THE BEHAVIOR OF HORMONALLY-ACTIVE PROTEINS AND PEPTIDES OF THE ANTERIOR PITUITARY ON CROSS-LINKED DEXTRAN POLYMER GELS*

PETER M. SANFELIPPO** AND JOHN G. SURAK
Marquette University, Chemistry Department, Milwaukee, Wis. (U.S.A.)

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INTRODUCTION

The development of the techniques of gel filtration by PORATH AND FLODIN[1] offered a simple and rapid method for the fractionation of water-soluble substances. These workers demonstrated that with carbohydrates the separation was dependent on the molecular size of the substances. PORATH[2,3] demonstrated that these techniques were applicable to the separation of proteins, peptides, and amino acids. LINDNER, ELMQVIST AND PORATH[4] utilized the technique to fractionate an extract of posterior lobes of hog pituitaries into a hormonally-active fraction and a fraction of hormonally-inert material. They subsequently utilized the technique to further purify the active fraction and obtained an approximately twenty-fold increase in specific activity. PORATH AND SCHALLY[5] have utilized gel filtration to effect separations of a number of posterior pituitary hormones. They were able to separate α-melanocyte stimulating hormone (MSH) from lysine vasopressin and α-from β-MSH.

WILHELMI, FISHMAN AND RUSSELL[6], WILHELMI[7,8], and ELLIS[9,10] have described conditions for the preparation of extracts of the pituitary gland which contained a number of the gland's hormones. Originally the single extracts were separated into the various hormonal activities by salt or ethanol fractionation and by chromatography. WILHELMI[8] and ELLIS[10] have suggested schemes for separation during the initial extraction of the pituitary glands based on varying conditions of pH and ionic strength.

This communication reports the results of a study of the behavior of a number of the individual anterior pituitary hormones when subjected to gel filtration. This investigation was carried out under the pH and ionic strength conditions encountered in initial extracts of the pituitary. This study is an evaluation of the individual hormones, prior to the utilization of gel filtration in the fractionation of crude pituitary extracts.

METHODS

Gel filtration experiments were carried out using the cross-linked polysaccharide Sephadex G-50*** (medium particle size). The bed material was washed with distilled

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** Present address: Marquette University School of Medicine.
*** Pharmacia, Uppsala, Sweden.

water. Fine particles were removed by repeated washing and decantation. The Sephadex was suspended in the particular eluent to be used in the experiment and poured into columns of 1 cm^2 cross-sectional area to a height of 10 cm.

The following eluents were used in these experiments: (1) 0.15 *M* sodium chloride; (2) 0.02 *M* acetic acid; (3) 0.10 *M* hydrochloric acid; (4) pH 4.0, 0.02 *M* acetate buffer with 0.10 *M* ammonium sulphate; (5) pH 5.5, 0.02 *M* acetate buffer; (6) pH 5.5, 0.02 *M* acetate buffer with 0.30 *M* potassium chloride.

The following anterior pituitary hormone preparations* were used: (1) porcine (oxycellulose purified) adrenocorticotrophic hormone (ACTH) No. 216174-3; (2) bovine thyrotrophic hormone (TSH) No. R-216-174-1; (3) equine lactogenic hormone (LH) No. 216-171-15; (4) bovine somatotrophic hormone (STH) No. 216-176-4; (5) porcine follicle stimulating hormone (FSH) No. 216-175-6. Solutions containing 1 mg/ml were prepared and 100 μl aliquots used in the experiments.

All experiments were run at room temperature. Volumetric fractions of 1 ml were collected. These fractions were immediately analyzed using the LOWRY modification of the Folin reaction[11]. All readings were made at 740 mμ using a Coleman Universal Spectrophotometer. Void volume determinations were performed using 100 μl aliquots of a 5 % hemoglobin solution. The internal volume was determined using the following relation:

$$V_i = (V_t - V_0) \frac{W_r d}{1 + W_r}$$

where V_i is the internal volume; V_t is the total bed volume; V_0 is the void volume; W_r is the water regain (equal to 5.0 g/g); and d is the wet density (equal to 1.06 g/ml).

It was then possible to evaluate the degree of diffusion for the different hormone preparations using the following relation:

$$K_D = \frac{V_{el} - V_0}{V_i}$$

where K_D is the distribution coefficient for a solute between the internal and external solvent; V_{el} is the volume necessary to elute the solute from the polymer gel; V_i is the internal volume or the volume of solvent contained within the polymer gel; and V_0 is the void volume or volume of solvent external to the polymer gel.

RESULTS

Table I summarizes the K_D values obtained in this study for these hormones. As the K_D approaches 0 this indicates increasing exclusion of solute from the internal solvent phase. As the K_D approaches 1, this indicates complete diffusion of the solute within the internal solvent phase. The techniques employed permitted replicate determinations of the K_D to be made with an average deviation of $9.8 \cdot 10^{-3}$ K_D units.

Fig. 1 indicates the result of plotting the K_D value against the reciprocal of the Briggsian logarithm of the molecular weight. The linear relation which exists between these two sets of data is described by the equation:

$$K_D = \frac{k}{\log \text{M.W.}} - C$$

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TABLE I

K_D VALUES OF ANTERIOR PITUITARY HORMONES

Hormone	*Molecular weight*	*K_D with different eluents*					
		1	*2*	*3*	*4*	*5*	*6*
ACTH	3,500	0.95	1.00	—	0.87	—	0.30
TSH	10,000	—	0.87	—	—	0.27	—
LH	40,000	0.49	—	0.34	0.32	0.20	—
STH	45,000	—	—	0.25	—	—	0.07
FSH	70,000	0.40	0.68	0.17	0.00	0.17	0.04

where K_D is the distribution coefficient or ratio between the concentrations of the solute in the void volume and internal volume; K is a constant evaluated from the slope; C is a constant equal to the value of the Y-intercept. The values obtained for K for the various eluent systems were determined to be: 7.25 with 0.15 M sodium chloride; 4.24 with 0.02 M acetic acid; 14.48 with 0.10 M hydrochloric acid; 11.90 with

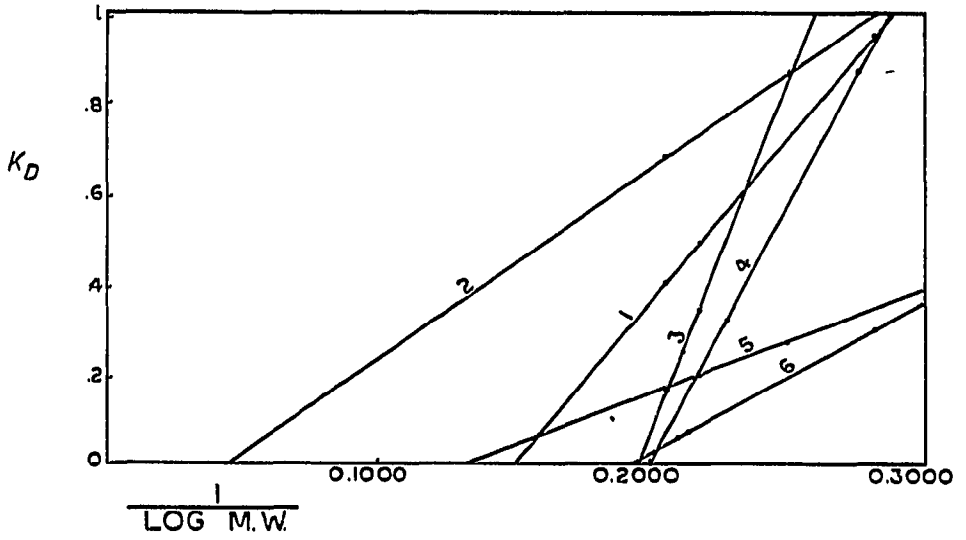

Fig. 1. Plot of K_D *versus* 1/log M.W. Eluent: (1) 0.15 M sodium chloride; (2) 0.02 M acetic acid; (3) 0.10 M hydrochloric acid; (4) pH 4.0, 0.02 M acetate buffer with 0.10 M ammonium sulphate; (5) pH 5.5, 0.02 M acetate buffer; (6) pH 5.5, 0.02 M acetate buffer with 0.30 M potassium chloride.

pH 4.0, 0.02 M acetate buffer with 0.10 M ammonium sulphate; 2.23 with pH 5.5, 0.02 M acetate buffer; 3.44 with pH 5.5, 0.02 M acetate buffer with 0.30 M potassium chloride.

The above results indicate that the individual anterior pituitary hormones studied (ACTH, TSH, LH, STH, and FSH-) behave on Sephadex G-50, under conditions of pH ranging from 1.0 to 6.8 and of salt concentration from 0.02 to 0.32 M, in a manner related to their molecular weights. The results further indicate that the distribution coefficients for these hormones on Sephadex G-50 can be predicted by the equation:

$$K_D = \frac{k}{\log \text{M.W.}} - C$$

DISCUSSION

Application of gel filtration, using materials such as Sephadex, seems suited to the fractionation of extracts of anterior pituitary. It is simpler and more economical with respect to time and to the valuable hormone products than salt or ethanol fractionation or multiple step chromatography. The possibility of interference with the observed, well-defined behavior in the presence of multiple hormonal protein components exists. This is presently being evaluated. The results obtained in these purely model systems do suggest that gel filtration on cross-linked dextran polymers can profitably be applied to the problem of fractionation and purification of extracts of anterior pituitary tissue for its hormonal activities.

SUMMARY

The possible application to the fractionation and purification of the various hormonal activities of the anterior pituitary has been examined. This investigation has dealt solely with the behavior of a number of the anterior pituitary hormones in model systems which parallel the conditions of pH and salt concentration found in extracts of anterior pituitary tissue. The behavior of these hormones, over the pH range 1.0–6.8 and salt concentration range of 0.02–0.32, is linearly related to the reciprocal of the log of their molecular weight. The distribution coefficient for a given hormone in a given system can be predicted by the equation:

$$K_D = \frac{k}{\log \text{M.W.}} - C$$

REFERENCES

1 J. PORATH AND P. FLODIN, *Nature*, 183 (1959) 1657.
2 J. PORATH, *Clin. Chim. Acta*, 4 (1959) 776.
3 J. PORATH, *Biochim. Biophys. Acta*, 39 (1960) 193.
4 E. B. LINDNER, A. ELMQVIST AND J. PORATH, *Nature*, 184 (1959) 1565.
5 J. PORATH AND A. V. SCHALLY, *Endocrinology*, 70 (1962) 738.
6 A. E. WILHELMI, J. B. FISHMAN AND J. A. RUSSELL, J. *Biol. Chem.*, 176 (1948) 735.
7 A. E. WILHELMI, *Ciba Found. Colloq. Endocrinol.*, 13 (1960) 25.
8 A. E. WILHELMI, *Can. J. Biochem. Physiol.*, 39 (1961) 1659.
9 S. ELLIS, *J. Biol. Chem.*, 233 (1958) 63.
10 S. ELLIS, *Endocrinology*, 69 (1960) 554.
11 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.