[CONTRIBUTION FROM THE VIRAL AND RICKETTSIAL RESEARCH SECTION, LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID CO.]

Studies with Corticotropin. IV. Ultracentrifugal Characterization of β-Corticotropin

BY RAYMOND A. BROWN, MAURICE DAVIES, MARY ENGLERT AND HERALD R. COX

RECEIVED APRIL 13, 1956

β-Corticotropin has been analyzed in the ultracentrifuge by means of the Archibald procedure and the synthetic boundary cell. β-Corticotropin behaves as a homogeneous substance in the ultracentrifuge. The molecular weights obtained by both procedures are in reasonable agreement with the minimum molecular weight of 4567 predicted from the amino acid ratios.

The isolation from porcine anterior pituitary of several fractions with corticotropin activity has been previously described.[1] A major portion of the corticotropin activity was found to be associated with one fraction, β-corticotropin. The ultracentrifugal characterization of this fraction is the subject of this paper.

Since it was important to determine the homogeneity of the preparation, it was necessary to use a procedure which would detect small deviations from homogeneity. The method of ultracentrifugal analysis developed by Archibald[2] provides a sensitive means for detecting heterogeneity, and recently has been shown to give reliable results when applied to data obtained with a standard substance in the Spinco ultracentrifuge.[3] Consequently this procedure was used to analyze β-corticotropin in the ultracentrifuge. For purposes of comparison, the substance was also examined in the synthetic boundary cell developed by Pickels, *et al.*[4]

Experimental

β-Corticotropin has a solubility which decreases with increasing salt concentration. The material has a tendency to aggregate when the limiting solubility is approached. Although it would have been desirable to include sufficient potassium chloride in the ultracentrifuge medium to minimize electrical effects, it was necessary to compromise this objective in order to obtain sufficient solubility and avoid aggregation. Solutions for analysis were prepared by dissolving a definite weight of β-corticotropin in the form of the acetate salt in a solution containing 0.05 mole of KCl and 0.05 mole of HCl per liter.[5] The actual concentration of the β-corticotropin was calculated from the weight of material added after correcting for the 10% water and 1% salt present in the sample.

Details of the experimental procedures used in determining molecular weights have been given in a previous paper.[3] In this experiment, the centrifuge was run at 37,020 r.p.m. with refrigeration, pictures being taken at 32-minute intervals. The thermocouple reading was 20.8° at the start and 20.6° at the end of the run. In order to convert the refractive index gradient to concentration gradient, the refractive index increment of corticotropin was assumed to be the same as that of bovine serum albumin corrected for the refractive index of the solvent (1.89×10^{-1} cm.^{3}/g.).

All the runs in the synthetic boundary cell were at 59,780 r.p.m., pictures being taken at 8-minute intervals. Base line pictures were obtained by using equivalent volumes of the solvent. The standard deviations of the gradient curves were calculated from the half-widths at the inflection point of traced projections.

(1) Part I, R. G. Shepherd, K. S. Howard, P. H. Bell, A. R. Cacciola, R. G. Child, M. C. Davies, J. P. English, B. M. Finn, J. H. Meisenhelder, A. W. Moyer and J. van der Scheer, THIS JOURNAL, **78**, 5051 (1956).

(2) W. J. Archibald, *J. Phys. Colloid Chem.*, **51**, 1204 (1947).

(3) R. A. Brown, D. Kritchevsky and M. Davies, *ibid.*, **76**, 3342 (1954).

(4) E. G. Pickels, W. F. Harrington and H. K. Schachman, *Proc. Nat. Acad. Sci.*, **38**, 943 (1952).

(5) Some etching of the cell centerpieces was observed with this solution. However, it was not enough to interfere with the analysis.

Results

Figure 1 is a plot of $1/rn(\partial n/\partial r)$ *vs.* r calculated from the 384- and 504-minute pictures. There is a trace of high molecular weight impurity in β-corticotropin which sediments rather rapidly at 59,780 r.p.m. This material is undoubtedly responsible for the deviation of the points from the curve at the bottom of the cell. Analysis of the 384-, 472- and 504-minute data gave a value of 0.51 for δ at the bottom of the cell and 0.50 at the meniscus. The material behaves as a homogeneous substance in the ultracentrifuge within the limits of experimental error.

A plot of $1/rn_0\ (\partial n/\partial t)$ calculated from the 384- and 504-minute pictures is shown in Fig. 2. In agreement with theory, the difference between the areas below and above the zero axis is small, being less than 1% of their sum. The sedimentation and diffusion constants were calculated from the average of the two areas.

The results obtained in the synthetic boundary cell

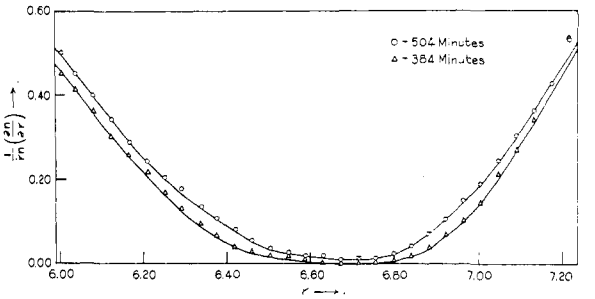

Fig. 1.—Plot of the function $\frac{1}{rn}\ (\partial n/\partial r)$ against r.

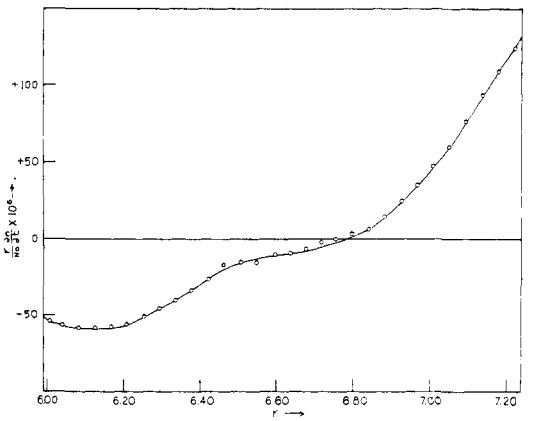

Fig. 2.—Plot of the function $r/n_0(\partial n/\partial t)$ calculated from the 384- and 504-minute picture against r.

with a 0.83% solution of β-corticotropin are shown in Figs. 3 and 4. The sedimentation and diffusion constants were calculated from the slopes of the best straight lines drawn through the experimental points.

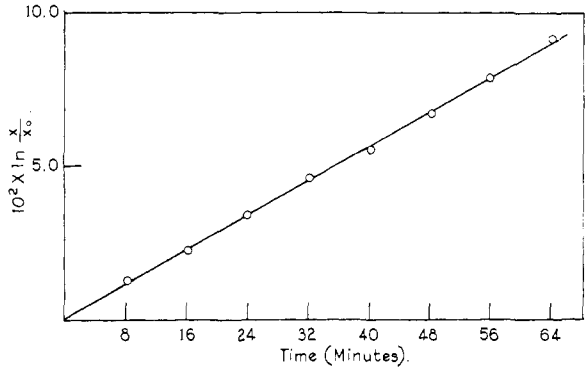

Fig. 3.—Plot of ln x/x_0 as a function of time for β-ACTH; initial concentration 0.826 g./100 ml.

Table I contains a summary of all the data. The partial specific volume of the acetate salt has been calculated from the volume given for the amino acid residues in Cohn and Edsall.[6,7] The change in electrostriction resulting from the suppression of the ionization of the carboxyl groups at low *p*H has been taken into account in the calculation.

TABLE I

Procedure	Initial concn. g./100 m.	$S_{20°}$	$10^6 \times D_{20°}$	Mol. wt. ($\bar{v}$ = 0.735
Archibald	0.455	0.49	1.05	4290
Velocity sed.	.826	.56	1.16	4400
Velocity sed.	.451	.54	1.13	4390

Discussion

Since β-corticotropin has been shown to behave as a homogeneous substance in the ultracentrifuge, the determination of the diffusion constants from the patterns obtained with the synthetic boundary cell is a valid procedure and the results obtained by the two procedures should agree. There is in fact excellent agreement between the molecular weights obtained by the two methods. The agreement between the observed sedimentation and diffusion constants is not as good, but these constants are obtained as auxiliary data in the Archibald procedure and cannot be measured as accurately as the molecular weight.

The formula weight of the free base form of β-corticotropin is 4567. One obtains a value of 4360 for the acetate salt in the ultracentrifuge. This corresponds to a value of 4240 for the free base. It is obvious that charge effects in the ultracentrifuge[8] will account for a large part of the discrepancy between the formula weight and the observed molecular weight. If one assumes that the extrapolated values obtained by the Archibald procedure are equivalent to equilibrium sedimentation results, and that the peptide concentration is small compared to that of the supporting electrolyte, he may use equation 8 of Johnson, *et al.*,[8b] and the known charge for β-corticotropin to correct the value of the molecular weight for charge effects. The corrected value for the free base is 4720.[9] The difference of 4% between observed and theoretical molecular weight is within the experimental error of the method.[3]

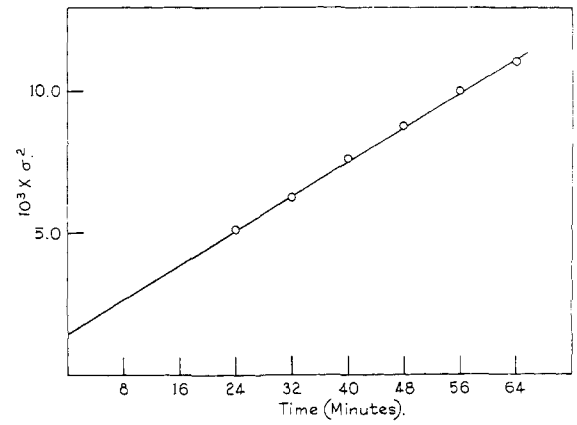

Fig. 4.—Standard deviations of centrifuge pattern calculated from the half-width at the inflection point; initial concentration 0.826 g./100 ml.

The good agreement between the observed and theoretical molecular weights may be in part fortuitous. The correction for charge effects is based on an assumption which has not been experimentally verified. It has not been demonstrated that one can use the residue values in Cohn and Edsall[6] to calculate the partial specific volume of a highly charged peptide.

The observed frictional ratio of approximately 1.7 corresponds to a rather asymmetrical molecule. This observed large asymmetry may indicate that one is working with a highly charged flexible molecule.

PEARL RIVER, N. Y.

(6) E. J. Cohn and John T. Edsall, "Proteins, Amino Acids and Peptides," Chapter 16, Reinhold Publ. Corp., New York, N. Y., 1943.

(7) The data given in Table IV of paper I indicates that there are two moles of acetic acid for each mole of peptide in β-corticotropin. With the two moles of acetic acid there is an equal number of basic and acidic groups in the molecule.

(8) (a) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford Press, London, 1940, pages 23–27; (b) A. S. Johnson, K. O. Kraus and G. Scatchard, *J. Phys. Chem.*, **58**, 1034 (1954).

(9) The molecular weights quoted in paper II of this series for the pepsin fragments of β have been adjusted for charge effects. In the calculations it was assumed that the material contained 10% water and sufficient acetate to make the substance neutral.