RICHARD L.M. SYNGE

Applications of partition chromatography

Nobel Lecture, December 12, 1952

Atrayee Datta
10th May, 2025

While still at school I heard tell of the science of biochemistry, which should draw together two aspects of nature into a common study. I had long been fascinated by living things (particularly the wild plants), and more recently by chemistry. This last was probably more for the appeal to the senses of the chemist's activities than for the knowledge of the nature of things which chemistry imparts. It was particularly reading in the newspaper Sir Frederick ideas. The latter outnumbered the former, usually. However, at an early stage in the course the student engaged in some quite rigorous isolative work under the guidance of Mr. N. W. Pirie. Pirie used to enliven the long hours at the bench with caustic anecdotes from the history of biochemistry which helped, quite as much as the isolative work itself and his comments thereon, to develop the critical faculty in those who possessed a rudiment of it. On graduating in 1936 I continued in the laboratory as a research student under

Pirie's supervision, and he suggested that I should make a chemical study of the glycoproteins, a class of substances then, as now, obscure in chemical nature and of great physiological interest. Soon I found my knowledge both of carbohydrate and protein chemistry was inadequate to the task, and began some synthetic work with Dr. D. J. Bell which involved preparing partially substituted derivatives of glucose. Among many useful things, I learnt from Bell the power of liquid-liquid extraction, with and without salting out, for separating methylated sugars according to the extent of their methylation. I

About this time, in 1937, Dr. Hedley R. Marston (then, as now, director of the C. S. I. R. Nutrition Laboratory at Adelaide, South Australia) came to the Biochemical Laboratory in Cambridge for a year as a guest. He was given bench space in the room where Pirie and I were working. He brought with him apparatus much more complicated than most workers in that laboratory were accustomed to, including an "artificial rumen" for microbial digestion of cellulose and a long fractionating column for distilling the esters of the resulting fatty acids. This by no means occupied his whole time, and

to be effective substitutes for wool. Marston's advice was to apply some of their money to fundamental studies of the nature of wool, and he suggested that part should be given to me as a Studentship to study in detail the amino-acid composition of wool, beginning by improving the methods of amino-acid analysis. "If you work steadily at that for five years, you will revolutionize the whole of protein chemistry" he said. The Studentship was on unusually generous financial terms, and as I also thought it would fit in with acquiring a more detailed knowledge of the protein side of the glycoprotein problem, I readily agreed. I began work in 1938 by studying the distribution

I will discuss in most detail the application of partition chromatography to the study of proteins. Partly because of intense interest of biochemists in proteins and partly because the earliest work with the method was in this field, the results obtained with partition chromatography have been especially striking here, although the method has similar capabilities in other branches of biochemistry.

First, the method has given the possibility of exhaustive analysis of complete hydrolysates of proteins. For qualitative work the two-dimensional procedures using paper have been most convenient. For quantitative work the procedures using starch grains elaborated by S. Moore and W. H. Stein have permitted accurate and complete analyses on a few mg of hydrolysed protein (Fig. 1). Latterly these workers have found ion-exchange resins to have advantages over starch. Apart from the positive value to protein chemistry of having convenient methods for carrying out the fundamental operations, the use of partition chromatography has helped firmly to establish that the vast majority of proteins yield on hydrolysis only the well-known amino acids and that these account for the entire substance of the protein molecule. This generalization is fundamental for the future devel-

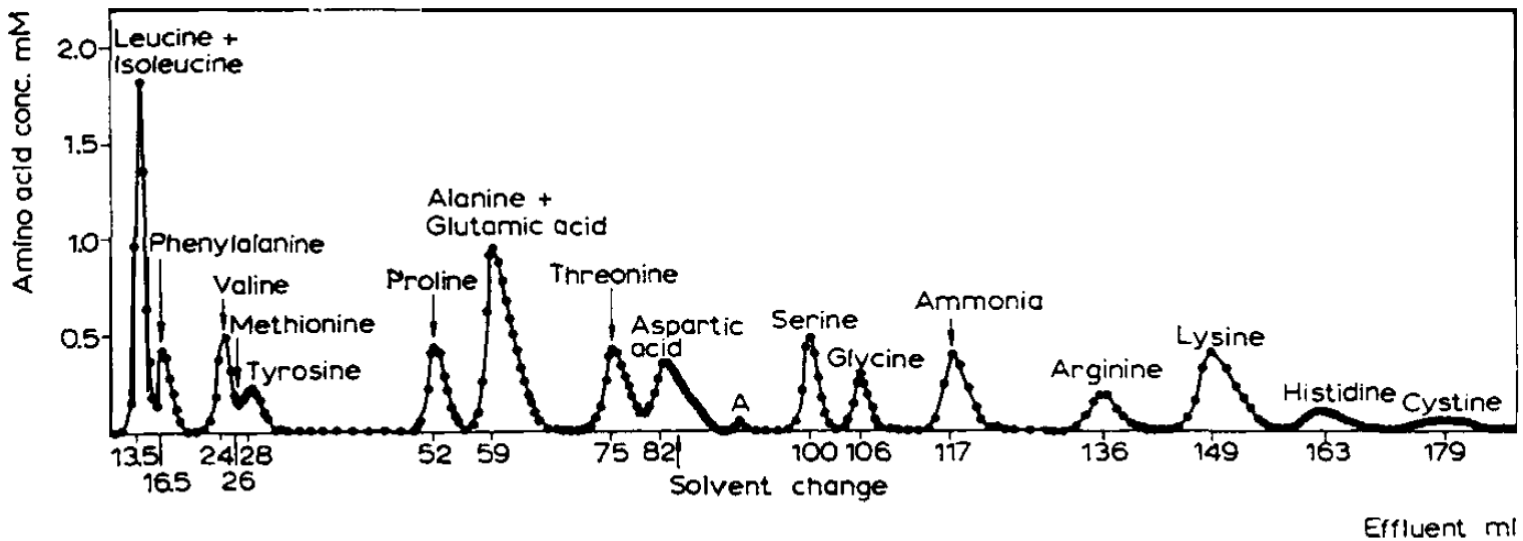

Fig. 1. Separation of a mixture of amino-acids on a starch chromatogram, as shown by analysis of the effluent. (S. Moore and W. H. Stein, *J. Biol. Chem.*, 178 (1949) 53.)

Fig. 2. Two-dimensional paper chromatogram of an extract of potato, coloured with ninhydrin showing free amino-acids, etc. (C. E. Dent, W. Stepka, and F. C. Steward, *Nature,* 160 (1947) 682.)

Partition chromatography has likewise been valuable for assessing the purity of amino acids and simple peptides and for studying the actions of enzymes so far as such simpler molecules are concerned. Previously unsuspected "transpeptidations" and syntheses have been revealed by this means.

The method has proved useful for the various procedures for allocating free functional groups within molecules of proteins or peptides; thus at present we have procedures for recognition of free amino groups by substitution with dinitrophenyl and other radicals, and of carboxyl groups by reduction to the corresponding alcohols or by enzymic hydrolysis of terminal amino-acids. These methods nearly always use partition chromatography as a final stage in the identification.

C-D, and D-E, permitting an unequivocal reconstruction. Martin and I, with R. Consden and A. H. Gordon, were able in this way, mainly using partition-chromatographic methods, to determine the amino-acid sequence in gramicidin-S, which is probably a cyclic decapeptide (Fig. 3). Subsequently F. Sanger and colleagues have elucidated by similar methods what may be the entire peptide sequences in the structure of ox insulin, the minimum molecule of which embodies 51 amino-acid residues. One of the two sequences established is shown in Fig. 4.

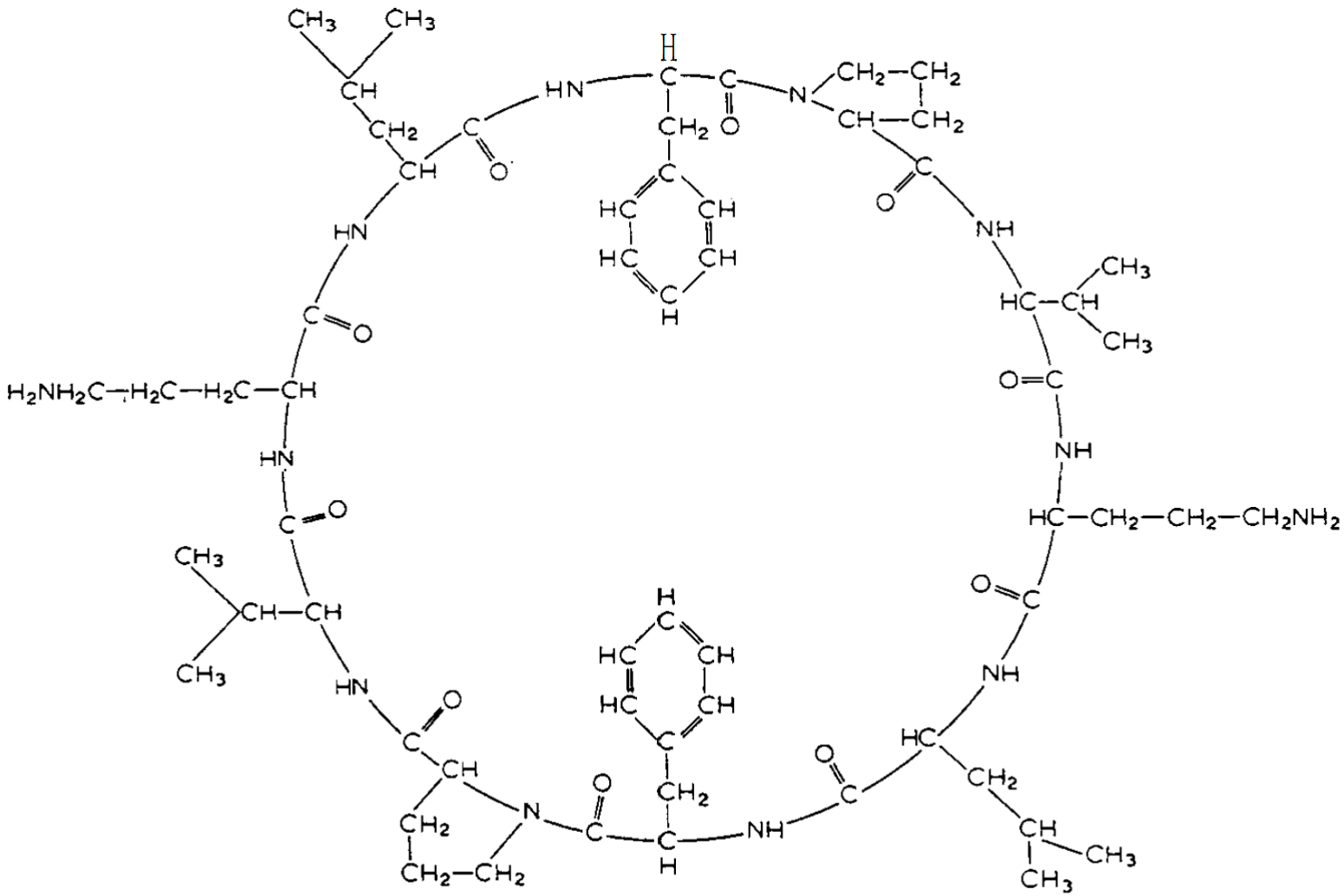

Fig. 3. Probable formula of gramicidin-S.

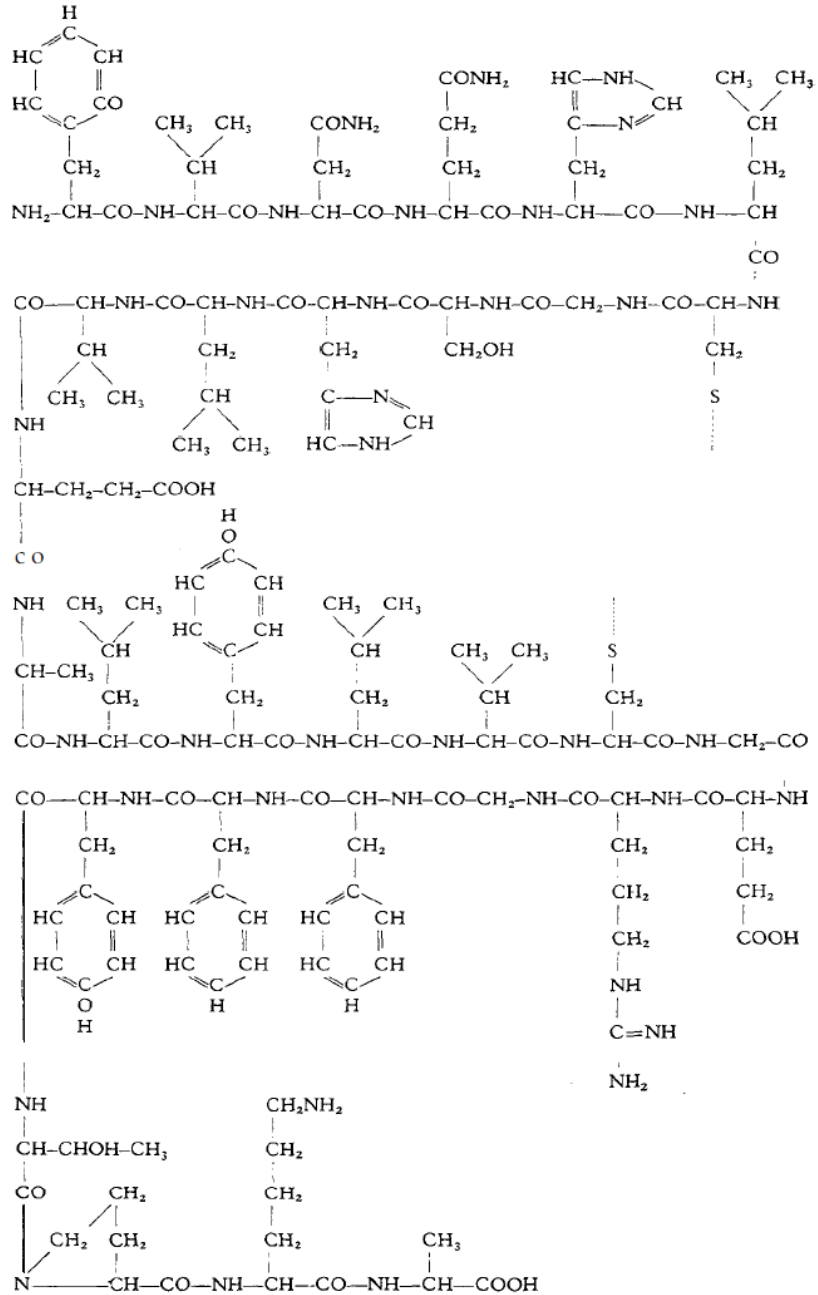

Fig. 4. Probable formula of the phenylalanyl chain in ox insulin.

mode of linkage of the component parts of more complicated molecules by substitution methods or by partial hydrolysis methods. Special mention should be made of the discovery of series of complicated fructosan-like oligosaccharides in the juices of various plants by means of paper partition chromatography (cf. Fig. 5). Partition chromatography has also proved almost ideally adapted for analysing the hydrolysis products of methylated polysaccharides, and permits more accurate analysis with much smaller quantities of material, thereby greatly increasing the scope of the methylation method as a step in determining the structure of natural polysaccharides. It gives me great pleasure that my teacher D. J. Bell was the first to use the method for this purpose.

It is evident that partition chromatography has considerable application in the study of the nucleic acids and their breakdown products, the third great chemical family of biological importance. Here the method can fulfil a sim-

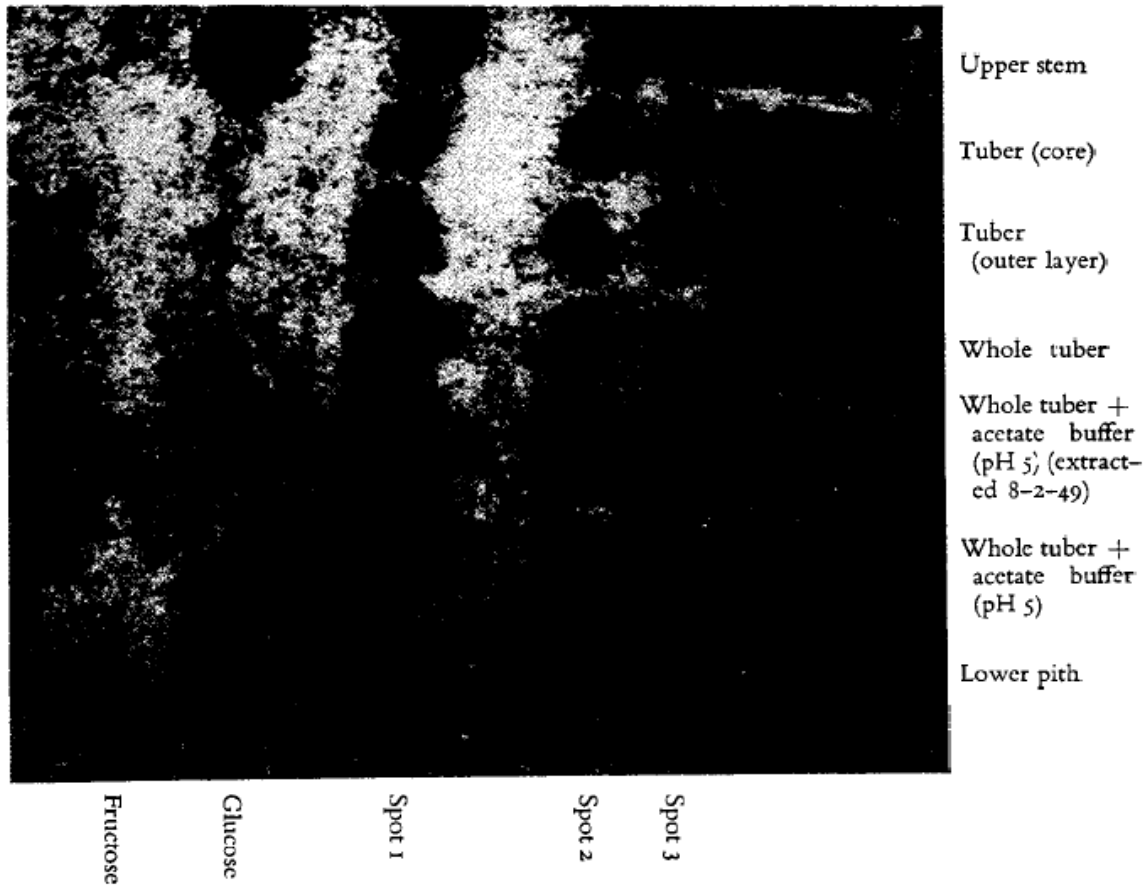

Fig. 5. One-dimensional paper chromatograms of extracts from the Jerusalem artichoke (*Heliunthus tuberosus* L.) showing fructosan-like oligosaccharides. (J. S. D. Bacon and J. Edelman, *Biochem. J., 48* (1951) 114.)

Partition chromatography has now been used for studying a wide variety of other substances of biological interest. Time prevents detailed discussion,

It must further be remembered that these partition methods are only one group in a wide family of refined physicochemical separation procedures developed during the present century and especially during the past decade. Adsorption chromatography, using M. S. Tsvett's elution development and such agents as alumina and charcoal, is a very powerful method giving useful separations, in many cases where partition chromatography is not very effective. Its field of application has been considerably extended by the displacement and carrier-displacement development procedures originated by A. Tiselius and colleagues. Further, it is only in recent years that the potentialities of the newer ion-exchange materials for chromatography have at all fully been realized. And leaving aside chromatography altogether, there are a variety of electrical-transport procedures which will effect refined separations of charged substances. With all these methods at his disposal it has become, for the analyst, essentially a routine matter to separate any pair of substances of molecular weight lower than, say, 500.

Biography

Richard Laurence Millington Synge

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Born	28 October 1914 Liverpool, England
Died	18 August 1994 (aged 79) Norwich, England
Alma mater	Winchester College Trinity College, Cambridge
Known for	Paper chromatography Partition chromatography
Awards	FRS (1950) Nobel Prize in Chemistry (1952) John Price Wetherill Medal (1959)
	Scientific career
Fields	biochemist