The Molecular Size of Enzymically Active Aldolase A

By C. J. MASTERS and D. J. WINZOR

Department of Biochemistry, University of Queensland, St Lucia, Qld. 4067, Australia

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From equilibrium-sedimentation studies Kawahara & Tanford (1966) have established unequivocally that under specified conditions aldolase A (EC 4.1.2.13), the muscle enzyme, has a molecular weight of 160 000, with four subunits of approximately equal size contributing to the quaternary structure of the enzyme. However, their observation of partial dissociation of aldolase at protein concentrations of about 0.2 mg/ml casts doubt on the assumption that the tetrameric entity still prevails at physiological enzymic concentrations, which are 10-100-fold lower than this value. The question of the size of the active enzyme assumes added importance as a result of the observation that aldolase may play a significant role in the metabolic regulation of glycolysis *in vivo* by virtue of its reversible adsorption on cell-membrane structures (Masters, Sheedy, Winzor & Nichol, 1969; Clarke, Masters & Winzor, 1970). If enzymically active aldolase is the tetramer, with four active sites per molecule, the partition of enzyme between solid and liquid phases may well effect a sigmoidal response of reaction velocity to substrate concentration. This communication presents velocity-sedimentation data that favour the tetramic species as the active form of aldolase.

Though it would be desirable to investigate this problem under conditions approximating to those employed in kinetic experiments, the low enzyme concentrations required to induce partial dissociation of native aldolase severely restrict the experimental procedures that may be used. We have therefore added 1.5 M-urea to aldolase solutions to observe dissociation of the enzyme at concentrations that may be studied by velocity sedimentation (Stellwagen & Schachman, 1962): such solutions retain full enzymic activity (Deal, Rutter & Van Holde, 1963).

A crystalline suspension of rabbit muscle aldolase (from Sigma Chemical Co., St. Louis, Mo., U.S.A.) was first dialysed at 4°C against an acetate buffer (0.2 M-sodium chloride, 50 mM-sodium acetate, 1 mM-EDTA, 0.1 mM-dithiothreitol, pH adjusted to 5.0 with acetic acid) to remove ammonium sulphate. At 1 h before the start of the ultracentrifugal analyses, aldolase solutions 1.5 M with respect to urea were prepared by adding 0.2 ml of buffer

containing 7.5 M-urea to each 0.8 ml portion of dialysed enzyme: the final conditions (1.5 M-urea, pH 5.0) thus approximated to those reported for the second frame of Fig. 1B of Stellwagen & Schachman (1962). Sufficient fructose 1,6-diphosphate or fructose 1-phosphate (both from Sigma Chemical Co.) was then added to make half of the enzyme solutions 10 mM with respect to substrate, the remainder being used as controls. This substrate concentration defines the initial value, not that pertaining at the time of ultracentrifugal analysis: the fructose 1,6-diphosphate concentration would still have been about 9 mM should the enzymic reaction have proceeded to equilibrium within the duration of the experiment. Velocity sedimentation was performed at 20°C and 59 780 rev./min in a Spinco model E ultracentrifuge, one-cell assembly with wedge window being used in each run so that the patterns obtained in the presence and in the absence of substrate could be observed simultaneously.

Representative ultracentrifuge patterns are shown in Fig. 1, the upper schlieren profile referring in each case to aldolase in the presence of substrate, fructose 1,6-diphosphate (Fig. 1a) or fructose 1-phosphate (Fig. 1b), the lower pattern depicting the sedimentation behaviour of enzyme alone. Inspection of the latter confirms the report by Stellwagen & Schachman (1962) that the presence of 1.5 M-urea leads to detectable dissociation of aldolase. Comparison of the upper and the lower patterns of Fig. 1 shows that in each case the mean rate of migration is faster in the sample containing substrate. For example, in the experiment with 10 mM-fructose 1,6-diphosphate added, only a single boundary is observed, the $s_{20,w}$ value of 7.2 S being in close proximity to that of 7.4 S reported for the tetramic enzyme (Stellwagen & Schachman, 1962). Since 10 mM-fructose 1,6-diphosphate suffices to ensure saturation of aldolase with respect to substrate, it appears that the enzyme-substrate complex exists predominantly as a tetrameric structure. We attribute the shift towards tetramer to an effect of substrate addition rather than of product formation because no such effect was observed with samples containing 1 mM concentrations of either D-glyceraldehyde 3-phosphate or dihydroxyacetone

Fig. 1. Effect of substrate on velocity-sedimentation patterns obtained with aldolase A in an acetate buffer, pH 5.0, containing 1.5M-urea. In these exposures, taken 35 min after attainment of speed (59780 rev./min), the lower pattern refers to aldolase alone, the upper to its migration in the presence of substrate: (a) 10 mM-fructose 1,6-diphosphate; (b) 10 mM-fructose 1-phosphate. The protein concentration was 6 mg/ml.

phosphate, the maximum product concentrations in the reaction mixtures containing fructose 1,6-diphosphate.

Deal *et al.* (1963) have reported that dissociation of aldolase by 2.3M-urea has no effect on the maximal rate of fructose 1,6-diphosphate cleavage, which at first sight suggests that the tetrameric and dissociated species possess equivalent affinities for substrate. However, from the present study it

appears that their observation reflects reconversion of the dissociated aldolase into tetramer as the result of preferential substrate binding by the latter species: in this sense the behaviour of aldolase parallels that of the heart form of chicken lactate dehydrogenase (Hathaway & Criddle, 1966) and the muscle form of the corresponding rabbit enzyme (Wurster & Hess, 1970). Thus, despite the possibility that aldolase exists in a state of reversible dissociation at enzymic concentrations (Kawahara & Tanford, 1966), we conclude that the tetrameric entity is the favoured enzymically active form of aldolase.

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