Assay of amino acids

Introduction：

Twenty common amino acids are found in animal, plant and microbial protein. They are glycine, alanine, valine, leucine, isoleucine, serine, threonine, cysteine, methionine, aspartic acid, asparagine, glutamic acid, glutamine, arginine, lysine, histidine, phenylalanine, tyrosine, tryptophan and proline.

Special chemical properties of certain amino acids and detection of these amino acids in proteins are as followed：

A. Ninhydrin reaction:

This reaction is given by primary amines whose α-carbon posses a hydrogen atom. Thus, all proteins and protein derivatives give this test, as well as other non-protein compounds containing the $–NH_2$ group. So it is also given by primary amines and ammonia but without the liberation of CO_2.

Ninhydrin (triketohydrindence hydrate), a powerful oxidizing agent, reacts with all α-amino acids between pH 4 and 8 to give a purple colored compound. The imino acids, proline and hydroxyproline, also react with ninhydrin, but a yellow color is obtained. Not all amino acids give exactly the same intensity of color.

B. Lead acetate test:

Sulfur is present in proteins as cysteine, cystine, methionine. The

thiomethyl group of methionine is much more stable toward alkaline lead acetate than the sulfhydryl group of cysteine or the disulfide group of cystine and requires fusion with an oxidizing mixture to give a sulfur test.

C. The Xanthoproteic test:

This test is specific for benzene ring which can be nitrated with conc. HNO_3 to form yellow derivatives of nitrobenzene. After the addition of conc. NH_4OH, yellow color is turned to orange.

The amino acids in protein or protein derivatives containing this group are tyrosine and tryptophan. It is more difficult to nitrate phenylalanine which requires H_2SO_4 as a catalyst.

Reagents:

1. 1% solutions of amino acids and proteins：

   cysteine、glycine、 tryptophan 、proline、egg albumin
2. Ninhydrin reagent
3. 10% NaOH
4. 10% lead acetate
5. conc. HNO_3
6. conc. NH_4OH

Procedure：

A. Ninhydrin reaction: (Perform in the hood!)

Add 1ml of the sample solutions into separate test tubes, next add 5 drops of ninhydrin reagent into each tube, mix, and heat in boiling water bath for ~5 minutes. Investigate the color change and record. Do these proteins contain primary amines?

B. Lead acetate test: (Perform in the hood!)

Add 1ml of the sample solutions into separate test tubes, next add 2ml of 10% NaOH and 5 drops of 10% lead acetate solution into each test tube, mix, and heat in boiling water bath for 10 minutes. (The color of the solution is seen to darken.) Record the results in the table. Do these proteins contain cysteine or cystine sulfur?

C. The Xanthoproteic Test: (Perform in the hood!)

Add 1ml of the sample solutions into separate test tubes, next add 1ml of conc.HNO_3 into each tube, mix, and heat in boiling water bath for ~3 minutes. (The color of the solutions becomes yellow.) After cooling, add slowly excess amount of conc.NH_4OH till orange color appeared. Record the results in the table. Do these proteins contain tyrosine or tryptophan?

Results：

Record your results: (請以“＋”或“－”記錄及顏色)

Assay / AAs or protein		A	B	C	
		Ninhydrin Test	Lead acetate Test	Xanthoprpteic Test	
				Add HNO_3	Add NH_4OH
1	Cysteine(胱胺酸) Cys	(+) 紫			
2	Glycine(甘胺酸) Gly				
3	Tryptophan(色胺酸) Trp				
4	Proline(脯胺酸) Pro				
5	H_2O				
6	Egg albumin				

Discussions：