Comparison of Aldolases Extracted from Rabbit Muscle at Low and High Ionic Strength

By I. GIBBONS and R. N. PERHAM

Department of Biochemistry, University of Cambridge, Cambridge CB2 1QW, U.K.

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The binding of glycolytic enzymes to structural proteins is of interest to the functioning of the glycolytic pathway *in vivo*. Arnold & Pette (1968) have reported that only a fraction of the fructose 1,6-diphosphate aldolase (EC 4.1.2.13) in rabbit muscle can be released during extraction with aqueous media of low ionic strength. They showed that aldolase binds reversibly to structural proteins of muscle, especially F-actin, which has one strong and one weak binding site for aldolase. The binding was completely reversed by 0.15M-KCl. Rabbit muscle aldolase is also thought to be a tetramer containing two types of polypeptide chain (references in Morse & Horecker, 1968). The present experiments were designed to test the possibility that there might be structural differences in the aldolase released from rabbit muscle in low-ionic-strength and high-ionic-strength media.

Purification of aldolase. White muscle from a mature female rabbit was minced and extracted first with two successive batches of 2 vol. of 5mM-EDTA, pH 6.0 (*I* 0.025), and then once with 2 vol. of 5mM-EDTA, pH 6.0, containing 0.15mM-NaCl (*I* 0.175). Extractions were carried out with mechanical stirring for 30 min. After each extraction the muscle was squeezed through muslin to separate it from the extract. This and all subsequent steps were carried out at 4°C. When assayed by the method of Blostein & Rutter (1963) 76% of the total aldolase activity extracted was found in the first extract, 6% in the second and 16% in the third (high-ionic-strength) extract. The second extract was discarded and the other two fractions (called 1 and 3) were worked up separately as described by Gibbons & Perham (1970). The yields and specific activities of the two fractions at each stage of the purification showed no differences. Final specific activities were about 14 units/mg.

Properties of the purified enzymes. Cellulose acetate electrophoresis was carried out on Gelman Sephadex III strips in 40mM-barbitone sodium buffer, pH 8.5, containing 2mM-2-mercaptoethanol at 12V/cm for 2h. Protein was stained with 0.25% (w/v) Ponceau S in 3% (w/v) trichloroacetic acid–3% (w/v) sulphosalicylic acid. Both proteins separately and when mixed gave a single protein band. The ratio of fructose 1,6-diphosphate aldolase activity to fructose 1-phosphatase activity in the

assay method of Blostein & Rutter (1963) was determined by using fructose diphosphate at 1.5mM (27 times its K_m ; Tung, Ling, Byrne & Lardy, 1954) and fructose 1-phosphate at 10.2mM (2.9 times its K_m ; Tung *et al.* 1954). The ratio obtained was 7.8 and 8.1 for fractions 1 and 3 respectively. The sedimentation coefficient of the enzymes dissolved in 20mM-*N*-ethylmorpholine, pH 8.2, containing 0.5M-NaCl, was found as a function of protein

Fig. 1. Polyacrylamide-gel electrophoresis of *S*-carboxymethylated rabbit muscle aldolase. 1, Fraction 1; 3, fraction 3; M, mixture of fractions 1 and 3. Electrophoresis was towards the anode (+) in 3.5% gels containing 8M-urea at pH 8.9 as described by Davis (1964).

concentration in the range 2–6 mg/ml by using a Beckman Spinco model E ultracentrifuge with schlieren optics. Runs were carried out at 60 000 rev/min at 20°C. Both proteins gave single symmetrical peaks. The sedimentation coefficient was virtually independent of protein concentration; extrapolation to zero protein concentration gave values of 8.2S for both proteins.

Both enzymes were *S*-carboxymethylated in 8M-urea with iodo[2-¹⁴C]acetic acid as described by Gibbons & Perham (1970). Two-dimensional peptide 'maps' of tryptic digests of the carboxymethylated protein were prepared as described by Anderson, Gibbons & Perham (1969). There were no differences in the 'maps' after staining with ninhydrin nor in radioautographs locating specifically peptides containing *S*-carboxymethyleysteine. Amino acid analysis of the proteins after hydrolysis in 6M-HCl in sealed evacuated tubes at 105°C for 24 h was carried out on a Beckman 120C amino acid analyser (Anderson *et al.* 1969). The compositions of the proteins were identical within experimental error ($\pm 3\%$).

Polyacrylamide-gel electrophoresis at pH 8.9 in 3.5% gels containing 8M-urea was carried out by the procedure of Davis (1964). Samples of the *S*-carboxymethylated proteins were applied dissolved in 6M-urea and the gels were stained with 1% Amido Black in 7% (v/v) acetic acid and destained electrophoretically. Both proteins separately and when mixed gave two bands (Fig. 1). The intensities of the stained protein bands were estimated in a Joyce-Loebl microdensitometer. In both cases the faster band represented 45% and the slower band 55% of the total. The presence of two bands on polyacrylamide-gel electrophoresis in 8M-urea is a common feature of mammalian muscle aldolases, and the asymmetry of staining has received comment (Anderson *et al.* 1969).

By all the methods used, no significant differences could be detected between the aldolases purified from the fractions 1 and 3. The partition of aldolase during extraction can therefore be attributed simply to reversible binding to other muscle proteins. Presumably endogenous salt is sufficient to release the bulk of the aldolase during the first aqueous

extraction, but during a second extraction the ionic strength falls so low that little or no more aldolase is extracted. Thus for preparative purposes it is desirable to extract the muscle with a buffer of sufficiently high ionic strength ($I > 0.2$) to release all the aldolase.

The two bands of rabbit muscle aldolase observed on polyacrylamide-gel electrophoresis in 8M-urea are thought to represent two chain types (Chan, Morse & Horecker, 1967), although it is clear that the difference between the chains cannot be large and may even reflect a chemical modification of one chain to form the other (Anderson *et al.* 1969). Moreover, there appears to be an age-dependent change in the ratio of the two chains, the α - (slower) chain predominating in the skeletal-muscle aldolase of young rabbits, whereas the ratio approaches unity in adult rabbits (Koida, Lai & Horecker, 1969), as found in the experiments reported here. It has been suggested that crystalline rabbit muscle aldolase is a mixture of the five possible forms obtained by association of two chain types to form a tetramer (Susor, Kochman & Rutter, 1969). In this context it is noteworthy that the ionic strength of the buffer used in extracting the aldolase has no significant effect on the ratio of the two chain types observable in gel electrophoresis.

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- Anderson, P., Gibbons, I. & Perham, R. N. (1969). *Eur. J. Biochem.* **11**, 503.
Arnold, H. & Pette, D. (1968). *Eur. J. Biochem.* **6**, 163.
Blostein, R. & Rutter, W. J. (1963). *J. biol. Chem.* **238**, 3280.
Chan, W., Morse, D. E. & Horecker, B. L. (1967). *Proc. natn. Acad. Sci. U.S.A.* **57**, 1013.
Davis, B. J. (1964). *Ann. N.Y. Acad. Sci.* **121**, 404.
Gibbons, I. & Perham, R. N. (1970). *Biochem. J.* **116**, 843.
Koida, M., Lai, C. Y. & Horecker, B. L. (1969). *Archs Biochem. Biophys.* **134**, 623.
Morse, D. E. & Horecker, B. L. (1968). *Adv. Enzymol.* **31**, 125.
Susor, W. A., Kochman, M. & Rutter, W. J. (1969). *Science, N.Y.*, **165**, 1260.
Tung, T.-C., Ling, K.-H., Byrne, W. L. & Lardy, H. A. (1954). *Biochim. biophys. Acta*, **14**, 488.