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Effect of ionic strength on poly(D-Asp-L-Lys) in Sephadex gel filtration

Poly(D-Asp-L-Lys), a polypeptide, with a repeating sequence of trifunctional amino acids was found to behave anomalously on Sephadex G-50 fine with changes in ionic strength of the eluent. This kind of behavior of the protein model studied here is new and different from that reported in the literature for the other proteins[1]. In Sephadex gel filtration of proteins it has been observed[2] that the ionic strength of the eluent is independent of the elution volume of proteins. However, GELOTTE[3] and PORATH[4] have shown that phenylalanine, tyrosine and tryptophan are adsorbed to dextran gels. A protein with high contents of these amino acids might behave anomalously on Sephadex columns.

Poly(D-Asp-L-Lys), an unknown protein model[5] was synthesized for biological investigation. Polypeptides, with a repeating sequence of amino acids are usually prepared by the self-condensation of the active ester of the tri- or tetrapeptide. Normally this results in a mixture of higher and lower molecular weight polymers. The polymer obtained in this study showed an average molecular weight of 6600, as determined by ultracentrifugation.

For biological investigation we needed a polymer with the highest molecular weight. This was done by separating the fractions of polymer by using the Sephadex gel filtration technique. We wish to report here the anomalous results shown by poly(D-Asp-L-Lys) on Sephadex G-50 fine on changing the ionic strength of the eluent. The best separation was obtained with an aqueous buffer solution containing ammonium carbonate of an ionic strength of 0.03.

The column was standardized with proteins of accurately known molecular

TABLE I

ELUTION VOLUME (ml) OF POLYPEPTIDES

Conditions: 1.5 × 90 cm column at 25°–27° with a flow rate of 23 ml/h for all ionic strengths. Void volume (V_0) was determined by using Blue Dextran 2000 and was found to be 51.5 ml.

Protein	*Ionic strength*			*Mol. wt.*
	0.0	*0.03*	*0.3*	
Chymotrypsin[a] (beef pancreas)	58.5	57.0	56.0	22 500
Myoglobin[a] (horse heart)	67.0	68.0	69.5	17 800
Cytochrome[a] (horse heart)	78.0	78.5	78.7	12 400
Insulin[a] (bovine pancreas)	107.0	107.8	108.0	6 000
Adrenocorticotropic[a]	116.0	117.3	119.0	4 566
Poly(D-Asp-L-Lys)	58–89[b]	96.0	125–145[b]	6 900[c]
	—	112.0	—	5 400[c]

[a] These proteins were obtained from Mann Research Laboratories, New York.

[b] All the fractions showed the mean mol. wt. to be 6600 when determined by ultracentrifugation. But on the basis of standardization of the column (a graph of elution volume (V_1)/void volume (V_0) against the logarithm of molecular weight for all the standard proteins was drawn and was found to be a linear function), molecular weights of 24 000–12 000 were found when the ionic strength was 0.0 and 3000 to 1500 when the ionic strength was 0.3.

[c] These values, determined by ultracentrifugation, were in perfect agreement with standardization of the column when ionic strength was 0.03.

weights using glass distilled water (ionic strength thus zero) and aqueous ammonium carbonate buffer solutions with ionic strengths of 0.03 and 0.3, respectively. In all the cases the protein was eluted within the range of 3 to 5 ml.

Poly(D-Asp-L-Lys) was eluted from the column at all three ionic strengths. In each case thirty fractions of 3 ml each were collected and the presence of the polymer was detected by spectrophotometric analysis using a Cary-14 spectrophotometer. Only two major fractions showed the presence of polymer at an ionic strength of 0.03. Both the fractions showed the expected molecular weights on the basis of the standardization of the column and by ultracentrifugation. But, at ionic strength of zero, when glass distilled water was used as eluent the separation was found to be over a wide range. Twelve fractions which came immediately after the void volume (V_0), showed the presence of polymer. When the ionic strength was changed to 0.3 the polymer was eluted at a higher elution volume and over a wide range. In both the later cases each fraction was lyophilized and the molecular weight of each fraction was determined separately by ultracentrifugation. These values differ very much from the values obtained on the basis of the standardization of the column. The results are given in Table I. Currently we are investigating this problem further using protein models containing other amino acids, synthesized in this laboratory.

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