Flow-birefringence properties of various myosin B preparations

It has been generally accepted that on prolonged extraction of minced muscle with strong salt solution, myosin A or pure myosin first dissolves and then gradually turns into myosin B or natural actomyosin by combination with actin[1]. In a recent article[2], we have attempted to elucidate the interaction between the so-called 24-h myosin B and ATP, using the flow-birefringence technique. The present report is concerned with the variation in flow-birefringence properties of myosin B preparations obtained by extraction with the Weber-Edsall solution for various times, with special reference to the effect of ATP. The details of the experimental procedures are described in our previous paper[2].

Both total extraction from minced rabbit-skeletal muscle for various times, as well as fractional extractions were carried out. In the latter case, the Weber-Edsall solution was adjusted to the same pH as reached in the total extraction at the corresponding time and added to the muscle residue with gentle stirring. The supernatant separated from the residue was diluted in 0.6 *M* KCl, neutralized to pH 7 and clarified for 2 h at 24,000 × *g*. The protein concentration was measured by the refractive index, checked by the Kjeldahl procedure. The results of measurements of the extinction angle (χ) are summarized in Table I. It should be noted the in all cases the flow-birefringence properties of the crude extracts agreed well with those of the purified protein solutions.

TABLE I

EFFECT OF ATP ON THE EXTINCTION ANGLE OF THE STRUCTURAL PROTEINS EXTRACTED WITH THE WEBER-EDSALL SOLUTION FROM RABBIT-SKELETAL MUSCLE

0.6 *M* KCl; 0.02 *M* tris(hydroxymethyl)aminomethane, pH 7.0; approx 0.1 % protein solution; 20°.

Extraction time	*Extinction angle (χ°)*							
G:*	*100*		*500*		*1200*		*2000*	
ATP:	—	+	—	+	—	+	—	+
0–10 min	—	—	40	39	41	40	41	41
10–60 min	23	26	32	40	39	42	40	41
1– 5 h	14	21	9	16	8	14	9	16
5–24 h	9	14	6	12	8	14	9	15
0– 1 h	—	—	30	38	35	40	36	40
0– 5 h	22	30	30	36	35	40	36	40
0–24 h	13	20	9	16	8	15	9	18

* Velocity gradient (*G*) is given as sec^{-1}.

The 10-min extract behaved just as pure myosin in a field of shear stress, and ATP gave no effect. In the 1-h and 5-h extract, myosin A appeared to be the main component, although fractional extraction between 1 and 5 h yielded a relatively small amount of protein of the 24-h myosin B type.

In connection with the thorough work of the Morales school on 5-h myosin B[3], the flow-birefringence properties of 5-h myosin B, purified according to these workers, are described here in some detail. As shown in Fig. 1, flow birefringence (Δn) was very small at velocity gradients (*G*) less than about 200 sec^{-1} and certainly decreased on addition of ATP at these ranges of *G*. However, the ATP effect was practically

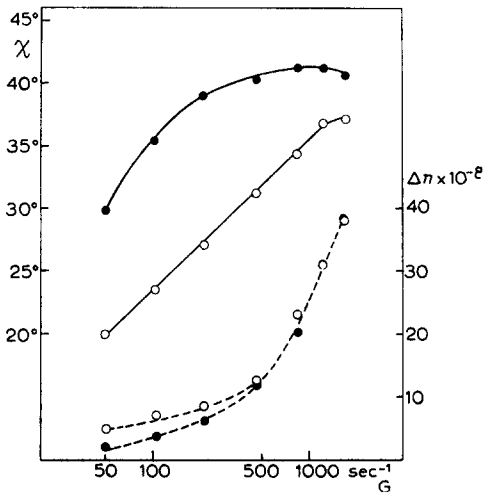

Fig. 1. Extinction angle (χ) and flow birefringence (Δn) of 5-h myosin B at varied velocity gradient (G). 0.1 % protein solution. χ: ———; Δn: – – – –; without ATP: ○; with 1 mM ATP: ●.

negligible at higher G. χ was considerably increased by ATP. It is of some interest to note that at high protein concentrations (0.3–0.5 %), ATP caused a decrease in χ at very low values of G, *viz.* < 5 sec^{-1} (*cf.* "anomalous phenomenon"[2]). Taking the Δn values of myosin A and 24-h myosin B into account[2], our 5-h myosin B preparations probably consisted of more than 90 % of myosin A, whereas MORALES *et al.*[3] reported that their preparations contained about 70 % myosin A. The discrepancy might be due to the preparation technique. The larger particles in 5-h myosin B had an apparent rotary diffusion constant of about 6 sec^{-1} at $G = 0$. Although the viscosity at high G of 5-h myosin B was little affected by ATP, the high structural viscosity at very low G (< 2 sec^{-1}) was greatly reduced.

There was evidence that a large part of the myosin A underwent polymerization in the Weber-Edsall solution between 5 and 24 h. Fractional extraction between these time limits yielded some typical "natural actomyosin", the particle length of which was somewhat larger than that of 24-h myosin B. F-actin could not be obtained. However, when purified 10-min myosin A in 0.6 M KCl was used as the extractant for the same muscle residue, from which 5-h myosin B had been separated, in place of the neutralized Weber-Edsall solution, protein of the type of 24-h myosin B was obtained in good yield after the 19-h contact. Preliminary determinations of the proline, glycine and arginine contents suggest the participation of actin in the myosin polymerization. Further investigations are required to elucidate the manner in which "natural" F-actin was solubilized by myosin A, forming actomyosin.

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[1] A. SZENT-GYÖRGYI, *Acta Physiol. Scand.*, 9 (1945) Suppl. 25.
[2] H. NODA AND K. MARUYAMA, *Biochim. Biophys. Acta*, 30 (1958) 598.
[3] P. H. VON HIPPEL, M. F. GELLERT AND M. F. MORALES, *Conference on the Chemistry of Muscle Contraction, Tokyo*, 1958, p. 1.

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