



Fig. 1. Purification of the modulator protein by DEAE-cellulose column chromatography. Crude modulator protein (9.5 ml, $A_{280}=2.0$ cm⁻¹) dialyzed against 0.25 M NaCl and 0.01 M Tris-HCl (pH 8.0) was applied to a DEAE-cellulose column (1.2 cm \times 28.5 cm) equilibrated with the same solvent. After application, the column was eluted with the same solvent. The flow rate was 15 ml/h, and 5 ml fractions were collected. O, A_{280} ; x, ^{32}P incorporated into myosin (mol/mol). Incorporation of ^{32}P into myosin was measured under the following conditions in a total volume of 0.1 ml: 0.04 mg/ml modulator protein-deficient myosin light-chain kinase, 0.05 mg/ml from one of tube numbers 5 to 20, 4.1 mg/ml myosin, 5.2 mM [γ - ^{32}P]ATP, 20 mM MgCl₂, 1 mM CaCl₂, 0.2 M KCl, and 45 mM His-KOH (pH 6.8) at 25°C. At 20 min after addition of [γ - ^{32}P]ATP, 70 μ l of the sample was assayed. Two SDS-PAGE (10% gel) patterns show the results for 10 μ g of the combined modulator protein fractions (a) and a mixture of 5 μ g of the modulator protein and 200 μ g of rabbit skeletal myosin (b). The gels were stained with Coomassie brilliant blue.

protein fraction is homogeneous. All of the following experiments were performed using this combined fraction. Gel b shows the pattern of a mixture of 5 μ g of the modulator protein and 200 μ g of rabbit skeletal myosin. The modu-

lator protein migrated with a mobility between those of g_2 and g_3 light chains. The molecular weight of the modulator protein was 16,500 as estimated previously using rabbit skeletal actin (MW 43,000) (11), g_1 (MW 25,000), g_2 (MW 18,000), and g_3 (MW 15,000) (12) as molecular weight standards.

Preparation Method B—The modulator protein prepared by this method was not as homogeneous as that prepared by method A. However, a fairly pure modulator protein can be obtained in 2 days by this method and the modulator protein preparation can be used for affinity chromatography as described in the following paper (7). The modulator protein was prepared from 1.8 kg of frozen mince of rabbit skeletal muscle. It was homogenized with 4 liters of a solution containing 5 mM Na EDTA and 50 mM sodium phosphate buffer (pH 5.7 at 0°C) as described above. Fifty percent trichloroacetic acid (240 ml) was added to the supernatant (about 4.1 liters) at 4°C with vigorous stirring to give a final trichloroacetic acid concentration of 3%. After 10 min of gentle stirring, the pH of the suspension was adjusted to 5.2 by the addition of 6 N NaOH. After 30 min of gentle stirring the suspension was centrifuged (5,000 rpm \times 15 min) and the supernatant was filtered (Toyo No. 2 filter paper) to remove small floating particles. Next, 1.14 kg of solid ammonium sulfate was dissolved into 3.8 liters of the filtrate. After standing for 30 min, the precipitate was removed by centrifugation (5,000 rpm \times 25 min) and a further 1.20 kg of ammonium sulfate was added to the supernatant. The resulting suspension was allowed to stand for 1 h. The precipitate was collected by centrifugation (5,000 rpm \times 30 min) and dissolved in 30 ml of 10 mM Tris-HCl (pH 8.0). The solution was dialyzed overnight against 1 liter of 10 mM Tris-HCl (pH 8.0). Into the dialysate (100 ml containing 1.6 g protein), 49 g of solid urea was dissolved to make 6 M urea solution. The solution was dialyzed against 400 ml of 6 M urea and 50 mM Tris-HCl (pH 8.0) for 3 h, and applied to a DEAE-cellulose column (2.2 cm \times 34 cm) equilibrated with 6 M urea and 50 mM Tris-HCl (pH 8.0). After washing the column with 140 ml of the solvent, the modulator protein was separated by a linear concentration gradient of NaCl from 0 to 0.3 M in a total volume of 800 ml. The modulator protein was eluted at

