MOLECULAR WEIGHT AND FAD CONTENT OF DIHYDROLIPOIC DEHYDROGENASE FROM ESCHERICHIA COLI

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Preparations of dihydrolipoic dehydrogenase obtained from extracts of E. coli by a procedure reported previously (Koike et al., 1960) showed an FAD content of 13-14 mμmoles per mg of protein, corresponding to a minimal molecular weight of 72,000-77,000. Ultracentrifugation experiments indicated a homogeneously sedimenting boundary, and the molecular weight was found to be about 88,000, suggesting the presence of one FAD per molecule of enzyme.

Preparations of dihydrolipoic dehydrogenase obtained by urea resolution of the highly purified pyruvate dehydrogenation complex showed an FAD content of 17-18 mμmoles per mg of protein (Koike and Reed, 1961), corresponding to a minimal molecular weight of 56,000-59,000. This value is incompatible with the older value of 88,000. The sedimentation coefficients ($s_{20,w}$) of the preparations of the flavoprotein obtained by the two procedures were in good agreement (approximately 6.3 S). The lower flavin content of the older preparations appeared to be due to a loss of flavin during the isolation procedure. It was suspected that the previously reported value of the diffusion coefficient ($D_{20,w} = 6.7 \times 10^{-7}$ cm^2 sec^{-1}), which had been determined using a synthetic boundary cell and Schlieren optics in the Spinco model E ultracentrifuge, was too high.

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A diffusion study of the flavoprotein obtained by urea resolution was carried out at 6.3° in a Rayleigh synthetic boundary cell in the Spinco model E ultracentrifuge. The solution used contained 8.0 mg of protein per ml of 0.05 M potassium phosphate buffer (pH 7.0) and had been dialyzed with stirring against the same buffer for 9 hours. The rotor speed was set at 4,000 r.p.m. and photographs of the fringes of the diffusing boundary were taken at intervals over a period of 5 hours. The fringe spacings were measured on a microcomparator and the diffusion coefficient was calculated by the method of Longsworth (1952). The corrected diffusion coefficient, $D_{20,w}$, of this preparation was 5.01×10^{-7} cm^2 sec^{-1}. The sedimentation pattern of this preparation showed a single component, $s_{20,w} = 6.24$ S. From these data and an assumed partial specific volume of 0.73 ml per g the molecular weight was calculated to be 112,000. It is thus apparent that the flavoprotein contains 2 FAD per molecule. Further understanding of the implications of this finding could conceivably require modification of the proposed reaction mechanism of dihydrolipoic dehydrogenase (Massey and Veeger, 1961; Searls et al., 1961).

It is pertinent to note that Straub's diaphorase (Straub, 1939), which is apparently identical with the dihydrolipoic dehydrogenase component of the pig heart α-ketoglutarate dehydrogenation complex (Massey, 1960; Searls and Sanadi, 1961), is reported (Savage, 1957) to have a molecular weight of 67,000 based on flavin content and 81,000 based on sedimentation and diffusion measurements, indicating one FAD per molecule. Reinvestigation of these measurements would appear to be in order in view of the present findings.

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