Lab (6): Protein fractionation by ammonium sulphate and dialysis

Aim:

- Fractionation of animal crude extract by ammonium sulphate.
- Removing of salts ions using dialysis.

Introduction:

Purification should yield a sample of protein containing only one type of molecule, the protein in which the biochemist is interested. This protein sample may be only a fraction of 1% of the starting material, whether that starting material consists of cells in culture or a particular organ from a plant or animal. How is the biochemist able to isolate a particular protein from a complex mixture of proteins? (1)

Several thousand proteins have been purified in active form on the basis of such characteristics as solubility, size, charge, and specific binding affinity. Usually, protein mixtures are subjected to a series of separations, each based on a different property to yield a pure protein. At each step in the purification, the preparation is assayed and the protein concentration is determined. (1)

The purification of proteins by altering the solubility achieved by what called *salting out*. Most proteins are less soluble at high salt concentrations, an effect called salting out. The salt concentration at which a protein precipitates differs from one protein to another. Hence, salting out can be used to fractionate proteins (as proteins will precipitate at different points with increases in salt concentration). Salting out is also useful for concentrating dilute solutions of proteins, including active fractions obtained from other purification steps. (1) In Fact, salting out is an effective means for initial molecule purification, but lacks the ability for precise isolation of a specific protein. Ideally, the type of salt being used, and the concentration of the salt can be varied to selectively precipitate the molecule. (2) Ammonium sulphate is common substance used to precipitate proteins selectively since it is very soluble in water, its relative freedom from temperature effects and harmful effects of proteins like irreversible denaturation.(3)

Proteins can be separated from small molecules (salts) by *dialysis* through a semipermeable membrane, such as a cellulose membrane with pores. Molecules having dimensions significantly greater than the pore diameter are retained inside the dialysis bag, whereas smaller molecules and ions traverse the pores of such a membrane and emerge in the dialysate outside the bag. (1)

Principle:

The most effective region of salting out is at the isoelectric point of the protein, because all proteins exhibit minimum solubility in solutions of constant ionic strength at their isoelectric points. Different proteins will precipitate at different salt concentration, where protein size is inversely correlated with salt concentration. A typical protocol consists of adding ammonium sulphate to give specific percentage saturation, followed by a period of time for proteins to precipitate and a centrifugation step to collect the precipitate. Precipitation of proteins is conventionally carried out at 0°C to avoid possible denaturation of proteins. Following fractionation by ammonium sulphate, dialysis is applied to remove salts. During dialysis, the small, unwanted salts ions removed from proteins in a solution by selective and passive diffusion through a semi-permeable membrane. Sample molecules (proteins) that are larger than the membrane-pores are retained on the sample side of the membrane, but small molecules and buffer salts pass freely through the membrane, where the salt molecules move from the more concentrated solution (from inside the dialysis bag) to the less concentrated solution (e.g. buffer). The movement of the salt molecules will stop, when the solution reaches the equilibrium. At this point, the buffer is changed to drive the diffusion and salts movements. (1,4,5,6)

Materials:

Chemical

Prepared crude extract, Ammonium sulphate, 0.1 M Tris-HCl, pH 7.4, distal water.

Equipment and Glassware

Beakers, measuring cylinder, centrifuge tubes, dialysis bags, electronic balance, centrifuge, magnetic stirrer.

Protocol:

A. Salting out of protein A by 40% ammonium sulphate saturation:

1. Measure the volume of your crude extraction and calculate the weight in g of ammonium sulphate needed to saturate the solution 40% using **Table 1**.
2. Add the required salt to the solution slowly and gradually with small quantities and mix well continuously using magnetic stirrer while the sample is placed in ice.
3. After the addition is completed and the salt is completely dissolved, centrifuge at 3500 rpm for 10 min.
4. Discard the supernatant and dissolve the pellet in 10 ml of extraction buffer (0.1 M Tris-HCl, pH 7.4).

B. Removing of salts molecules by dialysis:

1. Pre-wet the membrane by soaking the dialysis bag in dialysis buffer.
2. Close the dialysis bag from one side and load the sample.
3. Close the other side and place the bag in a beaker filled with 0.1 M Tris-HCl, pH 7.4 buffer.
4. Dialyze for 1 to 2 h at room temperature.
5. Change the dialysis buffer and dialyze for another 1 to 2 h.
6. Change the dialysis buffer and dialyze overnight at 4°C.

Table 1. Quantities of ammonium sulphate required in (g) to reach given degrees of saturation in one litre of solution. (7)

%	10	15	20	25	30	33	35	40	45	50	55	60	65	70	75	80	85	90	95	100
0	56	84	114	144	176	196	209	243	277	313	351	390	430	472	516	561	610	662	713	767
10		28	57	86	118	137	150	183	216	251	288	326	365	406	449	494	540	592	640	694
15			28	57	88	107	120	153	185	220	256	294	333	373	415	459	506	556	605	657
20				29	59	78	91	123	155	189	225	262	300	340	382	424	471	520	569	619
25					30	49	61	93	125	158	193	230	267	307	348	390	436	485	533	583
30						19	30	62	94	127	162	198	235	273	314	356	401	449	496	546
33							12	43	74	107	142	177	214	252	292	333	378	426	472	522
35								31	63	94	129	164	200	238	278	319	364	411	457	506
40									31	63	97	132	168	205	245	285	328	375	420	469
45										32	65	99	134	171	210	250	293	339	383	431
50											33	66	101	137	176	214	256	302	345	392
55												33	67	103	141	179	220	264	307	353
60													34	69	105	143	183	227	269	314
65														34	70	107	147	190	232	275
70															35	72	110	153	194	237
75																36	74	115	155	198
80																	38	77	117	157
85																		39	77	118
90																			38	77
95																				39

Supporting material:

- A video shows how to dialysis a sample by using dialysis tube: https://f1.media.brightcove.com/4/3663210762001/3663210762001_5214982793001_5214973220001.mp4?pubId=3663210762001&videoId=5214973220001
- How to make a dialysis bag: https://www.youtube.com/watch?v=mWN-eE6fmpM

References:

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