Salting out is a purification method that utilizes the reduced solubility of certain molecules in a solution of very high ionic strength. Salting out is typically, but not limited to, the precipitation of large biomolecules such as proteins. In contrast to salting in, salting out occurs in aqueous solutions of high ionic strength that reduce the molecule's solubility causing certain proteins to precipitate. Ideally, the type of salt being used and the concentration of the salt can be varied to selectively precipitate a the molecule. In reality, salting out is an effective means for initial molecule purification, but lacks the ability for precise isolation of a specific protein.

The Mechanism Behind Salting Out

The conformation of large biomolecules in vivo is typically controlled by hydrophobic and hydrophilic interactions with the cellular environment. These interactions largely govern the molecule's final conformation by folding in such a way that most hydrophobic functional groups are shielded from the polar cellular environment. To achieve this conformation the molecule folds in such a way that all of the hydrophobic parts of a molecule are aggregated together and the hydrophilic groups are left to interact with the water. In the case of proteins it is the charged amino acids that allow selective salting out to occur. Charged and polar amino acids such as glutamate, lysine, and tyrosine require water molecules to surround them to remain dissolved. In an aqueous environment with a high ionic strength, the water molecules surround the charges of the ions and proteins. At a certain ionic strength, the water molecules are no longer able to support the charges of both the ions and the proteins. The result is the precipitation of the least soluble solute, such as proteins and large organic molecules.

The Hoffmeister Series

Salting out can be a powerful tool to separate classes of proteins that vary in size, charge, and surface area among other characteristics. One method of controlling the precipitation is the utilize the different effects of various salts and their respective concentrations. A salt's ability to induce selective precipitation is dependent on many interactions with the water and solutes. Research by Franz Hofmeister in the early 20th century organized various anions and cations by their ability to salt out.

The ordering of cations and anions is called the Hoffmeister Series (1). The cations are arranged as follows

\[
\text{NH}_4^+ > K^+ > Na^+ > Li^+ > Mg^{2+} > Ca^{2+}
\]

where ammonium has the highest ability to precipitate other proteinaceous solutes. Likewise, the order for anions is

\[
\text{F}^- \geq \text{SO}_4^{2-} > \text{H}_2\text{PO}_4^- > \text{H}_3\text{CCOO}^- > \text{Cl}^- > \text{NO}_3^- > \text{Br}^- > \text{ClO}_3^- > \text{I}^- > \text{ClO}_4^-
\]

Between cations and anions in solution the concentration of the anion typically has the greatest effect on protein precipitation.

One of the most commonly used salts is ammonium sulfate, which is typically used because the ions produced in an aqueous solution are very high on the Hofmeister series, and their interaction with the protein itself is relatively low. Other ions such as iodide are very good at precipitating proteins, but are not used due to their propensity to denature or modify the protein.
Salting out relies on changes in solubility based on ionic strength. The ionic strength of a solution, I, is defined as

$$I = \frac{1}{2} \sum_i m_i z_i^2$$  \[1\]

where
- \(m_i\) is the concentration of the ion and
- \(z_i\) is the charge of the ion.

Total ionic strength of multiple ions is the sum of the ionic strengths of all of the ions. Using the Debye-Hückel limiting law given by

$$\log \gamma_\pm = -\frac{1.824 \times 10^6}{(\epsilon T)^{3/2}} |z_+ z_-| \sqrt{I}$$  \[2\]

where
- \((I)\) is the ionic Strength
- \((z_+)\) is the catonic charge of the electrolyte for \(\gamma_\pm\)
- \((z_-)\) is the anionic charge of the electrolyte for \(\gamma_\pm\)
- \((\gamma)\) is the mean ionic activity coefficient
- \((T)\) is the temperature of the electrolyte solution
- \((\epsilon)\) is the relative dielectric constant for the solution

which can be adapted for for an aqueous solution at 298 K,

$$\log \gamma_\pm = -0.509 |z_+ z_-| \sqrt{I}$$  \[3\]

the solubility, \((S)\), of a particular aqueous solute can be defined as

$$\log \frac{S}{S_0} = -0.509 |z_+ z_-| \sqrt{I} - K' I$$  \[4\]

where
- \((K')\) is the is a constant dependent on the size of the solute and the ions present,
- \((S)\) is the solubility and
- \((S_0)\) is the solubility in pure solvent.

**Example \(\PageIndex{1}\)**

A common salt used in protein precipitation is ammonium sulfate, calculate the ionic strength of a 4g being added to 1230 ml 0.1M NaCl.

**Solution**

0.191M
Example (PageIndex{2})

Which of the following polypeptides would likely precipitate first at pH of 4: AAVKI or DDEKVK

Solution

Although ionic strength matters, one cannot forget that normal solubility rules still hold and the polypeptide AAVKI would likely precipitate first given its almost complete non-polar nature.

Example (PageIndex{3})

Since protein precipitation is dependent on which salt is used, which of the following salts would precipitate protein at the lowest concentration of the salt solution?

a. LiI
b. NaBr
c. K_2SO_4
d. LiF

Solution

c

Example (PageIndex{4})

Protein Y was just discovered by scientists at a national lab. The scientists managed to purify the protein with some precipitate in their flask. However, their yields were very low, to solve their problem they added 58 g of NaCl to 1 L of their protein solution in order to salt out the protein Y. After addition of the NaCl, they noticed that the solution no longer had some of the previously precipitated protein. What is the reason for the disappearance of the precipitate?

Solution

The ionic strength of the solution after addition of 58g of NaCl was about 1. In this case the ionic strength was in the region where salting in occurs. Hence the disappearance of the precipitate.

References
