C or colder whenever possible. Avoid the use of frost-free freezers, which vary enormously in temperature during the frequent defrosting cycles. Repeated freeze-thaw cycles can also damage peptides.

Chemical Changes in Your Peptides

Peptides vary in stability, and a peptide as supplied may soon be degraded if care is not taken to ensure proper storage. In addition to the risk of degradation from proteolytic enzymes, other chemical changes can occur. The short section below is meant to help with situations that will commonly arise.

1. Oxidation

A characteristic of cysteine- and methionine-containing peptides is the tendency of these residues to oxidise. Susceptibility to oxidation is sequence-dependent and sometimes even minimal exposure to air of peptides containing these amino acids can lead to oxidation. If you wish to avoid oxidation, always work with degassed or deoxygenated solvents and solutions. If possible, maintain peptide solutions at acidic pH (<7). Rate of oxidation increases with pH, so even if the peptide is in the fully reduced form initially, some oxidation will occur if the peptide is maintained under neutral or basic conditions.

Normally, single peptides from Mimotopes are consigned in the fully reduced form. If handled properly, they will remain so, but if you need to carry out reduction of a peptide the procedures are as follows:

- Reduction of oxidised cysteine. Dissolve the peptide in 0.1M ammonium bicarbonate containing dithiothreitol (approximately 10-50 fold molar excess) and hold for 4h at room temperature. This procedure is a reasonable starting point but certain sequences may require more forcing conditions of temperature and time. After reduction, lyophilise the solution, or de-salt using size exclusion gel chromatography (e.g. Pharmacia Sephadex G-25) or reverse-phase chromatography (e.g. Millipore/Waters Sep-Pak). To prevent re-oxidation, follow the handling procedures mentioned above and store the peptide powder under nitrogen gas and in a freezer.
- Reduction of oxidised methionine by the method of Houghten and Li [1]. Dissolve the peptide in 10% acetic acid in water, and add N-methylmercaptoacetamide to 10%(v/v). Incubate at 37°C for 24h or more, then lyophilise or de-salt as for cysteine-containing peptides.

Please note that lyophilization of the above solutions will not remove the excess reducing agent, which may cause toxicity in subsequent usage.

2. Other reactions

Peptides have a variety of reactive side chains, and side reactions can occur under both acidic and basic conditions. For example, if glutamine occurs at the N-terminus of a peptide, cyclisation to form pyroglutamate is likely under acidic conditions (10% acetic acid). Mild basic solutions (0.1M ammonium bicarbonate) will lead to imide formation in asparagine-containing peptides. Peptides containing an Asp-Pro bond may cleave under acidic conditions such as 10% acetic acid in water. When dissolving single peptides, avoid conditions known to promote side reactions with the residues present.

Degradation due to microbial growth should not occur provided sterile distilled water or buffers are used, and solutions are frozen for storage. Sterilizing filtration of the peptide solution is another option, which also removes traces of insoluble or particulate materials. If filtration is chosen, ensure that the filters used are resistant to the solvent in which the peptide is dissolved, and have low peptide-binding properties.

Conclusion

In trying to convey a set of methods you can use to manage your peptide(s); we have necessarily concentrated on the problems. Please don't be discouraged by the mass of possible problems - if one approach doesn't work, try another! Chances are your experiments will go smoothly and you'll wonder what all the fuss was about!

References

[1] Houghten, R.A. and Li, C.H. (1979) Anal. Biochem. 98; 36-46.

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