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ACTIVE ENZYME SEDIMENTATION OF PIG HEART FUMARASE

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Summary

Active band sedimentation studies of pig heart fumarase indicate that the enzyme is predominantly tetrameric at enzyme concentrations between 0.0125 and 0.25 mg/ml and at a fumarate concentration of 2.5 mM. At enzyme concentrations of 0.25—1.0 mg/ml and fumarate concentrations known to activate and inhibit the enzyme, the sedimentation band of fumarase becomes disperse and indicates the presence of polymers greater than tetramers.

Physical measurements have shown that fumarase has a molecular weight of 220 000 and that it consists of four identical or nearly identical subunits [1,2]. Several different isoenzymes of fumarase have been detected and three major and three minor types of subunits have been resolved from pig heart [3]. Active tetramers can be reconstituted from each of these subunits in vitro [4]. The occurrence of these isoenzymes might explain the complex steady state kinetic behavior [5,6] of the enzyme. Although the enzyme occurs as a tetramer at relatively high protein concentration [1,2], the state of aggregation, at the low concentrations used in steady state kinetic study, has not been established. The research reported here addresses this question.

Because it is sufficiently sensitive to detect active enzyme at the low concentrations ordinarily used in initial rate kinetic studies, the "active band" sedimentation method [7] was used. The location of the sedimenting, catalytically active, enzyme band was observed indirectly through changes in fumarate concentration, determined by the use of the ultraviolet absorption optical system. The ultraviolet wavelength was varied with changes in

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fumarate concentration in order to remain within the linear sensitivity range of the film. A graphic display of the absorbance from top to bottom of the centrifuge cell was generated from each frame of developed film with a Joyce-Loebl densitometer. The derivative peak, obtained by subtracting a sigmoid absorbance curve from one immediately following it in time, was taken as the position of the active enzyme band at the average of the two times.

Table I shows the sedimentation coefficient values for fumarase over a 20-fold range of enzyme concentration. The calculated value of the sedimentation coefficient remains constant, clustering around $9.1 \cdot 10^{-3}$ s, which is in agreement with the values of 9.24 S and 9.09 S reported by Johnson and Massey [8] and Frieden et al. [9], respectively, for the tetrameric form of fumarase.

Our data do not exclude the possibility, shown in other tetrameric enzymes [10—12], that a dimeric and monomeric form of the enzyme may have catalytic activity. We interpret these results to indicate that the enzyme occurs as a tetramer, if not exclusively a tetramer, under conditions in which ideal initial rate kinetic behavior is observed [5].

Our unpublished data indicate that fumarase aggregates, at fumarate concentrations greater than the Michaelis constant value, to form polymers larger than tetramers. The conditions promoting aggregation, 10—100 mM fumarate, approximate those in which substrate activation and inhibition have been reported [5].

The conclusion by Crabbe and Bardsley [6] that the most likely explanation for their observed fourth order behavior of fumarase involves cooperative subunit interactions is consistent with our data which show the enzyme to be a tetramer under similar experimental conditions.

TABLE I

SEDIMENTATION COEFFICIENTS OF ACTIVE FUMARASE AT VARIOUS ENZYME AND SUBSTRATE CONCENTRATIONS

Fumarase purchased from Cal Biochem and dialyzed against 0.05 M phosphate, pH 7.0, and 0.01 M NaCl, was layered onto fumarate solutions containing 0.05 M phosphate, pH 7.0 and 0.1 M NaCl in a Vinograd synthetic boundary centerpiece. The following wavelengths were used for each fumarate concentration: 2.5 mM fumarate, 253 nm (H or Xe light source — Br/Cl filter or monochromator); 10 mM fumarate, 288 nm (H or Xe); 33 mM fumarate, 296 nm (Xe); 100 mM fumarate, 305 nm (Xe). The distance of the band from the axis of rotation was determined optically at 8—9 different times during each sedimentation. The slope of the line relating these two parameters was calculated by linear least squares analysis; the sedimentation coefficient was calculated from the slope of this line [13]. Sedimentation coefficients were corrected to the solvent water by a factor of 1.014 (the ratio of the viscosity of buffer to that of water) and to 20°C by the factor 0.889 (the ratio of viscosity of water at 25°C to that at 20°C).

Fumarase concentration (mg/ml)	Fumarate concentration (mM)	$s_{20,w}$ of active fumarase
0.0125	2.5	8.84 ± 0.31
0.025	2.5	9.19 ± 0.61
0.05	2.5	9.36 ± 0.51
0.10	2.5	9.14 ± 0.33
0.20	2.5	9.39 ± 0.40
0.25	2.5	8.95 ± 0.24
0.25	10.0	All boundaries polydisperse
0.25	33.3	
1.00	100.0	

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