ON THE INTERACTION BETWEEN ACTH AND POLYPHLORETIN PHOSPHATE

by

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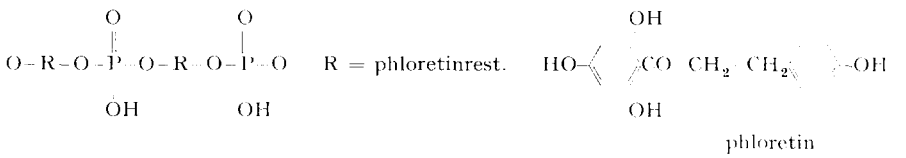
It has been demonstrated that ACTH can be linked to certain negatively-charged enzyme inhibitors and that administration of ACTH in such a form offers therapeutic advantages, *e.g.*, prolonged (retarded) effect[5,4]. These inhibitors are of the type polymeric phosphates of either phloretin or aromatic hydroxy and amino compounds. On the basis of biological and clinical experiments, polyphloretin phosphate (POFF)



phloretin

proved most suitable for medical use because of its very low toxicity.

In addition to the pharmaceutical, clinical and biological experiments a study of the complex formation between ACTH and POFF was performed, the results of which are presented in this paper. Both ACTH and POFF are charged compounds of a relatively high molecular weight. The basic groups of ACTH and the acidic groups of POFF are considered to form some sort of a salt-like "addition compound". On account of these properties of ACTH and POFF, the authors applied the electrophoresis and ultracentrifugation techniques.

EXPERIMENTAL

ACTH: A pig ACTH preparation with low activity, 1.0 I.U./mg, but homogeneous in free electrophoresis was used (lot No. 5-1954).

POFF: Polyphloretin phosphate was prepared according to DICZFALUSY *et al.*[2] and the product (lot Fl 1023) was further purified by electrodialysis and lyophilized. Average molecular weight 15,000.

Electrophoresis. The electrophoresis experiments were performed according to the well-known technique of TISELIUS AND SVENSSON[8,7] and also as zone electrophoresis on starch. The electrophoretic mobility, u is expressed in units of 10^{-5} cm^2/volt sec.

Ultracentrifugation. The sedimentation in the Svedberg ultracentrifuge was recorded by the scale method. The sedimentation constant is given in Svedberg units (10^{-13} c.g.s.).

Measurements on the ACTH activity. Biological assay of the corticotrophic activity was carried out by the adrenal ascorbic acid depletion method of SAYERS, SAYERS AND WOODBURY[6].

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RESULTS

Electrophoresis. A series of experiments on samples of varying ratios of ACTH: POFF is recorded in Table I. Medium: phosphate buffer of ionic strength 0.1 and pH 6.8.

The ACTH and POFF preparation separately (Expts. 1 and 8) exhibit only one peak, u_1 and u_3 respectively. In Experiments 2–7 there is also another peak belonging to a new compound of these two constituents. If the excess of POFF is large, the ACTH peak disappears, Fig. 1. It is, however, possible to determine the amount of free and bound ACTH from the electrophoretic patterns. The refractive index increment, dn/dc, of POFF is $180 \cdot 10^{-5}$ which also is the mean value adopted for proteins. Assuming the "addition" compound to have the same refractive index increment as its constituents the values of the last column of Table I have been computed.

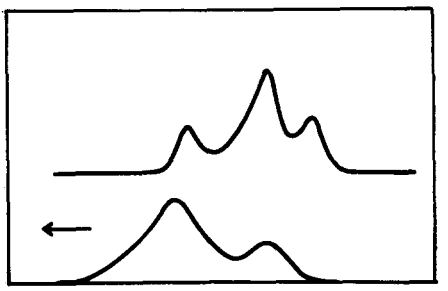

Fig. 1. Upper curve. Electrophoretic pattern 32 min after start in Expt. 2. Lower curve. Electrophoretic pattern 98 min after start in Expt. 7. (*Cf.* Table I). Direction of migration indicated by the arrow.

TABLE I

ELECTROPHORESIS OF MIXTURES OF ACTH AND POFF IN VARYING PROPORTIONS, IN PHOSPHATE BUFFER, pH 6.8

Expt.	*Concentration in per cent*		*Ratio*	*Mobility*			*Bound ACTH in per cent of total ACTH*
	ACTH	*POFF*	*ACTH: POFF*	u_1	u_2	u_3	
1	0.30	—		5.4	—	—	
2	0.60	0.20	3:1	6.1	10.0	16.6	73
3	0.80	0.27	3:1	5.8	10.0	18.2	
4	0.30	0.30	1:1	6.2	10.2	15.7	80
5	0.40	0.40	1:1	6.9	12.0	18.0	85
6	0.20	0.40	1:2	5.2	12.2	—	
7	0.30	0.90	1:3	—	11.0	18.1	100
8	—	0.30		—	—	18.8	

The usual electrophoresis technique is not suitable for isolation and purification of relative large amounts of material. Zone electrophoresis, however, permits isolation of the "addition" compound in a fairly pure state. A mixture of 100 mg of ACTH and 100 mg of POFF was run on a horizontal starch column (3.5 × 26 cm) in sodium citrate-sodium hydroxide buffer of pH 6.8 for 48 hours. On the basis of spot test on a filter paper the column was divided in four fractions separately eluated with buffer. The eluates were dialyzed, lyophilized and the material thus obtained was analysed for nitrogen and Sayers activity, Table II.

Fraction II contains the main part of the ACTH activity and the electrophoretic mobility of this fraction measured in free electrophoresis at pH 6.8 and ionic strength 0.1 is identical with that of the presumed "addition" compound, $u = 10.3$ (*cf.* Table I).

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TABLE II

ZONE ELECTROPHORESIS ON STARCH OF A MIXTURE OF EQUAL AMOUNTS OF ACTH AND POFF IN SODIUM CITRATE-SODIUM HYDROXIDE BUFFER, pH 6.8

Fraction No.	*Migration in cm*	*Yield in mg*	*Nitrogen content per cent*	*ACTH activity Units per mg N*
I	0–3	15	14.0	0.21
II	3–13	56	2.1	57
III	13–18	33	0.5	10
IV	18–24	9	0.2	10

Sedimentation in the ultracentrifuge. The sedimentation experiments were performed in phosphate buffer of pH 6.8 at 200,000 *g*. The sedimentation diagram of ACTH as well as of the POFF preparation alone showed only one polydisperse component the sedimentation rate being only slightly dependent on the concentration. Also in mixtures of ACTH and POFF, only one polydisperse component was detected (*cf.* Table III).

TABLE III

SEDIMENTATION EXPERIMENTS

Concentration in per cent		
ACTH	*POFF*	*s*
0.50		1.14
0.25		1.20
	0.50	0.94
	0.25	1.10
0.50	0.75	1.02

DISCUSSION

Electrophoresis experiments indicate some sort of equilibrium between ACTH and POFF on the one side and an addition compound of these two constituents on the other (*cf.* Table I). As expected, an increase of the relative amount of POFF results in an increased amount of complexed ACTH. The "hormone part" of ACTH is of a much more basic nature than the carrier proteins, properties which also have been utilised by ASTWOOD, RABEN AND PAYNE in the purification of ACTH on oxycellulose[7]. Similar results were obtained by DIXON *et al.*[8] with ion exchange. Consequently, the addition compound preferably contains the "hormone" part, a fact which is shown by the results of zone electrophoresis of ACTH and POFF. Fraction II (Table II) contained only about 12% of the total protein but almost all of the total ACTH activity in the Sayers test, *i.e.*, with these experimental conditions most of the Sayers activity was recovered as bound to POFF.

Sedimentation in the ultracentrifuge failed to reveal the existence of a addition compound of ACTH and POFF probably due to the fact that the differences in particle size are too small.

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SUMMARY

1. The interaction between ACTH and POFF (polyphloretin phosphate) was studied by means of electrophoresis and ultracentrifugation.

2. Electrophoresis experiments indicate that in solution there exists some sort of equilibrium between ACTH and POFF and an addition compound of these constituents.

3. By means of zone electrophoresis on starch, most of the active ACTH was recovered bound to POFF.

4. The difference in particle size between the addition compound and the constituents is too small to permit sedimentation analysis of the system.

RÉSUMÉ

1. L'interaction entre l'ACTH et le POFF (polyphlorétine phosphate) a été étudiée par électrophorèse et ultracentrifugation.

2. Les électrophorèses montrent qu'en solution il y a un certain équilibre entre l'ACTH et le POFF et que les deux corps forment un produit d'addition.

3. Par électrophorèse de zone sur amidon, la majeure partie de l'ACTH active a été récupérée liée au POFF.

4. La différence de taille des particules entre le produit d'addition et ses constituants est trop petite pour permettre une étude du système par sédimentation.

ZUSAMMENFASSUNG

1. Die Interaktion zwischen ACTH und POFF (Polyphloretinphosphat) wurde an Hand der Elektrophorese und der Ultrazentrifugierung untersucht.

2. Elektrophoretische Versuche deuten darauf hin, dass in Lösung eine Art Gleichgewicht zwischen ACTH und POFF, sowie einem Additionsprodukt dieser Konstituenten besteht.

3. Durch Zonenelektrophorese auf Stärke wurde der grösste Teil des aktiven ACTH in Verbindung mit POFF wiedergewonnen.

4. Der Grössenunterschied zwischen Partikeln des Additionsproduktes und der Konstituenten ist zu gering, um eine Sedimentierungsanalyse des Systems zu ermöglichen.

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