MOLECULAR WEIGHT STUDIES OF CANINE CARDIAC MYOSINS

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1. Introduction

Only recently were conditions developed for determining accurately the molecular weight of myosin [1,2]. Conditions selective for obtaining myosin in a monomeric form were used such as, analyses of myosin at a low protein concentration (< 2 mg/ml), relatively high equilibrium speed (14 200 *g*), a phosphate buffer (0.2 M, pH 7.3), and high salt concentration (> 0.3 M KCl) [1–3]. Using these conditions the molecular weight of rabbit skeletal muscle myosin was 458 000 [1] and 468 000 [2], molecular weights considerably lower than that reported earlier for rabbit skeletal muscle myosins [4,5]

Optimal conditions for analyses of the molecular weight of myosin were used in the following studies for determination of the molecular weight of canine cardiac left and right ventricular myosins. Earlier studies on the molecular weight of myosin obtained from dog heart was described as 225 000 [6] and 758 000 [7].

2. Materials and methods

Myosin was purified as described earlier [8,9] and purity established for right and left ventricular myosin [10]. Only preparations which were less than 1 week old were used for the studies reported here.

Sedimentation equilibrium experiments were performed in a Beckman Model E ultracentrifuge equipped with an RTIC temperature control and electronic speed controls [11]. Photoelectric scanning was used with the ultraviolet optical system (280 mμ). Long column meniscus depletion equilibrium techniques were used [12] so as to attain equilibrium in a shorter period of time and to eliminate baseline problems which may occur in the usual sedimentation equilibrium procedures due to the presence of low concentrations of nonsedimenting impurities [13]. A 12 mm double sector capillary synthetic boundary cell with Epon filled centerpiece was used with an AN-J four-place rotor [11]. Cells were filled according to Chervenka [12], i.e., 0.05 ml protein solution in the sample sector and 0.45 ml dialysate in the reference sector. All other conditions are explained in figs. 1 and 2.

3. Results and discussion

The long column method of Chervenka [12] was used to reduce centrifugation time and thus possible aggregation of myosin. Furthermore, high sedimentation equilibrium speed (9 000 rpm), of myosin at low protein concentrations (0.3–0.8 mg/ml) in a phosphate buffer containing a high concentration of salt (0.5 M KCl–0.2 M PO_4^{-2}) reduced association of myosin and gave excellent precision in molecular weight determinations.

At the protein concentrations of this study (0.3–0.8 mg/ml) the reciprocal apparent molecular weight increases with decreasing 'c', typical non-ideal behavior [14] (fig. 1). Rabbit skeletal muscle myosin showed a more complex behavior [15] indicative of rapid monomer–dimer equilibrium. We obtained similar results with rabbit skeletal muscle myosin, however this did not occur with canine cardiac myosins under similar conditions. Under the conditions chosen for this study the extrapolated weight average mole-

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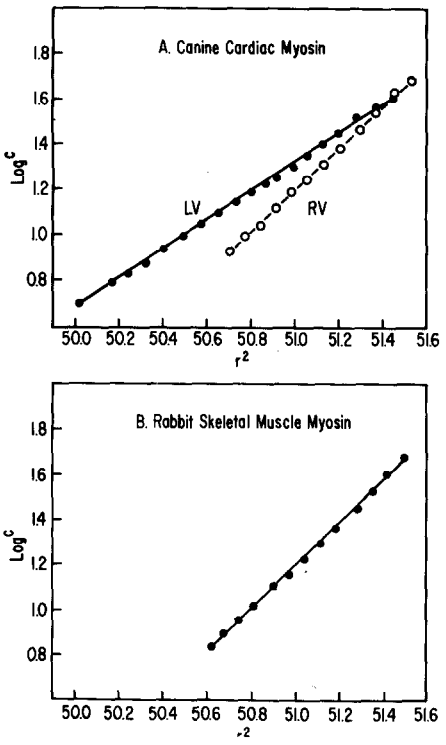

Fig. 1. Typical log c vs r^2 plots of data obtained from high speed sedimentation equilibrium studies [1,2] (9000 rpm for 18–24 hr) of myosin in the presence of 0.2 M Na_2HPO_4, pH 7.5, 0.5 M KCl, and 0.05 M Tris, 5°C [4]. Samples were dialyzed against above buffer 24 hr prior to analyses (5°C). Proteins were determined by Lowry [18]. (A) Canine left ventricular myosin (●-●-●), right ventricular myosin (o-o-o) and (B) rabbit skeletal muscle myosin. The concentration of myosin was 0.54 ± 0.04 mg/ml for the three myosins. A partial specific volume ($\bar{v}$) of 0.728 was used [1]. Calculations were made on the Wang 720 C computer. This gave weight average molecular weights and standard deviations by least squares analyses from the linear slope of log c against r^2 plots. Calculations were made as described by Chervenka [12].

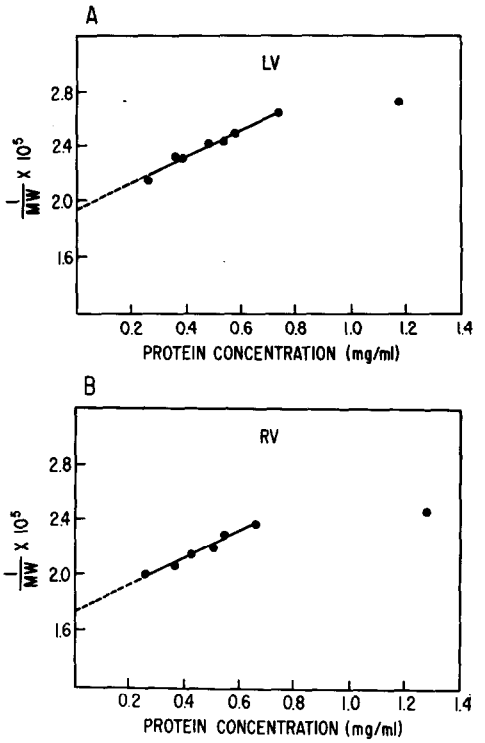

Fig. 2. Average reciprocal molecular weight vs concentration plots from high speed sedimentation equilibrium analyses. Sedimentation equilibrium analyses of myosin were analyzed at concentrations from 0.3–0.8 mg/ml. Data shown are those from three different preparations of myosin, both for left and right ventricular myosin. Purity of myosin was shown earlier (8–10).

cular weights 574 000 ± 9000 and 524 000 ± 9000 for canine cardiac right and left ventricular myosins respectively were obtained (fig. 2). These weights of 574 000 and 524 000 differ considerably from the previously reported value of 758 000 for the whole heart of the dog [7]. However, the conditions employed in that study, i.e., no phosphate, low centrifugation speed and myosin analyzed in a protein concentration range of 2–10 mg/ml could lead to a significant amount of aggregation. This would result in higher molecular weight values as in the earlier studies of rabbit skeletal muscle myosin [4,5].

The difference in molecular weights of the myosins from left and right ventricles is statistically significant. The ATPase activity of left ventricular K^+- and Ca^{2+}- activated myosin was significantly higher, approximately 30%, than that of right ventricular myosin [16]. When proportion of light to heavy chains were examined, molecular weight determined and proportions converted to moles, there was one mole of myosin light chains per mole of myosin heavy chains in left ventricular myosin and two moles of myosin light chains per mole of myosin heavy chains in right ventricular myosin [16,17]. It is proposed that the difference obtained here in the mole-

cular weight of right and left ventricular myosin is directly related to the variance in number of light chains present in the two myosins.

Acknowlegement

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