J. SOC. COS. CHEM. 15, 459–463 (1964)

# TWO-DIMENSIONAL DESCENDING PAPER CHROMATOGRAPHY AS A METHOD FOR THE SEPARATION OF THE AMINO ACIDS IN HAIR

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ABSTRACT

**Results of paper chromatographic analyses of the amino acid composition of normal, of reduced, and of reduced and oxidized human hair are presented. These preliminary data suggest that reduction of hair with alkaline thioglycolate not only splits disulfide linkages of cystine but causes chemical changes in other amino acids.**

Human hair can be subjected to many chemical treatments in order to produce various physical effects. It appears likely that the changes taking place at the various bonds which structurally support the keratin molecule can be elucidated through amino acid analyses. A particularly promising analytical tool is two-dimensional paper chromatography (1) which can be applied readily to the qualitative microanalysis of protein hydrolysates or other amino acid mixtures. In the studies reported here, the protein hydrolysate was first separated into fractions comprising acidic, basic and neutral amino acids with the aid of ion exchange resins. These three fractions were then individually subjected to paper chromatography.

## MATERIALS AND EQUIPMENT

Whatman No. 1 filter paper was used (standard sheet, 18 × 22.5 in.). The advancing front of liquid is yellowish-brown, but this contaminant of the paper moves so rapidly that it does not usually interfere. A commercially available trough for two-dimensional paper chromatography was employed. The chamber, a glass-sided lead box (about 75 × 75 × 12.5 cm.) was made airtight with a lead cover.

The first solvent used was a phenol mixture which is prepared as follows: To a mixture of 100 ml. of water and 400 ml. of liquid phenol, made homogeneous by warming, is added 20 mg. of 8-quinolinol. During chroma-

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tography with this solvent, a beaker containing 30 ml. of 0.3% ammonia is placed into the chamber.

The second solvent is prepared as follows: To 55 parts of 2,6-lutidine are added 20 parts of isopropanol and 25 parts of water. To each 500 ml. of this mixture is added 3.3 ml. of diethylamine. During chromatography with this solvent a beaker containing 100 mg. of sodium cyanide in 4–6 ml. of water is placed into the chamber.

## Experimental

Various hair swatches were exposed to the following treatments for comparative study:

A—untreated hair.
B—hair subjected to three minutes of total immersion in a reducing solution of pH = 9.3 (alkali = 0.67 *N*; thioglycolate = 6.62%).
C—hair subjected to treatment B followed by a four-minute total immersion in 1.5% $H_2O_2$ at pH = 4.8.
D—hair subjected to 8 minutes of total immersion in solution of B followed by a four-minute total immersion in 1.5% $H_2O_2$ at pH = 4.8.

The hair swatches in all cases above were given final water rinses in order to remove residual matter and then were dried with a hair dryer.

Two hundred mg. hair charges were placed in 5 ml. of 1:1 HCl in Pyrex test tubes. The tubes were sealed, and the hair was hydrolyzed in a paraffin oven for 6 hours at 120°C. At the end of the hydrolysis, the tubes were allowed to cool, then cracked open. HCl removal was accomplished by repeated evaporation in a vacuum oven. The hydrolysates were then filtered and made up to 5 ml. with deionized water.

The filtered hydrolysates were separated into their acidic, basic and neutral fractions by means of ion exchange columns. The hydrolysate was first passed through a column of Amberlite IR-4B®*; the acidic amino acids are adsorbed, and the neutral and basic amino acids pass through. The adsorbed acidic amino acids were eluted with N/10 HCl and collected separately. (The column is conveniently regenerated with 4% NaOH.) The basic and neutral hydrolysate fraction was then passed through a column of Amberlite IR-50-C which was previously buffered at pH 4.0 with an acetate buffer. The basic amino acids are adsorbed, and the neutral amino acids pass through. The basic amino acids were eluted with N/10 HCl. (The column is regenerated with 4% NaOH and buffered at pH 4.0 with acetate buffer.) By buffering the Amberlite IR-50-C at pH 4.0, cystine appears in the neutral fraction of amino acids. That cystine

* Amberlite is a trade name of Rohm & Haas Co., Philadelphia, Pa.

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was found exclusively in the neutral fraction was demonstrated by the platinic iodide test (2).*

The solution (6 to 12 ml.), corresponding to 200–400 mg. of protein hydrolysate, was placed near a corner of the filter paper sheet, 6 cm. from either edge. The paper was held with one edge slightly overlapping the opening of the trough and pressed into it with a strip of sheet glass somewhat longer than the paper. This assembly was then transferred to the chamber which had been prepared as follows:

A removable lead tray, the bottom of which was covered with a two-phase layer of water and the first solvent, was placed on the floor of the box in order to secure a saturated atmosphere. The chromatogram was allowed to develop for 24 to 72 hours. The paper was dried in a drying cupboard, turned through a right-angle and returned to the trough in order to be developed by the second solvent. After drying, the paper was sprayed with 0.1% ninhydrin in *n*-butanol, again dried, and then heated at 80° for 5 minutes. The spots were outlined with pencil because of eventual fading.

## Results

*A—Untreated Hair.* It was shown that all the amino acids expected to be present in untreated hair could be accounted for. There were twelve spots on the chromatogram for the neutral fraction, two spots on the chromatogram for the acidic fraction, and four spots on the chromatogram for the basic fraction. The spots for methionine, leucine, phenylalanine and proline in the neutral fraction were so close together that they could be regarded as one spot. No special effort was made to separate the individual members making up this spot.

The amino acid composition of each fraction is considered to be as follows:

| *Acidic* | $R_f$ |
|---|---|
| Aspartic acid | 0.14 |
| Glutamic acid | 0.24 |
| *Basic* | |
| Lysine | 0.50 |
| Histidine | 0.72 |
| Hydroxylysine | 0.76 |
| Arginine | 0.67 |
| *Neutral* | |
| Glycine | 0.40 |
| Alanine | 0.57 |

* Another—less conclusive—proof of the presence of cystine in the neutral fraction is the formation of typical cystine crystals when the neutral fraction of untreated hair was permitted to remain over a weekend at refrigerator temperature. This crystalline precipitate must be cystine because it is the only amino acid in this fraction which exhibits such high insolubility.

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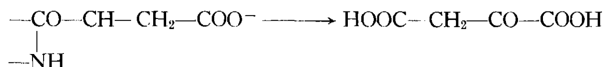
| | |
|---|---|
| Serine | 0.33 |
| Proline | 0.87 |
| Valine | 0.78 |
| Threonine | 0.50 |
| Leucine | 0.84 |
| Methionine | 0.82 |
| Phenylalamine | 0.86 |
| Tyrosine | 0.59 |
| Tryptophan—theoretical | 0.76 |
| Cystine | 0.13 |

The acidic fraction contained a ninhydrin spot at the solvent "frontal." This was considered to be due to inorganic salt impurities collected during the ion exchange separation. The spot was not characteristic of a typical amino acid and was, therefore, discounted.

*B—Hair Treated Three Minutes in Reducing Solution.* Ninhydrin development of the chromatograms of the three amino acid fractions of treated hair disclosed that a marked change had taken place: The acidic fraction was completely void of amino acids. However, two new spots appeared in the neutral fraction, and it is possible that they are due to the presence of some degradation product of the native acidic amino acids. The basic fraction, however, was completely accounted for. This finding was so unexpected that replication of this series of experiments was necessary to exclude the possibility of experimental error. The results of the second experimental series were identical with the first one and suggest that the acidic amino acids were altered during the reducing treatment. It is believed that this change may be a result of deamination by the reducing solution to produce oxaloacetic acid from the aspartic acid:



and α-keto glutaric acid from glutamic acid:

*C & D—Hair Reduced by Method "B" for Four and Eight Minutes Respectively Then Oxidized in 1.5% $H_2O_2$ at pH 4.8.* Both methods of treatment (C & D) yielded identical chromatograms. The chromatograms of the acidic fractions for both of these treatments had a new spot which was not encountered in untreated hair. For reasons not clearly understood, the spots for glutamic and aspartic acids reappeared in the hydrolysates from reduced-and-oxidized hair. The basic fractions of both treatments revealed chromatograms which could account for all amino acids. The neutral fractions of both treatments contained two new spots which were not encountered before. A spot at an $R_f$ value of 0.10 appears to match

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the position of a known chromatogram of lanthionine. In addition, there appeared a very large spot or grouping of spots at an $R_f$ value of 0.50.

Since these new spots appeared only as a result of treatments C & D, it is assumed that their presence is the result of some changes which took place during reduction and oxidation of the hair. These changes probably occurred at the disulfide linkage since lanthionine was produced; other changes probably occurred at the polar linkage as well since the new spots are some modification of the acidic amino acids.

## Discussion and Conclusion

In all treated hair swatches there seemed to be a distinct reduction of the total quantity of aspartic and glutamic acids. The appearance of a new spot in the neutral fraction as a result of a typical cold wave procedure (total immersion notwithstanding) suggests formation of lanthionine.

It is known that acid hydrolysis of proteins destroys only tryptophan by polymerizing its residue with a prosthetic carbohydrate to form humin. On the other hand, it is known that alkaline hydrolysis of proteins destroys cystine and hydroxy amino acids, converts arginine to ornithine, causes loss of ammonia, and rapidly racemizes all the amino acids. It is likely, therefore, that the changes in amino acid composition of hair keratin reported here take place not during hydrolysis but during (alkaline) cold wave treatment.

It is proposed that, during cold waving, the reducing solution not only converts disulfide linkages to sulfhydryl groups, but also causes a variety of reactions due to its high alkalinity. Thus, the conversion of cystine (to lanthionine) and deamination of acidic amino acids (glutamic and aspartic acids) and of basic amino acids (arginine and lysine) might well account for the unusual findings reported here. Evidently these reactions are not quantitative, and considerably more work will be required to elucidate completely the chemistry of the interaction between keratin and cold waving preparations.

(Received February 7, 1964)

## References

(1) R. Consden, A. H. Gordon, and A. J. P. Martin, *Biochem. J.*, **38**, 224 (1944).
(2) J. W. Sease, *et al.*, *Anal. Chem.*, **20**, 431 (1948).

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