PAPER PARTITION CHROMATOGRAPHY FOR THE SEPARATION OF ANALOGUES OF SULFUR AMINO ACIDS[1]

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In 1943 the British biochemists Gordon, Martin and Synge, in attempts to separate amino acids of a wool hydrolysate by means of a silica gel partition chromatogram column, were unsuccessful due to adsorption of the amino acids by the gel. It was found, however, that a good separation could be achieved by use of filter paper (cellulose) as an inert support. Separation depends on differences in coefficients of partition, between the slightly miscible aqueous and non-aqueous phases, of the components of a mixture. In this method, a filter paper strip carrying a mixture of amino acids (a few micrograms of each) near the upper end is hung from a trough containing water-saturated solvent (e.g., n-butanol, phenol, s-collidine), with the whole system in an atmosphere saturated with respect to solvent and water vapors. The solvent siphons down the strip as a front, and after a suitable time the spots of amino acids are revealed by drying the paper, spraying with 0.1% aqueous ninhydrin solution and then gently heating. A simplification of this method, the capillary ascent technique, as reported by Williams and Kirby (1) of the University of Texas was used throughout our work.

Several possibilities as to the organic solvent were mentioned by Consden, Gordon and Martin (2) in their initial paper, which is the primary reference in the study and application of paper chromatography. The early literature was, however, extremely vague as to the ratios of solvent to water. Nothing was accomplished in the present work prior to a private communication from Williams and Kirby, who advised using phenol (100 g. phenol: 20 ml. 10% aqueous sodium citrate: 0.5 g. ascorbic acid) or n-butanol (80 ml. n-butanol; 20 ml. ethanol: 20 ml. water) as solvents. In later literature, collidine, lutidine, or their 1:1 mixture, 1:1 with water were suggested as suitable solvents.

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The sheet of filter paper is formed into a cylinder by stapling. Drops (0.01 ml.) of 0.1% solutions of amino acids (10 micrograms) are placed and labeled at about one inch intervals, on the origin line, parallel to the base of the cylinder at a height of about one inch from the bottom. Drops should not be placed too close to the outer edges of the paper where it is fastened together to form the cylinder to avoid distorted rising, as the solvent may hold back near the edges. When the drops of samples are dry, the paper cylinder, if small, is set vertically in a beaker containing the developing solvent, enclosed in a tall, sealed jar, or set directly in the solvent contained in the bottom of the jar if the paper is large. The solvent should be about one-fourth inch deep in the beaker or jar. When the solvent has risen to the top of the paper or has reached a stationary level, the paper is removed and dried in the hood before drying in the oven at 100° for about five minutes or until excess solvent is removed. Locations of the dispersed amino acid spots are determined by spraying with a 0.1% aqueous ninhydrin solution, drying for a short time at room temperature and heating in the 100° C. oven for five minutes. The spots representing the locations of the various amino acids develop characteristic red colors.

As the solvent front rises, the spots of different amino acids rise to different heights, due to different travel rates, which are quite constant in the same solvent, so that amino acids can be identified on the R_f value (the ratio of the distance traveled by the amino acid to the distance traveled by the solvent.) Different solvents produce different rates of travel and, thus, different R_f values may be obtained in different solvents for one amino acid. We can separate amino acids still more by drying the paper, turning it through a right angle and developing with a different solvent after the first run, giving a two-dimensional chromatogram with a scattered pattern characteristic of the two solvents used.

Since this technique is being used in an attempt to

separate sulfur bearing amino acids from their selenium analogs occurring in seleniferous cereal proteins, the work has been done chiefly with cystine, selenium-cystine, methionine and selenium-methionine. The solvents, phenol, and n-butanol have been used for most of the work but a few of the trials have also been run using lutidine and collidine as solvents. As reported by other workers (3), the phenol solvent mixture (Mallinkrodt's phenol has been most satisfactory) gave the best results, in that the spots were more widely separated vertically. The n-butane mixture did not move cystine or selenium-cystine from the origin line, but was better for separating methionine from selenium-methionine.

Although the analogs were not paired in a high number of trials, and although the separations are not wide, as shown in Fig. I, and not always in the same order, we have

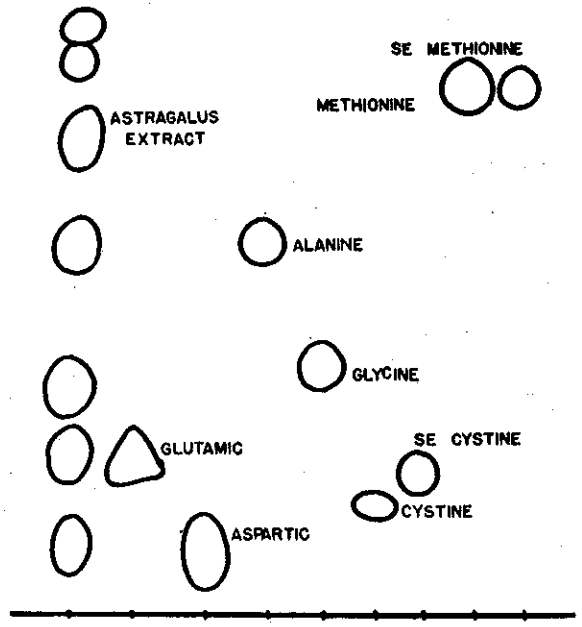

Fig. I. Tracing of representative paper chromatogram showing vertical separation of cystine and selenium cystine and some other amino acids. Selenium methionine is usually found slightly above methionine, also. A water extract of **Astragalus bisulcatus** occupies the first position on the left.

been able, by the average difference of R_f values, to show how the analogs may be separated. In phenol the average R_f differences between the cystines and between the methionines place the selenium analogs at an R_f value 0.02 higher than the sulfur compounds. In butanol the average R_f difference shows that of selenium-methionine to be 0.07 higher than that of methionine, with no separation of the cystines. Collidine and lutidine show greater R_f differences for both sets of analogs on one paper, but the actual vertical separation is small, since the compounds rise only a short distance.

In the study of methods of distinguishing the analogs on a chromatogram, it is apparent that some means other than the R_f difference would be more satisfactory. However attempts to distinguish them by use of ascorbic acid or sulfur dioxide reduction or the iodine-sodium azide test have not been successful. The McCarthy-Sullivan or the nitroprusside tests will distinguish separate spots of the analogs but will not show separations from a mixture.

Chromatograms of two more sulfur bearing amino acids, cystathionine and homolanthionine (R_f 0.17 and 0.22) have been run in phenol, showing a satisfactory separation from cystine and methionine. Selenium analogs of these compounds have not been chromatographed, however.

BIBLIOGRAPHY

1. Williams, R. J. and H. Kirby, Paper Chromatography Using Capillary Ascent. Science, 107, 481 (1948).
2. Consden, R., A. H. Gordon and A. J. P. Martin, Qualitative Analysis of Proteins: A Partition Chromatographic Method Using Paper. Biochem. J., **38**, 224 (1944).
3. Draper, J. O. and A. L. Pollard, The Purification of Phenol for Paper Partition Chromatography. Science, **109**, 448 (1949).
4. Dent, C. E., A Study of the Behavior of Some Sixty Amino-Acids and other Ninhydrin-reacting Substances on Phenol-'collidine' Filter-paper Chromatograms, with Notes as to the Occurrence of some of them in Biological Fluids. Biochem. J., 43, 169 (1948).