

COLOR REACTIONS OF AMINO ACIDS WITH PHENOLS AND HYPOCHLORITES, INCLUDING NEW TESTS FOR TRYPTOPHANE AND PHENYLALANINE

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INTRODUCTION

In 1875 Engel¹ discovered what he thought to be a very delicate color reaction for glycocholl. This consisted in adding to its solution a few drops of phenol and a little sodium hypochlorite, which after standing a few minutes developed a beautiful blue color. Later investigators² found that other amino acids gave the color as well, and thought the reaction was based upon the splitting off of ammonia through the action of the hypochlorite. Proceeding on the above theory it was believed that fixed conditions might be established under which some of the amino acids could be made to react specifically. Such conditions were thought possible by varying the base of the hypochlorite, by using different phenols, or by using different degrees of alkalinity in the hypochlorite solutions.

EXPERIMENTAL

Preparation of Reagents: The amino acids, obtained from the Eastman Kodak Company, were made up in aqueous solution on the basis of their amino group rather than by their molecular weight. Best results were obtained when the hypochlorites were prepared by passing chlorine through a suspension or solution of the corresponding oxide or hydroxide. For purposes of comparison the alkalinity of all solutions was calculated as Na_2O and the hypochlorite as NaOCl . Phenol ($\text{C}_6\text{H}_5\text{OH}$) solutions were made up with water to 5 per cent by weight. The higher phenols were made up in alcoholic solution.

General Procedure: To 2cc. of the one-fiftieth molal (M/50) amino acid contained in a test tube were added 8 drops of the phenol and 2cc. of the hypochlorite solution. The contents were well mixed and the color observed immediately and at regular intervals thereafter.

The Use of Various Phenols: The list included phenol, o-, m-, and p-cresol, alpha and beta naphthol, guaiacol, hydroquinone, pyrocatechinol, and resorcinol. The colors obtained were usually brown or dark red and in no case did the use of the higher phenols show any advantage over phenol itself.

Alkali Concentrations in the Hypochlorite Solution: For this purpose known strength solutions of NH_4OH and of amino acids were made up. The alkalinity of the hypochlorite solutions was controlled by adding

¹ Henninger, A., Ber. Chem. Ges., 8:699 (1875).

² Zimmerman, W., Z. Physiol. Chem., 189:4 (1930); Furth, O., and Fromm, F., Biochem. Z., 220:69 (1930).

various amounts of standardized NaOH. The general procedure was followed. The results showed that the lower concentrations of free alkali gave the more satisfactory results.

A Study of Hypochlorite Concentrations: A series of sodium-, calcium-, barium-, and magnesium-hypochlorites were prepared in which the NaOCl content was controlled in the preparation. It was found that NaOCl concentrations above 2 per cent were undesirable, and that the most practicable results were obtained with solutions of below 1 per cent.

Reactions with Various Hypochlorites: When a solution of calcium hypochlorite was used upon one-fiftieth molal amino acids the following results were obtained in from 30 to 60 minutes:

(M/50) Amino Acids	NaOCl-0.95% Na ₂ O-0.03%	NaOCl-0.32% Na ₂ O-0.01%
Glycocoll.....	blue.....	
Alanine.....	blue.....	blue-green
Tyrosine.....	brown.....	yellow
Tryptophane.....	reddish-brown.....	rose
Leucine.....	blue.....	
Caprine.....	blue.....	
Proline.....	blue.....	
Phenylalanine.....	white turbidity.....	white turbidity
Histidine.....	pale brown.....	
Glutamic acid.....	blue.....	
Aspartic acid.....	yellow.....	

Using magnesium hypochlorite the following observations were made in from 30 to 60 minutes:

(M/50) Amino Acids	NaOCl-1.08% Na ₂ O-0.05%	NaOCl-0.27% Na ₂ O-0.013%	NaOCl-0.11% Na ₂ O-0.005%	NaOCl-0.04% Na ₂ O-0.001%
Glycocoll.....	blue.....			
Alanine.....	blue.....	pale blue.....		
Tyrosine.....	light brown.....			
Tryptophane.....	light brown.....	rose.....	rose.....	pink
Leucine.....	blue-green.....			
Proline.....	blue-green.....			
Caprine.....	blue-green.....			
Aspartic acid.....	yellow.....			
Asparagin.....	blue.....			
Phenylalanine.....	w. turbidity.....	w. turbidity.....		
Histidine.....	faint brown.....			
Glutamic acid.....	blue.....			

The immediate, specific color reaction of tryptophane should be noted. It is best shown when conditions are such that other amino acids fail to respond. The maximum color develops in from 30 to 60 minutes at room temperature, but can be speeded up by gentle heating.

The test for phenylalanine is no less specific but requires from 8 to 10 hours of standing at room temperature for the development of the white turbidity in the greater dilutions.

When sodium hypochlorite is used in the scheme outlined above similar results are obtained. Barium hypochlorite solutions react likewise but have a tendency to form precipitates.

The Departments of Indole and Skatole: When indole and skatole are allowed to react under the same conditions as tryptophane, indole produces a white flocculent precipitate and skatole a white turbidity as compared with the rose color for tryptophane. By this means it is seen that indole and skatole may be differentiated and that their presence does not interfere with the specificity of the test for tryptophane.

The Department of Egg Albumin: A solution of egg albumin was prepared which contained as much tryptophane in the combined form as is present in a one one-hundredth molal solution of pure tryptophane. Aliquot portions of this solution were diluted with water in various proportions. The reagents were added as for the regular tryptophane test.

In a limited number of cases a faint greenish blue color developed, but in no case was there a trace of pink or rose. From this it was concluded that the test does not apply to tryptophane combined in the protein molecule.

The Department of Mixtures of Amino Acids: Mixtures of the amino acids previously studied were made up in the ratio in which they are found in egg albumin and in casein according to the best authorities.³

Observations based upon a large number of tests indicated that best results are obtained when very dilute solutions of sodium hypochlorite were used, the NaOCl content ranging between 0.13 and 0.022 per cent. Under these conditions the phenylalanine did not produce its turbidity and the tryptophane color remained clear in every case.

The Qualitative Detection of Tryptophane: A positive test is indicated by a beautiful rose or pink color which develops immediately and reaches a quite stable condition in from 30 to 60 minutes. Low concentrations of hypochlorite are essential and the free alkalinity must be maintained at a minimum. Concentrations of from 0.5 to 0.01 per cent NaOCl and from 0.5 to 0.002 per cent Na₂O in the hypochlorite solution work successfully. The test is especially specific at the lower concentrations of hypochlorite, because under such limitations all other amino acids fail to respond. Positive results have been obtained in solutions containing 0.0008 grams per cc.

The Qualitative Detection of Phenylalanine: A positive test is indicated by the development of a white turbidity which develops within 30 minutes with the higher concentrations of hypochlorite, but is considerably slower for the lower ones. Best results are obtained with

³ The Biochemistry of the Amino Acids, A.C.S. Monograph No. 48, p. 191.

the more dilute solutions when allowed to stand from 8 to 10 hours. Concentrations of from 0.9 to 0.2 per cent NaOCl and from 0.25 to 0.003 per cent Na₂O work successfully.

The Quantitative Application to Tryptophane: The following amounts of reagents were added and in the order given: 2 cc. tryptophane, 4 cc. water, 8 drops phenol, and 2 cc. sodium hypochlorite. Color readings, using the Duboscq colorimeter, were taken at 30-minute intervals for 3 hours. A large number of samples were made up and 5 or more readings taken on each. Calculations showed that an average of 98.5 per cent of theory was obtained. The most suitable range was found to be between M/50 and M/150, and the time of standing from 2 to 3 hours. Gentle heating was found to accelerate the color equilibrium.

SUMMARY

The intensity of the blue color is given in no definite order by alanine, glutamic acid, carpine, asparagin, glycine, leucine, and proline. In general alanine gives the most intense color. Amino acids containing the benzene ring, such as tyrosine, tryptophane, and phenylalanine fail to give the blue color under ordinary conditions; histidine and aspartic acid likewise fail.

Among the various phenols C₆H₅OH is the most satisfactory. The reactions of the various hypochlorites are not specific.

A delicate test was worked out for phenylalanine, which is specific in that it produces a pronounced white turbidity with the more dilute solutions of hypochlorite.

Tryptophane yields a beautiful rose or pink at very low concentrations of hypochlorite. It can be detected in solutions containing 0.0008 grams per cc. Indole and skatole do not interfere. The test is not applicable to tryptophane when combined in the protein molecule but can be applied successfully when present in a mixture of amino acids. The characteristic color develops immediately and lends itself readily to quantitative applications.