involving the use of an insoluble trypsin derivative. Further, it has been shown that multiple species of ovomucoid exist in the hen oviduct before their secretion.

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The States of Aggregation of Citrate Lyase

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Citrate oxaloacetate-lyase (EC 4.1.3.6), also known as citrate lyase, cleaves citrate with the formation of oxaloacetate and acetate. The enzyme has been shown to exist as octamer, tetramer, dimer and monomer with molecular weights of 575 000, 273 000, 137 000 and 74 000 daltons respectively (Mahadik & SivaRaman, 1968). Only the octamer is enzymically active and requires Mg^{2+} , or other bivalent ions, for its activity and stability (Blair, Datta & Tate, 1967). The molecular weights referred to above were determined by the transient-state method in the ultracentrifuge and are therefore sensitive to conditions pertaining to the air/solution meniscus. This renders the method of particular value for the detection of size heterogeneity (Creeth & Pain, 1967).

The purpose of the present study was to re-examine the molecular weight of 314 000 reported by Bowen & Rogers (1963), who used the Ehrenberg modification of the transient-state method. In addition, use was made of the high-speed meniscus-depletion method for sedimentation equilibrium capable of yielding molecular weights independent of conditions at the meniscus and at much lower protein concentrations where thermodynamic conditions closer to ideality are possible (Van Holde &

Baldwin, 1958; Yphantis, 1960). The enzyme was prepared by a method recommended by S. P. Datta (personal communication), and a partial specific volume of 0.735 ml/g (Bowen & Rogers, 1963) was assumed. First, the conditions used (Bowen & Rogers, 1963) were closely repeated with a protein concentration of 4.5 mg/ml in a buffer containing 50 mM- KH_2PO_4 adjusted to pH 7.4 with 2M-KOH, containing 0.15M-KCl but no Mg^{2+} . The approach to equilibrium was studied at 8500 rev./min at 20°C. The Trautman plot constructed was mainly linear with a slope corresponding to a molecular weight of 290 000 daltons. However, a small region of greater slope for the earlier photographs indicated that the sample contained a heavier component, probably the octamer (Yphantis, 1959), which would be absent from the meniscus for the later photographs. Secondly, a repeat of the experiment in a buffer composed of 50 mM-tris adjusted to pH 7.4 with M-HCl containing 1 mM-EDTA and 5 mM-MgCl₂ gave a molecular weight of 570 000 from a Trautman plot, with no sign of the slight curvature mentioned above. In this experiment the simultaneous use of Mg^{2+} and EDTA was as in the purification scheme recommended by S. P. Datta (personal communication) although EDTA alone acts as an inhibitor.

The high-speed equilibrium meniscus-depletion method was applied to protein concentrations of 0.5 and 0.65 mg/ml, respectively yielding results of 573 000 and 570 000 daltons. In both cases the runs were carried out at 12°C in the Mg^{2+} -EDTA buffer described above at 11 272 rev./min.

The present results closely agree with those reported by Mahadik & SivaRaman (1968) for the active octameric form of the enzyme, although the experimental conditions were varied considerably.

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