REACTION OF DIHYDROXYACETONE (DHA) WITH HUMAN SKIN CALLUS AND AMINO COMPOUNDS*

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Dihydroxyacetone (DHA), a keto-sugar, $CH_2OHCOCH_2OH$, has been known for a number of years as an intermediate product in normal carbohydrate metabolism in humans and animals (1, 2, 3, 4). Recently it was shown that solutions of DHA are able to stain human skin (5). The present studies were undertaken to investigate the chemical mechanism responsible for the "tanning" phenomenon.

METHODS AND RESULTS

Callus from human skin was treated with solutions of DHA of varying concentration. By using ground callus the reactive surface area was enlarged. Control samples of callus were treated with normal saline. Specimens were also treated with fructose solutions for comparison. Fructose is also a keto-sugar which, like DHA, possesses hydroxyl groups on carbons adjacent to the carbonyl carbon. The callus developed a dark brown color at room temperature a few hours after being painted with 10% solution of DHA. A brown color developed when unpainted callus was ground into a powder and mixed with solutions of DHA. No immediate reaction occurred when callus was treated with 10% fructose solution. Ground callus treated with fructose solution developed a deep brown color when heated on a steam bath. Pieces of callus immersed in concentrated fructose solution turned brown when heated in a boiling water bath for 10 to 15 minutes.

Solutions of DHA dried on filter paper at room temperature turned brown when exposed to the laboratory atmosphere for several days. The brown substance was eluted from the filter paper. Spot tests using an equilibrium solution of zinc oxinate on the eluant showed the presence of ammonia (6). Control papers kept in an ammonia free atmosphere in tightly closed wrappings or in a desiccator over sulfuric acid did not develop color after several weeks. Eluant from these papers gave negative tests for ammonia.

Solutions of DHA and fructose (10%) were treated with ammonia, amino acids, or related compounds. These results are shown in Table 1. Arginine and DHA reacted almost immediately to produce a yellow color. A stable brown color developed within 30 minutes. Other basic amino acids and glycine reacted more slowly with a yellow or brown color after 6 to 12 hours. Further tests were performed on arginine and some substituted guanidine compounds to test whether the reactivity of the guanido group in arginine might be modified by substituents. Aminoguanidine bicarbonate when combined with DHA solution turned dark brown within 3 hours. 1,3-diphenylguanidine showed a faint color reaction with DHA when the mixture was allowed to stand 4 days at 3° C. 1,2,3-triphenylguanidine and DHA developed a slight yellow color only after three weeks at 30° C. Guanidine nitrate and DHA reacted to produce a faint yellow color after 3 weeks. No color formation occurred when DHA was combined with urea. The reactivity of the DHA-guanidine mixture appears to be directly related to the availability of the guanidine hydrogens for reaction.

The reaction mixtures of DHA with ammonia and amino acids were subjected to paper chromatography and qualitative chemical tests for functional groups. In Figure 1 are shown the positions on chromatograms of DHA and intermediate products in the reaction between DHA and ammonia. Untreated DHA (300 μg) was added to the origin. The chromatogram was resolved in an ammoniacal atmosphere during which reaction of DHA and ammonia occurred. After removal of the first solvent, the reaction products were separated in butanol-acetic acid-water solvent. Three stable intermediate substances were produced. Two of these no longer gave reactions characteristic of sugars (see Table 2), but reacted with Pauly reagent (diazotized sulfanilic acid-sodium carbonate) to produce red color. If solutions containing the isolated intermediate compounds were heated, a brown color developed. When mixtures of DHA and amino acids were chromatographed, in addition to the unchanged amino acid, substances were detected which were positive both with ninhydrin (amino acid) and aniline-phthalate (sugar) reagents (7). The nature of these compounds produced by interaction of

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TABLE 1

Reactions of DHA and fructose with amino compounds

	DHA	Fructose
Ammonia vapor	immediate formation of a dark brown syrupy compound	brown colored syrup after approx. 1 hr.
Arginine	immediate yellow; dark brown after 30 minutes	brown color after 12 hr. at 30° or several minutes at 100°
Glycine	yellow after 60 min; dark brown after 12 hr.	brown color after 72 hr. at 30°
Histidine	Same as glycine	—
Lysine	Same as glycine	—
Tryptophane	yellow after 12 hr.	no color reaction after 12 hr.
Alanine	faint yellow after 12 hr.	—
Valine, Leucine, Phenylalanine	no color change after 12 hr.	no color reaction
Amino guanidine-bicarbonate	dark brown within 3 hr.	no color reaction
1,3-diphenyl-guanidine	faint yellow-gray after 96 hr. at 30°	—
1,2,3-triphenyl-guanidine	faint yellow after 3 weeks at 30°	—
Guanidine nitrate	faint yellow after 3 weeks at 30°	—
Urea	no color formation at 30° or 100°	no color formation

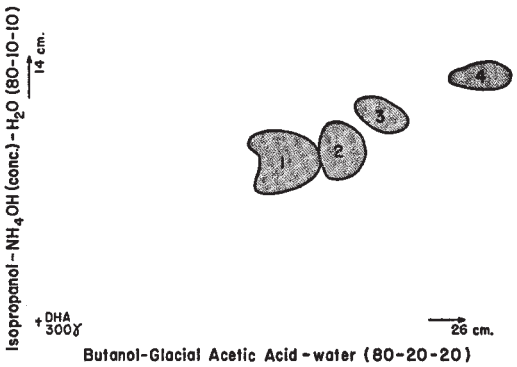

FIG. 1. Two dimensional chromatogram of DHA and unknown substances formed in reaction between DHA and ammonium hydroxide.

DHA and amino acids or ammonia is also under investigation.

Fructose and ammonia reacted to give a brown color after an hour. Fructose and arginine gave a brown color after 12 hours at 30° C, or after several minutes at 100° C.

When callus was treated with formaldehyde several minutes prior to the application of DHA, no color was observed. When equimolar amounts of DHA and formaldehyde were mixed in aqueous solution and the callus was painted with this mixture, a yellow or light brown color developed which never became as dark as under the influence of DHA alone. Callus painted with formaldehyde or ground callus mixed with formaldehyde gave no color reaction even upon heating. Addition of formaldehyde to the reaction mixture of DHA and arginine inhibited the color formation.

DISCUSSION

Ammonia and its derivatives such as amines have long been known to react readily with carbonyl compounds (10). Ketones generally are less reactive than aldehydes. Reducing sugars readily form hydrazones, oximes, ureides, and semicarbazones, for example. The interaction of amino acids and proteins with carbohydrates has been shown to be the cause of much of the browning that occurs during manufacture and storing of foods (8). Richards (9) investigating the interaction between D-glucose and glycine, succeeded in isolating an intermediate in the browning reaction, the enolic form of N-(carboxymethyl)-amino-1-deoxyfructose, which gave reactions of both a sugar and an amino acid. Carbonyl compounds with strongly electron-attracting groups are highly reactive. In the case of DHA, the normal electrophilic reactivity of the carbonyl group is enhanced by the hydroxyl groups on adjacent carbon atoms. Arginine with

TABLE 2

	UV Light	Ammonium molybdate	Phospho molybdate	Aniline phthalate	Pauly reagent
DHA (spot 2)	yellow-white	+	+	+	−
Unknown 1	−	−	−	−	+
Unknown 3	−	−	−	−	+
Unknown 4	faint yellow	ft +	ft +	ft +	yellow
Fructose	yellow-white	+	+ (slow)	+	−

Reactions of unknown intermediate compounds produced by combination of DHA and ammonium hydroxide. Compared with DHA and fructose.

its nucleophilic guanido group reacts readily with DHA, to form a stable dark brown color after 30 minutes.

The formation of stable intermediates in the reaction of DHA and ammonia suggests a similarity in the mechanism to that described by Richards in interaction between glucose and glycine. The reaction of fructose with arginine and skin to produce a brown color indicates that the browning of skin may be a general reaction characteristic of amino acids and sugars. The rapid development of color which occurs when skin is treated with DHA results from the high reactivity both of DHA and the basic groups of arginine. The decreased color noted when formaldehyde was added as a third reactant is consistent with the observation that formaldehyde is an unusually active carbonyl compound and reacts readily with amino acids by substitution of the amino group. It also combines with ammonium hydroxide to produce a brown syrupy substance. In the reaction of DHA with skin or arginine, formaldehyde probably competitively inhibits color formation.

Arginine is present in proteins of the skin in high concentration. It seems likely that this amino acid is responsible for most of the "tanning" effect of DHA. Variations in color development and the relation to individual variations in amino acid composition of skin should be investigated. The relatively slow rate of reaction of DHA on the skin (6 to 8 hours when applied as a cosmetic) as compared to the rapid rate of reaction of DHA and arginine in vitro (less than 30 minutes) might be due to any one of several factors, such as availability of free amino groups in skin protein, pH of skin, or presence of inhibitors. Further investigation will be required to clarify details of the staining mechanism.

SUMMARY

The reaction of dihydroxyacetone (DHA) with amino acids and with callus from human skin has been studied in relation to the "tanning" effect of DHA on human skin. Ground human skin was stained brown by solutions of DHA at room temperature; callus immersed in fructose turned brown when heated at boiling for 15 minutes. Arginine and DHA react rapidly to form a brown color. Fructose and arginine react similarly but at a much slower rate at room temperature. Chromatographic studies revealed the presence of several intermediate products which may be responsible for the browning phenomenon. From the point of view of these studies, arginine is one of the most important amino acid constituents of human skin. Combination of DHA and the basic groups of arginine is probably responsible for the browning which is observed when human skin is treated with DHA.

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