Conclusions

An increase of about 30% over the number of peptides detected in G-actin with previously reported techniques of peptide mapping on paper is obtained by means of two simple thin layer techniques described in this paper. The number of spots detected is close to the maximal value theoretically expected and thus indicates that G-actin must consist of either one unique[8] or two different polypeptide chains and not of two very similar subunits as proposed by JOHNSON *et al.*[4].

The present results suggest that both techniques might be advantageously used, independently or together, to reassess the so-far observed similarity of G-actin extracted from different sources, as well as to undertake comparative studies on any homologous proteins.

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Received September 5th, 1968

CHROM. 3772

Identification and quantitation of α-hydroxy and α-keto acids with a ceric ammonium nitrate reagent

The use of ceric ammonium nitrate for the identification of α-hydroxy acids, α-keto acids and mercaptans in paper chromatograms was reported[1]. In continuation of the above, the reagent has now been stabilized and modified for thin-layer chromatograms. A quantitative procedure has also been developed.

Experimental

Identification. The paper or thin-layer chromatogram is dried and sprayed with a fresh solution of 10 % ceric ammonium nitrate in methanol or ethanol. The chromatogram is allowed to dry at room temperature for approximately 10 min. α-Hydroxy acids, α-keto acids and mercaptans show up as light spots on an orange background. Tryptophan reacts to form a brown color, and tyrosine forms a yellow color. The chromatogram is now sprayed with 0.25 % indole in methanol or ethanol. The spots become clearer due to the greater contrast since the background turns dark brown with the spots retaining their original color. These spots are stable both on paper and on thin-layer plates for over 3 weeks.

Quantitative determination. The spots are cut from the paper or scraped from the thin-layer plate and are placed in a centrifuge tube. One ml nitric acid (1:3) is introduced. After mixing for 5 min, the material is centrifuged (low speed, clinical centrifuge). Any paper shreds or silica precipitate immediately.

One-half ml of the clear supernatant is transferred to the center compartment of a Warburg vessel. One-half ml of ceric ammonium nitrate (30 %, w/v) in the nitric acid (1:3) is placed in a side arm. The thermobarometer contains 0.5 ml of the above nitric acid and 0.5 ml of the ceric ammonium nitrate solution. The vessel is equilibrated at 30° for 10 min, and then the two solutions in the vessel are mixed. The volume of gas produced in 20 min is measured.

Results

Identification. The following acids reacted in the range of 0.1 to 1.0 μmoles.

α-Hydroxy acids: α-Hydroxyglutaric acid, lactic acid, ascorbic acid, erythorbic acid, glycolic acid, malic acid, β-methylmalic acid, citramalic acid, glucuronic acid, gluconic acid, glyceric acid and tartaric acid.

α-Keto acids: Glyoxylic acid, α-ketoglutaric acid, pyruvic acid, oxalacetic acid.

Mercaptans: Cysteine, cysteamine and thioglycolic acid.

The other acids mentioned previously[1] reacted as recorded in that paper.

Quantitative determination. The relative volumes of CO_2 released from the α-keto and α-hydroxy acids are listed in Table I.

TABLE I

VOLUME OF CARBON DIOXIDE RELEASED WHEN α-HYDROXY ACIDS OR α-KETO ACIDS REACT WITH CERIC AMMONIUM NITRATE IN A STRONGLY ACID MEDIUM

Acid	*Gas volume μl per μmole*	*Linear range μmole*
Pyruvic acid	21	0.3 –3.5
Lactic acid	22	0.3 –3.5
Glyoxylic acid	24	0.3 –3.5
Malic acid	25	0.2 –2.0
α-Ketoglutaric acid	25	0.2 –2.0
Oxalic acid	42	0.2 –1.5
Tartaric acid	45	0.15–2.0
Citramalic acid	48	0.15–2.0
Glucuronic acid	66	0.1 –1.4
Citric acid	68	0.1 –1.2

The results are linear in the range listed in the table. Other carboxylic acids (neither α-keto nor α-hydroxy) such as butyric acid, palmitic acid, linoleic acid, succinic acid and glutaric acid, did not give off any gas under identical conditions.

Discussion

The ceric ammonium nitrate reagent described in this paper was developed from reagent A of TROP *et al.*[1]. When the above reagent was used, however, the orange background color faded quite rapidly due to reduction by the cellulose of the paper. In addition, the method of drawing the paper through a solution of reagent resulted in a slight diffusion of the colored spots during the time the paper was soaked with the reagent. An aqueous solution was also not suitable for spraying in thin-layer chromatography. The present reagent can be sprayed on paper or on thin layers. It dries rapidly and there is little or no diffusion.

The orange background is quite striking at first. This is due to the complex of the hydroxyl groups of the cellulose with the ceric ammonium nitrate[2]. The orange color is stable as long as the alcohol does not evaporate on thin-layer plates which do not contain cellulose. The contrast is greatly increased by the indole spray, the indole reacting with the unreduced ceric ammonium nitrate to form a dark brown background. The indole does not react with the reduced ceric ammonium nitrate, the spots remaining light.

The oxidation of α-hydroxy and α-keto acids in a strongly acid solution causes the release of CO_2 which can be measured with a Warburg manometer. When sodium hydroxide is placed in the center well, no manometer change is noted. Simple α-hydroxy and α-carbonyl acids (lactic acid, malic acid, glyoxylic acid, pyruvic acid and α-ketoglutaric acid) release almost a stoichiometric volume of gas. Polyhydroxy acids, or acids with the carbonyl groups in a position to allow them to interact with the ceric ammonium nitrate, will release greater quantities of CO_2, the volume of gas not being stoichiometric.

The above is not a great disadvantage because the volume of CO_2 released is linear in the ranges listed, and the quantitative determination can be made with an accuracy of $\pm 6\%$.

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Received September 9th, 1968

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