Structural Analysis of a Dipeptide

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I. INTRODUCTION

Dipeptides are two amino acids joined together by a peptide bond. There are 20 universal amino acids that differ from each other in their side groups. They also all have an amino end and a carboxyl end. This information is the foundation to understanding how to identify an unknown dipeptide and what its individual components are. First, the dipeptide's peptide bond was hydrolyzed to separate the amino acids. The separated amino acids were developed on a paper chromatography along with standard amino acid samples to determine the identity of the unknown amino acids. Then, the N-terminus of the dipeptide was determined through dansyl chloride treatment and thin layer chromatography. Overall, R_f values were calculated to make a conclusion regarding the identity of the unknown sample.

II. METHODS

Peptide Hydrolysis

1. 1 mg of unknown peptide B was placed into a small vial and dissolved in 0.05 mL of 6 N HCl.
2. The vial was left at room temperature for 1 week.
3. The hydrosylate was transferred to a watch glass and evaporated under a heat lamp.
4. 0.1 mL of distilled H_2O was added and evaporated for two times.

Paper Chromatography

5. A 20x20 cm Whatman 3mm paper was marked the paper with pencil, identifying the unknown peptide and standard amino acids.
6. A total of three 5 uL drops was applied onto the corresponding spot of the paper for each sample with a capillary tube.
7. Solution I (60:40 of acetonitrile: 0.1 M ammonium acetate) was added to fill 1 cm of a chromatography tank.
8. 60 minutes passed until the mobile phase reached 1 cm from the top and the position was marked with a pencil.
9. The chromatography paper was dried in a hood and sprayed with ninhydrin solution. The paper was then placed in a 110 degree Celsius oven for 2 – 10 minutes until colored spots appeared and were circled with a pencil.

Dansyl Chloride Treatment

10. 1 mg of unknown peptide B was placed into an eppendorf tube and dissolved in 0.5 mL of 0.2 M sodium bicarbonate.
11. 0.2 mL of dansyl chloride in acetone was added to the tube and mixed. Then it was covered with Parafilm and incubated for 2 hours at room temperature.
12. The solution was mixed with N_2 gas and in a beaker of warm H_2O.
13. Once dried, the contents were dissolved in Solution II (0.5 mL acetone and 6 N HCl), mixed and evaporated in N_2 gas.
14. The vial was left at room temperature for 1 week.
15. The hydrolysate was decanted onto a watch glass and evaporated under a heat lamp. The residue was dissolved in 50% aqueous pyridine (100 uL).

Thin Layer Chromatography

16. A silica TLC plate was marked with the positions of the unknown and dansylated standards.
17. 3 uL of each standard and 5 uL of unknown were applied to the marked positions.
18. The plate was developed in Solution III (75:5:1 of chloroform: methanol: acetic acid) until the solvent front reached 1 cm from the top and marked with pencil. The solvent was spread throughout the tank using a filter paper.
19. The plate was viewed under UV light and the spots were circled with a pencil.
20. The measurements were made and the data was compiled and R_f values were calculated to identify the unknown dipeptide and which amino acid was at the N-terminus.

III. RESULTS

Paper Chromatography			
Sample	**Distance traveled (cm)**	**R_f**	**Color**
Solvent Front	15.5	–	–
Unknown B	10.4, 5.7	0.67, 0.37	Light Pink, Yellow
Gly (G)	6.1	0.39	Dark Purple
Tyr (Y)	8.1	0.52	Pink
Phe (F)	10.2	0.65	Dark Blue
Leu (L)	9.3	0.60	Purple

Table I. The paper chromatography measurements for distance traveled, R_f values and colors of ninhydrin spot for the unknown and the standards.

Thin Layer Chromatography			
Sample	**Distance traveled (cm)**	**R_f**	**Color**
Solvent Front	13.3	–	–
Unknown B	6.3	0.47	Green (UV)
Gly (G)	6.3	0.47	Green (UV)
Tyr (Y)	8.2	0.62	Green (UV)
Phe (F)	8.5	0.64	Green (UV)
Leu (L)	9.4	0.71	Green (UV)

Table II. The thin layer chromatography measurements for distance traveled, R_f values and colors of fluorescent spot for the unknown and the standards.

Calculations:

R_f = distance traveled by sample / distance traveled by solvent front

R_f = 6.3 cm / 13.3 cm = 0.47

IV. DISCUSSION

According to the data, unknown sample B is Gly-Phe with glycine at the N-terminus. The identity of the two amino acids that make up the dipeptide was

determined via paper chromatography and the amino acid at the N-terminus was determined via thin layer chromatography.

In the paper chromatography experiment, the R_f values were calculated by dividing the distance each sample migrated up the stationary phase by the distance of the solvent front. There were two R_f values for the unknown because the dipeptide was hydrolyzed into its individual amino acids. From the data, the unknown R_f values match with the R_f values of glycine and phenylalanine, which can be explained by how the unknown amino acids migrated via capillary action the same distance up the stationary phase as the standards glycine and phenylalanine. This also indicates that the unknown amino acids, glycine and phenylalanine have similar polarities with the mobile phase. However, the colors of the ninhydrin spots for the unknown and the standards were not the same because the concentration of the unknown sample may not have been strong enough for ninhydrin to react with.

Thin layer chromatography identifies the amino acid at the N-terminus with the use of dansyl chloride. The reagent dansyl chloride works by forming a bond between the dansyl group and the N-terminal amino acid, which resists acid hydrolysis whereas the rest of the peptide does not. Therefore, there is incomplete hydrolysis as opposed to the complete hydrolysis that occurred in the paper chromatography experiment. By matching R_f values between the unknown and the standard samples, glycine is the amino acid at the N-terminus because the R_f values are equivalent at 0.47.

The techniques used in this experiment are essential methods that are commonly used in laboratories. Specifically, dansyl chloride treatment is extremely sensitive at identifying amino acids and is used in peptide sequence determination. Nevertheless, the accuracy of experiments involving proteins—one of the building blocks of life—can only improve through the mastery and evolution of these techniques with trial and error. Only then will science as a discipline continue to progress and succeed.