CHROM. 3939

Note on a selective test for the detection of sulphur amino acids

Generally, detection of materials on chromatograms employs reagents that react with all members of a group or class[1]. However, the need sometimes arises, as in the study of nutritional problems of proteins, for a selective reagent to detect an individual amino acid, or a small group of acids, such as those containing sulphur.

Comparatively few selective reagents have been used for the detection of amino acids. The ninhydrin reagent has been modified[2], while sodium nitroprusside has been used for the detection of –SH containing compounds such as cysteine, and sodium cyanide for submicro amounts of cystine[3]. A chlorine–tolidine reagent[4] has been employed in studies on disulphide interchanges in proteins[5].

Incidental to other work on the detection of organo-sulphur compounds[6], it was observed that sulphur-containing amino acids responded to the test. The possibility of using it as a selective test, and of applying it to nutritional studies, suggested a more detailed study of the reaction should be made.

The principle of the test, which depends upon the reaction of the sulphur atom of the amino acid with a halogen in the presence of an aromatic amine, has been discussed previously[6], in establishing the need for the aromatic amine. Negative results were obtained with aliphatic amines, such as benzylamine, and sulphur amino acids with bromine alone do not respond. The ensuing co-oxidation results in the formation of a coloured species, and although an attempt has been made to explain the nature of this in one instance[7], its composition remains uncertain.

Experimental

Reagents. Aniline (redistilled) 3% v/v in light petroleum (b.p. 40–60°), ninhydrin 0.3% in ethanol.

Procedure. Solutions of amino acids, singly or in admixture, were applied to Whatman No. 4 paper and developed in the ascending manner using *n*-butanol–water–acetic acid (4:4:1) as solvent.

When the solvent had travelled 16–20 cm, the paper was removed, dried in a current of warm air and cut vertically into strips.

Each strip was drawn through the aniline solution and the petroleum solvent allowed to evaporate in the air. The strip was then exposed immediately to the vapours of bromine contained in an open dish, making sure the strip was thoroughly treated. Fumes of HBr were evolved at this stage. Sulphur amino acids were revealed as mauve to brown-mauve coloured spots within a few minutes.

Results and discussion

The test was applied to a short series of sulphur-containing amino acids, and for comparison, to three non-sulphur amino acids. In control tests the same series of seven compounds was chromatographed and treated with the ninhydrin reagent. Results obtained by both means of detection are shown in Table I.

Best results were obtained for cysteine and glutathione (detection limit, 10 μg/cm^2), but larger amounts of cystine (125 μg) and methionine (750 μg) were required for a positive test.

TABLE I

CHROMATOGRAPHY AND DETECTION OF AMINO ACIDS

Amino acid	*Aniline–bromine test*			*Ninhydrin test*
	Colour obtained	*Sensitivity ($\mu g/cm^2$)*	*R_F value*	*R_F value*
Glutathione	Brown-mauve	10	0.28	0.29
Cysteine	Brown-mauve	10	0.35	0.37
Cystine	Pale pink-brown	125	0.12	0.13
Methionine	Pale yellow-brown	750	0.61	0.50
Proline	No colour	—	—	0.36
Tyrosine	No colour	—	—	0.47
Leucine	No colour	—	—	0.63

The sensitivity of the test for cystine was improved by first reducing with metal and acid to cysteine. In trials, 250 μg, 125 μg, 50 μg, 25 μg, 10 μg and 5 μg were applied to the paper and chromatographed. Only the 250 μg spot could be readily detected, and the 125 μg spot with a little more difficulty. After reduction of the parent solution with zinc and dilute HCl, the same amounts of solution were again applied and chromatographed. In this case, all six spots could be readily detected by the test, and the coloured areas were now located at the position on the chromatogram appropriate for cysteine, *i.e.* closer to the solvent front.

Effects of residual acid on the paper after chromatography were overcome by double treatment of the cut out strips with the aniline solution.

The most reactive of the sulphur-containing amino acids are clearly those containing the –SH group. This is demonstrated by the positive reactions given by both cysteine and glutathione, and is reinforced by the lesser degree of sensitivity shown by cysteine containing the –S·S– group, and the comparatively insensitive reaction shown by methionine containing the thioether group.

Other amino acids, proline, tyrosine and leucine, in large amounts, did not respond to the test, thus confirming its selectivity for cysteine and glutathione, and for cystine when reduced. It appears to be a useful technique for detecting sulphur amino acids in the presence of other amino acids where overlapping R_F values are encountered (Table I).

The cyanide, iodoplatinate and iodopalladium reagents discussed by TOENNIES AND KOLB[3] are considerably more sensitive (by a factor of about 15 for cysteine). However, the tests are different in character and depend on inhibition of colour development at the site of the S-amino acid thus giving bleached spots on coloured backgrounds. By contrast in the present test colour is obtained at the site of the amino acid. The nitroprusside reagent[3] gives a brilliant red colour with cysteine but it was observed here that rapid fluctuation in the shade and intensity of colour occurs on standing.

In veterinary diagnostic work the need sometimes arises for the application of confirmatory tests to some particular biochemical investigation. Using the present test it was possible to confirm the identity of major and minor constituents of uncommon urinary calculi obtained from sheep in western New South Wales. Appli-

cation of the test to chromatograms of calculi extracts revealed the presence of cystine as a major component, with traces of methionine. The test also showed the presence of only traces of organic sulphur compounds in a calculus consisting predominantly of calcium carbonate.

Department of Agriculture,
Veterinary Research Station,
Glenfield, N.S.W. (Australia)
R. F. BAYFIELD

School of Chemistry,
University of New South Wales,
Kensington, N.S.W. (Australia)
E. R. COLE

1 H. N. RYDON AND P. W. G. SMITH, *Nature*, 169 (1952) 922.
2 C. E. DENT, *Biochem. J.*, 43 (1948) 169.
3 G. TOENNIES AND J. J. KOLB, *Anal. Chem.*, 23 (1951) 823.
4 F. REINDEL AND W. HOPPE, *Ber.*, 87 (1954) 1103.
5 L. A. SLUYTERMAN, *Biochim. Biophys. Acta*, 48 (1961) 429.
6 R. F. BAYFIELD, V. CLARKE AND E. R. COLE, *J. Chromatog.*, 19 (1965) 370.
7 R. F. BAYFIELD AND E. R. COLE, *Anal. Chim. Acta*, 34 (1966) 193.

First received December 10th, 1968; revised manuscript received January 9th, 1969

J. Chromatog., 40 (1969) 470–472