CHROM. 5101

Chromatography of plant constituents on crosslinked dextrans in phenol–acetic acid–water mixtures

Chromatography in phenol–acetic acid–water (PAW) mixtures on Sephadex* of the G-series (dextran crosslinked with epichlorhydrin) has proved useful for fractionating polypeptides according to their primary-valence molecular weight, without complications from association of subunits or retardation by aromatic amino acid residues[1,2]. However, the Sephadexes of lower degree of crosslinking (G->75) do not swell suitably in PAW, so that this technique has not proved useful for polypeptides above mol. wt. 5,000. The lipophilic O-hydroxypropylated LH-Sephadex[3] seemed to offer hope for extending upwards the range of molecular weight which could be handled. At present, only LH-20 is commercially available. The late Dr. B. GELOTTE (of AB Pharmacia, Uppsala) kindly provided some experimentally prepared higher members of the LH series for us to test in PAW. These showed promise with proteins in the lower molecular-weight range (5,000–20,000). However, we found considerable sorptive retention of phaeophytin by LH-Sephadexes in PAW, whereas with G-Sephadexes phaeophytin behaves normally (see below). This deterred us from using LH-Sephadexes for fractionating peptides and proteins of leaf extracts in PAW (*cf.* refs. 4, 5). Recently PUSZTAI AND WATT[6,7] have found Biogel P-100** (crosslinked polyacrylamide) suitable for handling a wide molecular-weight range of peptides and proteins in PAW.

More recently, we have become interested in fractionating those neutral and weakly acidic leaf constituents which do not migrate electrophoretically in PAW[5,8]. Most of these behaved as low-molecular-weight substances on Sephadex G-75*** (see ref. 5). We accordingly did model experiments with a number of typical plant constituents to assess whether Sephadex G-25 in PAW would give a further fractionation according to molecular weight. The results (Fig. 1) show that, whereas the amino acid and lipid derivatives studied, as well as phaeophytin, behaved more or less 'normally', there was appreciable sorptive retention of polyphenolic and polycarboxylic compounds.

Similar sorption has been noted by other authors[9–23] with various Sephadexes used in aqueous solvents, and has been to some extent mitigated by addition of organic solvents. We had hoped that the high concentrations of phenolic and carboxylic groups in PAW would antagonize such sorption, but have, on the whole, been disappointed.

Materials studied

These are listed in the legend to Fig. 1. The phaeophytin arose spontaneously in PAW from a mixture of chlorophylls *a* and *b* (kindly provided by Dr. R. HILL, F.R.S.). N-acetyl-DL-alloisoleucine[24], N,N'-dibenzoyl-DL-lysine[25] and N-2,4-dini-

* AB Pharmacia, Uppsala, Sweden.

** Bio-Rad Laboratories, Richmond, Calif., U.S.A.

*** Recoveries of bound amino acids after fractionation were good. The excess recoveries mentioned[5] have now been traced to contamination of the water used for evaporation of the phenol, and did not arise from the Sephadex.

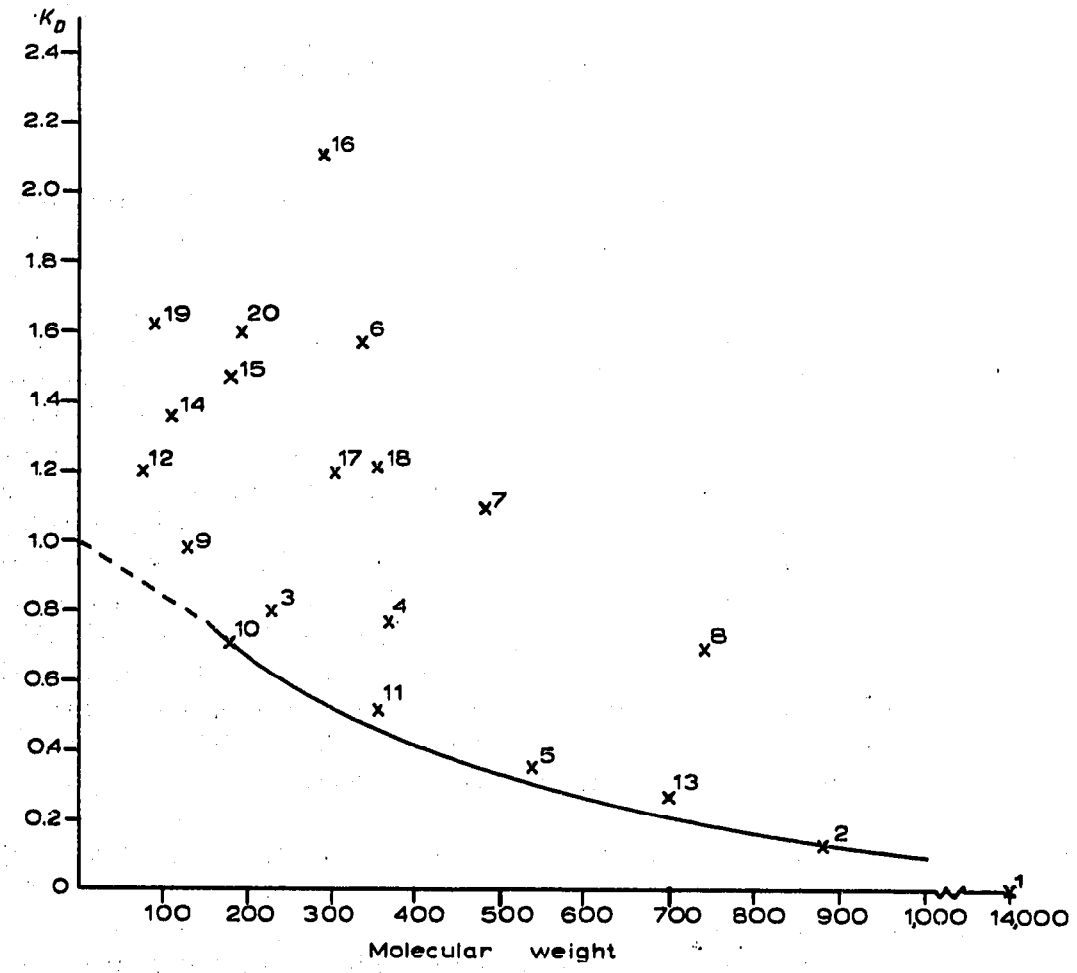

Fig. 1. Relation of K_D with molecular weight for: 1 = cytochrome *c*; 2 = phaeophytin; 3 = N-2,4-dinitrophenylethanolamine; 4 = N^{α}-2,4-dinitrophenyl-L-tryptophan; 5 = β-carotene; 6 = quercetin; 7 = quercitrin; 8 = robinin; 9 = L-pyrrolidonecarboxylic acid; 10 = N-acetyl-DL-alloisoleucine; 11 = N,N′-dibenzoyl-DL-lysine; 12 = glycine; 13 = phosphatidylethanolamine; 14 = pyrocatechol; 15 = caffeic acid; 16 = catechin; 17 = nordihydroguaiaretic acid; 18 = chlorogenic acid; 19 = oxalic acid; 20 = citric acid. The curve represents the function: K_D = 2.60 — 0.84 Log_{10} (mol. wt.) for mol. wts. 150–1000. The distance of each point above the curve serves as a measure of the sorption of each substance by the gel matrix.

trophenylethanolamine[26] were synthesised in our laboratory. The others were obtained commercially.

Procedure

Gel beds (9.4 cm^3) were prepared[4] using Sephadex G-25 (fine: Lot No 2726 or To 4150) in phenol–acetic acid–water (1:1:1, w/v/v). The elution volumes (V_e) for the coloured compounds (1–8) were estimated by eye for emergence of the centres of the zones. For the colourless compounds, small fractions were collected and the zone centres located by spray tests of aliquots of these spotted on filter paper as follows: RYDON AND SMITH reaction[27] (9); 0.1% (w/v) triketohydrindene hydrate in water-saturated butan-1-ol (12, 13); 0.4% (w/v) ferric chloride in dilute aqueous HCl (14–18); 0.04% (w/v) Bromocresol Green in 95% (v/v) aqueous ethanol (19, 20). Com-

pounds 10 and 11 gave poor RYDON AND SMITH reaction, and were located by evaporating aliquots of the fractions to dryness and titrating (Bromothymol Blue) against 0.01 N $Ba(OH)_2$.

The distribution coefficient (K_D) for each compound was calculated using eqn. A[28].

$$K_D = \frac{V_e - V_0}{V_i} \qquad \text{(A)}$$

where V_0 is the void volume (elution volume for cytochrome c) and V_i the volume of solvent within the grains. V_i was calculated from eqn. B.

$$V_i = V_t - V_0 - a\,v_g \qquad \text{(B)}$$

where V_t is the total bed volume, a is the wt. of dry Sephadex used (g) and v_g the partial specific volume of the Sephadex, assumed to be 0.6 $cm^3 \cdot g^{-1}$ (see ref. 29).

We thank Miss JANICE J. PAYNE for skilful assistance with this work.

Agricultural Research Council's Food Research Institute, Colney Lane, Norwich, NOR 70F (Great Britain)

W. M. LAIRD
R. L. M. SYNGE

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Received October 21st, 1970