APPENDIX

Sedimentation Behaviour of the Enzyme

By A. RODGERS

M.R.C. Cell Metabolism Research Unit, Department of Biochemistry, University of Oxford

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The sedimentation behaviour of the crystalline tartronic semialdehyde reductase was examined in a Spinco model E analytical ultracentrifuge. The enzyme was dissolved in, and dialysed against, 0.1 M-sodium potassium phosphate buffer, pH 7.60, for 21 hr. at 3°. The protein concentration of the dialysed solution was measured refractometrically and, assuming a specific refractive increment of 1.86×10^{-3} , was 0.765 g./100 ml. of solution.

The enzyme sedimented in the ultracentrifuge as a single symmetrical peak (Fig. 3, p. 277) with $S_{20,w}$ 6.14×10^{-13} sec. at a protein concentration of 0.765 g./100 ml. of solution. At least 90 % of the protein in the ultracentrifuge cell could be accounted for by the area under the schlieren peak at all times during the sedimentation. The areas were corrected for radial dilution. The variation of sedimentation coefficient ($S_{20,w}$) with protein concentration appeared anomalous, showing a maximum value of 6.68×10^{-13} sec. at a protein concentration of about 0.28 g./100 ml. This may be an indication of a possible association phenomenon, as found for chymotrypsin by Schwert (1949), although insufficient material was available for this point to be examined in detail.

The weight-average molecular weight (M_w) was measured by the Archibald (1947) technique under the following conditions: rotor speed 7447 rev./min., temperature 1.8°, protein concentration 0.765 g./100 ml. Photographs were taken at 10 min. intervals at a phase-plate angle on the instrument of 70°. The mean molecular weight (M_w) was found to be $90\,800 \pm 1300$ (S.E.M. four photographs). No significant difference was observed between measurements made at the top and at the bottom of the cell.

The preparation was examined for homogeneity by computing the apparent diffusion coefficient (D_{app}) from the sedimentation patterns and examining the variation of this quantity with time. D_{app} was computed from the equation

$$D_{app} = \frac{1}{4\pi t} \left(\frac{A}{H_{max.}} \right)^2 \cdot (1 - \omega^2 S \cdot t)$$

where A is the area under the schlieren peak, $H_{max.}$ the maximum ordinate of the peak, S the sedimentation coefficient ($S_{20,w}$) and t the time (sec.). Computed values of D_{app} did not show any variation with time and gave a value of 6.3×10^{-7} cm.² sec.⁻¹ (uncorr.) at a protein concentration of 0.096 g./100 ml. of solution. Measurement of D_{app} at a higher protein concentration (0.765 g./100 ml.) gave an anomalous result, decreasing rapidly with time and later levelling off, indicating that it is necessary to apply corrections for the effect of c upon S at this concentration (Baldwin, 1957; Schachman, 1959). It is concluded that, at low protein concentration, no inhomogeneity is revealed by this test.

Tests for the presence of metal ions were done after digestion of 2 mg. of the crystalline enzyme in 5 ml. of concentrated nitric acid for 20 min. at 100°. The digest, examined with the flame-photometer attachment of the Beckman DU spectrophotometer, was found not to contain significant amounts of Zn, Ca, Mn, Fe, Cu and Mg.

SUMMARY

1. A preparation of crystalline tartronic semialdehyde reductase was examined in the ultracentrifuge and appeared to be homogeneous at low protein concentration.

2. A value of $S_{20,w}$ of 6.14×10^{-13} sec. at 0.765 % (w/v) protein concentration was obtained; the molecular weight was found to be 90 800.

3. Zinc, calcium, manganese, iron, copper and magnesium were not detected in the preparation by flame photometry.

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