PREPARATION OF MYOSIN RODS FROM MYOFIBRILS

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SUMMARY

A simple and fast procedure is described for producing myosin rods with a good yield. The method is based on the digestion of myofibrils by soluble papain. After purification by alcoholic precipitation and gel filtration, rod segments exhibited physical-chemical parameters identical to previously reported data.

INTRODUCTION

From suspended myosin digested with insoluble papain, rod segments of the myosin molecules have been isolated by Lowey and coworkers (1). In this paper, the mechanism of the proteolytic action was extensively discussed and data were provided on the amino acid composition and the physical-chemical parameters of the rod segments. Molecular weight determinations on the isolated myosin rods and length measurements on single molecules by electron microscopy led to the conclusion that the rod segments were made of two α-helical polypeptide chains. More recently, Harrington and Burke (2) have studied the association behaviour of myosin rods at high ionic strength and deduced the geometry of myosin dimer from the overlap lengths of light meromyosin and rod dimers.

The present work reports a new method of preparation of myosin rods by digesting myofibrils with soluble papain. In addition to being faster than the previously described procedures, the new method supplies a good yield of rod segments.

METHODS

Myofibrils were obtained from rabbit legs and back muscles by the method of Perry (3) modified by Biró et al (4). According to the latter authors, minced muscles were washed and suspended in 0.1 M KCl - 0.02 M borate buffer, pH 7.2 (stock solution), containing 0.02 M EDTA in the first two washes.

Myofibrils were used immediately or stored for up to two months in a 50:50 (v/v) mixture of glycerol : stock solution.

The digestion of myofibrils (15 mg/ml) was carried out in 0.1 M KCl - 0.02 M borate - 0.01 M EDTA - 0.001 M dithiothreitol, pH 6.6 for 10 min. at 25° C with soluble papain activated by cysteine (Worthington Co) using a weight ratio of 1:100 (papain to myofibrils). Inactivation of papain was performed by bringing the final iodoacetic acid concentration to 1 mM and lowering the pH to 6.0 (1). The ionic strength of reaction mixture was adjusted to 0.6 by adding crystallized KCl and the solution was immediately centrifuged at 100,000 g for 3 hours to separate acto-subfragment 1. The supernatant was dialyzed against low ionic strength buffer (0.01 M phosphate buffer pH 6.8) and then the purification procedure of Harrington and Burke (2) was applied to the low ionic strength insoluble material to obtain rod segments. This procedure included alcoholic precipitation to discard the residual myosin followed by filtration through Sephadex G-200.

Protein concentrations were determined by the Folin-Ciocalteu and microbiuret methods, and in Schlieren patterns by the area delimited by the gradient concentration curve using a specific refraction increment of 0.185 ml/g at 546 nm.

All experiments were performed in 0.5 M KCl - 0.05 M phosphate - 0.01 M EDTA, pH 7.2 at 20° C, except as otherwise mentioned.

RESULTS AND DISCUSSION

With the direct digestion of myofibrils with papain in the presence of EDTA, this method combines Kominz and coworkers' data for one part (5) and Lowey et al (1) observations for the other part. The former pointed out the action of EDTA on papain digestion and the latter observed that the enzymic cleavage at "site A" of myosin was blocked when myosin is aggregated into filaments.

The time of digestion and the ratio of papain to substrate described above have been selected from among several experiments and found to be the best conditions for producing myosin rods with suitable yield and degree of purity. The yield of rod segments reaches 40 to 50 mg/g of myofibrils.

In line with previous observations, myosin rods obtained

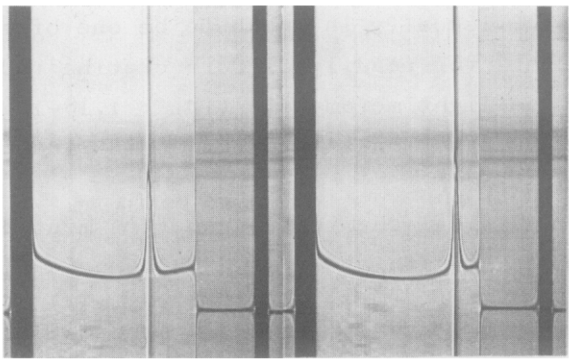

Fig. 1. Sedimentation patterns of purified myosin rod. The protein concentration is 3.9 mg / ml. Photographs are taken at 40 and 80 min after reaching a speed of 59 420 rev / min.

in the work reported here exhibited no enzymic activity, neither Ca^{2+}-activated ATPase at high or low ionic strength, nor Mg^{2+}-activated ATPase, nor K^{+}-activated ATPase. Because of the lack of enzymic activity, rod segments were characterized by the physical-chemical parameters.

The purified rod segments sedimented as a single hypersharp peak in the sedimentation velocity patterns (Fig. 1). The sedimentation coefficient was found to be $s^{o}_{20,w} = 3.4$ S. By high-speed equilibrium at 14,000 rev/min (Yphantis, 1964) (6) in 0.5 M KCl - 0.2 M phosphate - 0.01 M EDTA, pH 7.2 a value of 205,000 was obtained for the molecular weight. This value was in good agreement with the Harrington and Burke determination (2). In these equilibrium studies, a higher concentration of phosphate ion was employed, according to Godfrey and Harrington (7), in order to minimize the dimerization effect.

From optical rotatory dispersion measurements in the ultraviolet region a value of $[m']_{233} = -15{,}000^{\circ}$ characterized the through of the Cotton effect. Between 560 and 300 nm, the value of b_o in the Moffitt equation was found to be equal to -660. This value of the Moffitt parameter is the same as that determined by Lowey. This constant, which is lower than the value of -630, agrees with the assumptions of Chen et al (8) suggesting an helical b^{∞}_{o} for proteins in aqueous solution closer to the value -700 than to -630.

Intrinsic viscosity is known to be one of the best physical parameters for readily differenciating rod ($[\eta]$: 2.5-2.6 dl/g) from light meromyosin ($[\eta]$: 1.18-1.23 dl/g) (1, 2). Our rod preparations exhibited an intrinsic viscosity of at least 2.6 dl/g.

All the physical-chemical parameters mentioned above are in good agreement with previous data reports (1, 2).

In conclusion, myofibrils are a good material for the preparation of myosin rod segments. The ready digestion of myofibrils makes the intermediate preparation of myosin unnecessary and favours the homogeneity of the proteolytic action of papain on myosin molecules aggregated in organized filaments.

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