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The chromatography of polylysine

In the chromatography of partial hydrolysates of polylysine on columns of carboxymethyl-cellulose[1], conditions were found that would resolve the first 20 members of a homologous series of lysine polypeptides. The resolution of a partial acid hydrolysate of polylysine is shown in the upper chromatogram of Fig. 1. The first 5 peaks in order of their emergence from the column were identified as lysine, di-, tri-, tetra-, and penta-lysine respectively by comparative chromatography on paper[2] and carboxymethyl-cellulose columns using lysine, di-lysine[3], and the products of trypsin hydrolysis of poly-L-lysine[2] as reference compounds. In this way, the peak number was

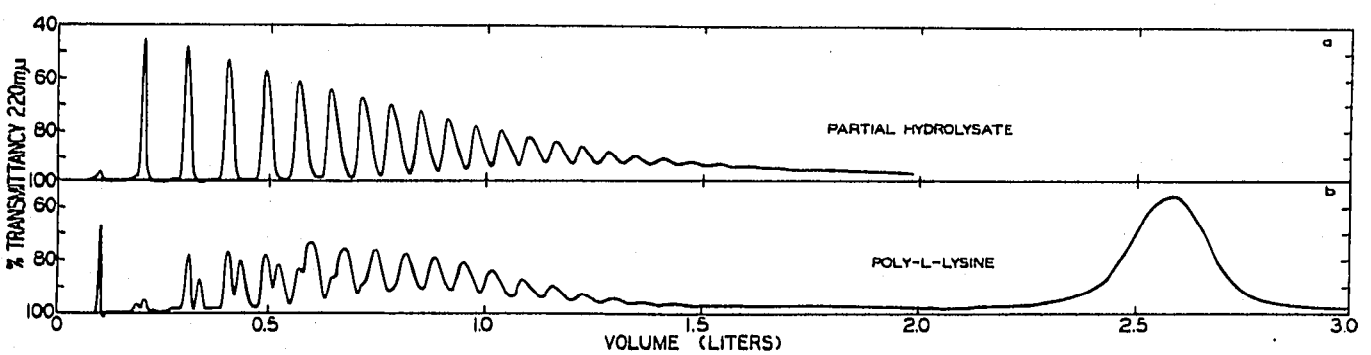

Fig. 1. Chromatograms of polylysine developed on a 40 × 0.9 cm column of 4 g carboxymethyl-cellulose (0.7 mequiv./g capacity, Na^+ form) with an exponential gradient of NaCl. The 1 l constant volume mixing flask, initially filled with water, was replenished with 0.82 *M* NaCl (after 2 l of flow, with 1.73 *M* NaCl) at 2.0 ml/min. Upper chromatogram is of a partial acid hydrolysate of 30 mg high molecular weight poly-L-lysine. Lower chromatogram is of 40 mg poly-L-lysine·HCl synthesized by initiating polymerization of ε-carbobenzoxy-L-lysine N-carboxy anhydride in dioxane (4% w/v) with NH_3 at a molar ratio of 12 and after 48 h reacting the polymerization mixture with anhydrous HCl at 90°.

shown to be identical with the number of lysine residues per molecule in each fraction.

A plot of the logarithm of the peak number or the number of lysine residues per molecule against the molarity of sodium chloride in the column at elution with a linear gradient disclosed a simple correlation between the molecular weight and the eluent concentration. This relationship is shown in the straight line labeled polylysine in Fig. 2. That this relationship may be a general one for the chromatography of other polyelectrolytes on substituted cellulose ion-exchange columns was suggested by plotting the published data on the chromatography of polythymidylic acid[4] and

polyglutamic acid[5] on DEAE-cellulose (Fig. 2). This linear relationship between the logarithm of the number of monomer residues per molecule larger than the trimer and the molarity of salt required for elution suggests a new method for the simultaneous separation and determination of both the molecular weight and the size distribution of the lower members of such homologous series of polyelectrolytes.

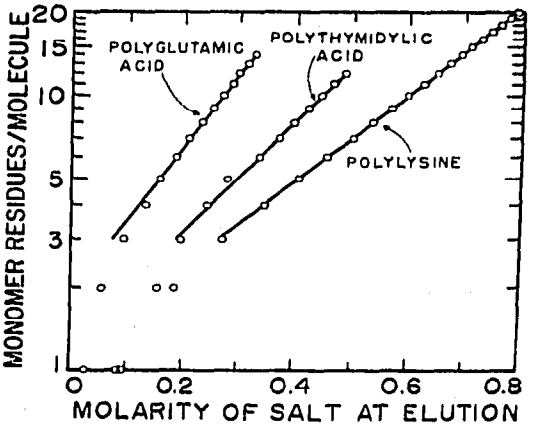

Fig. 2. Relationships of molecular weight to salt concentration in linear gradient elution analysis. Polylysine was developed on the column of Fig. 1 with a linear gradient (1 mM/min) of NaCl. Polythymidylic acid data are from KHORANA AND VIZSOLYI[4]. Polyglutamic acid data are from MILLER[5].

This linear correlation between the logarithm of the degree of polymerization and the molarity required for elution underwent nearly parallel translation towards higher molarities as the length of the carboxymethyl-cellulose column was changed from 16 to 150 cm or as the gradient rate was increased from 1 to 3 mM/min. Hence, in such chromatographic analyses, reproducible results are obtained only if conditions are carefully controlled.

The literature indicates that in the ion-exchange chromatography of polyelectrolyte molecules, the R_F would tend to be either 0 or 1, and would change abruptly from one extreme to the other when the composition of the eluent is changed within a very narrow range[6,7]. PORTER[8] has pointed out that the principal disadvantage of ion-exchange cellulose chromatography lies in the difficulty of finding conditions in which there is a finite partition coefficient of polyelectrolyte molecules between the ion-exchange cellulose and the eluent. These conditions were realized with polylysine by the use of an exponential gradient produced by a constant volume mixer. Selected members of the polylysine series from the dimer to the 15-mer moved through a 40 cm column of carboxymethyl-cellulose during gradient elution analysis (Fig. 1) with R_F values between 0 and 0.31. Thus, when the 15-mer was developed with 0.55 M sodium chloride (the composition of eluate in which it emerged during exponential gradient development) it was eluted at 4.8 liquid hold-up volumes or with an R_F value of 0.21.

These techniques have been used for the analysis of polylysine preparations, one of which is shown in the lower chromatogram of Fig. 1. Ninety-seven per cent of the 220 mμ absorbancy applied to the column was recovered in two widely separated regions. We conclude that this ammonia initiated polylysine preparation[9] had a bimodal molecular weight distribution. The members of the lower series emerged

in double peaks located near the position of each of the smaller peptides in the partial acid hydrolysate. At least two homologous polylysine series were present in the lower molecular weight fractions which probably differ in their amino or carboxyl terminal end. The lower molecular weight series contained peptides with from one to about twenty lysine residues per molecule. The degree of polymerization of the higher series was estimated at 250 from the intrinsic viscosity in dimethyl formamide of the recarbobenzoxylated fraction[10,11]. Such analyses have shown a bimodal molecular weight distribution in the products of polymerization of ε-carbobenzoxy-L-lysine N-carboxy anhydride initiated in dioxane solution with ammonia at molar ratios of monomer to initiator of from 6 to 50[12]. We believe that the new principles illustrated in this study will be of value in the isolation and analysis of polyamino acids, polynucleotides, proteins, and other polyelectrolytes.

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Detection of ketosteroids on chromatograms

The detection of ketosteroids may be carried out on untreated strips by means of contact photography in U.V.-light at about 253 mμ. This method, however, is specific for Δ^4-3-ketosteroids and therefore saturated ketosteroids are not detectable in this way. The strips may also be treated with various, more or less specific reagents; in this case fluorescence in U.V. light or colouring of the spots occurs. However, treated chromatograms cannot be used for further quantitative evaluation, owing to the