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Note

Separation of myosin light chains by high-speed gel filtration on TSK-GEL SW columns

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Myosin is composed of two heavy chains with a molecular weight of about 200,000 and four light chains with a molecular weight of about 20,000[1]. The number of light-chain species of a given myosin varys depending on muscle type[2–4]. Those light chains are very difficult to separate from each other, because of their close similarity in both molecular weight and charge. Only a poor resolution of these light chains has been attained with ion exchangers[5,6].

Gel filtration on several materials has also been attempted, resulting in a better resolution and a higher yield of myosin light chains [7,8]. Agarose gel filtration gave the best result among the materials tested, although it required a long time (up to 9 days) for rabbit skeletal muscle myosin.

In this connection, various column packings have recently been developed for high-speed gel filtration. Among them, TSK-GEL SW, which is a microparticulate silica gel chemically bonded with hydrophilic compounds, has been demonstrated to be suitable for the separation of proteins[9].

In this paper, we report a rapid separation of myosin light chains of rabbit skeletal muscle by high-speed gel filtration on TSK-GEL SW columns.

EXPERIMENTAL

Sample

Myosin was prepared from rabbit fast muscle according to Perry[10]. The myosin was treated with 4 *M* urea in 0.05 *M* Tris–HCl buffer (pH 8.0) after Lowey and Holt[6], resulting in the separation of two fractions: the light-chain fraction consisting of A_1, A_2 and 5,5′-dithiobis-2-nitrobenzoic acid (DNTB) light chains, and the heavy-chain fraction. After being concentrated by freeze-drying, the light-chain fraction was dissolved in 0.2 *M* potassium phosphate buffer (pH 6.0) containing 2 *M* urea and 0.1 m*M* dithiothreitol (DTT), at a final concentration of about 10 mg/ml, and applied to high-speed gel filtration.

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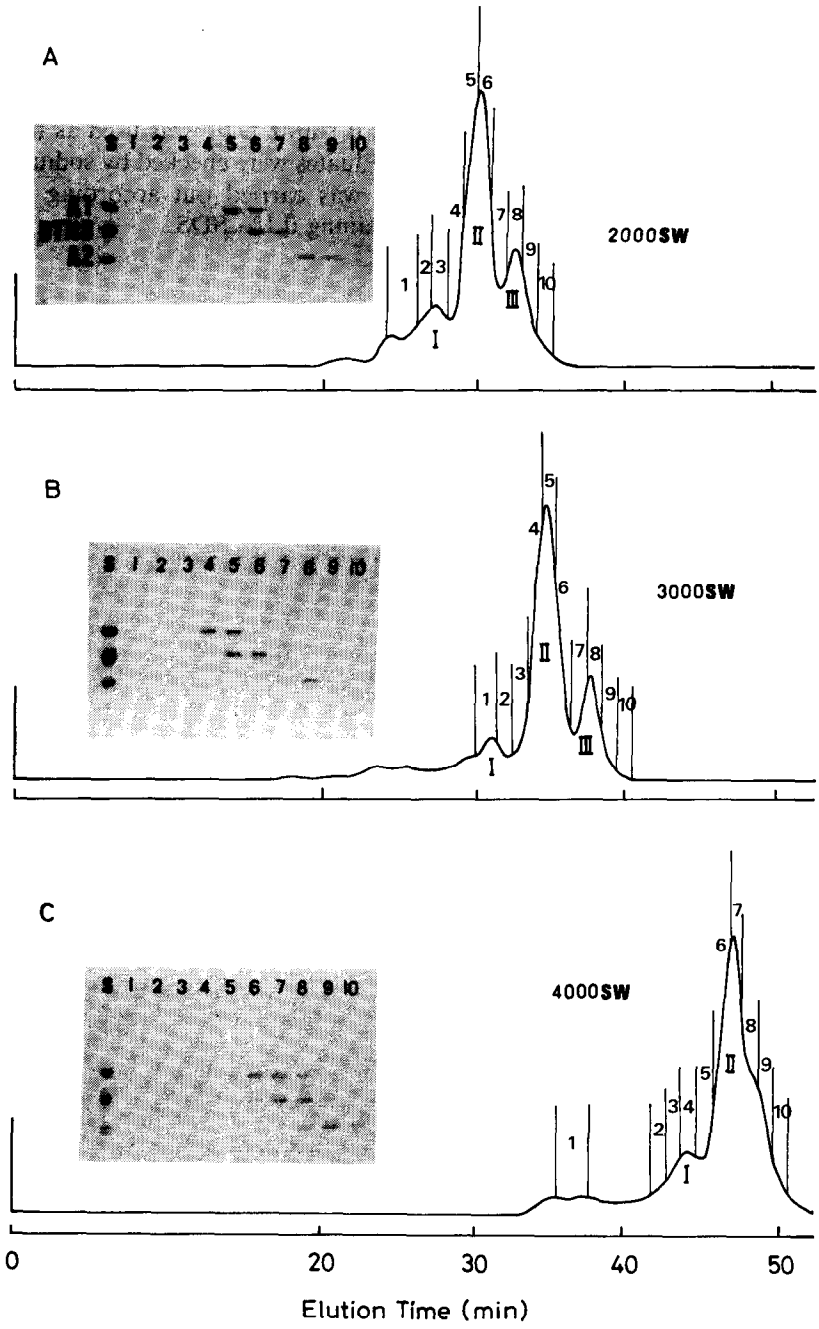

Fig. 1. Elution profiles of rabbit skeletal myosin light chains obtained by gel filtration on (A) G2000SW, (B) G3000SW and (C) G4000SW. A 0.2 *M* potassium phosphate buffer (pH 6.0) containing 2 *M* urea and 0.1 m*M* DTT was used as the eluent. Photographs show SDS gel electrophoretic patterns of the applied sample and selected fractions.

High-speed gel filtration

Gel filtration was carried out on a Spectra-Physics Model 8700 liquid chromatograph equipped with a UV monitor at 280 nm. Columns (60 cm × 7.5 mm I.D.) of G2000SW, G3000SW or G4000SW (Toyo Soda) were used. A 0.2 *M* potassium phosphate buffer (pH 6.0) containing 2 *M* urea and 0.1 m*M* DTT was used as the eluent. The flow-rate was maintained at 0.5 ml/min. Eluates were checked by sodium dodecyl sulphate (SDS) gel electrophoresis, which was carried out according to Laemmli[11] using 15% polyacrylamide slab gel containing 0.1% SDS.

RESULTS

High-speed gel filtration was carried out at 3°C on columns of G2000SW, G3000SW and G4000SW, resulting in the elution profiles shown in Fig. 1. The relatively large peak I, which appeared at about 28 min on the G2000SW column (Fig. 1A) or at about 32 min on the G3000SW column (Fig. 1B), seemed to be composed of aggregated light chains. The subsequent main peak II at about 31 min on the

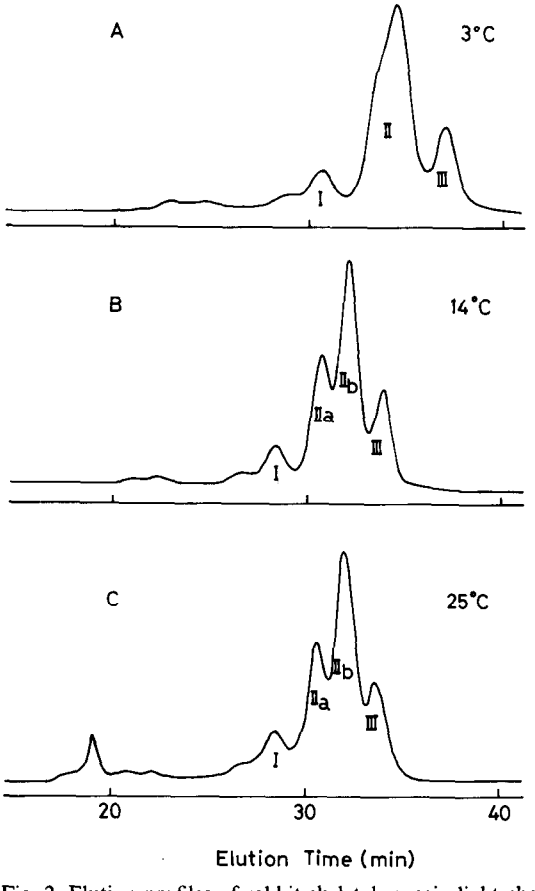

Fig. 2. Elution profiles of rabbit skeletal myosin light chains obtained by G3000SW gel filtration at (A) 3°C, (B) 14°C and (C) 25°C. Eluent as in Fig. 1.

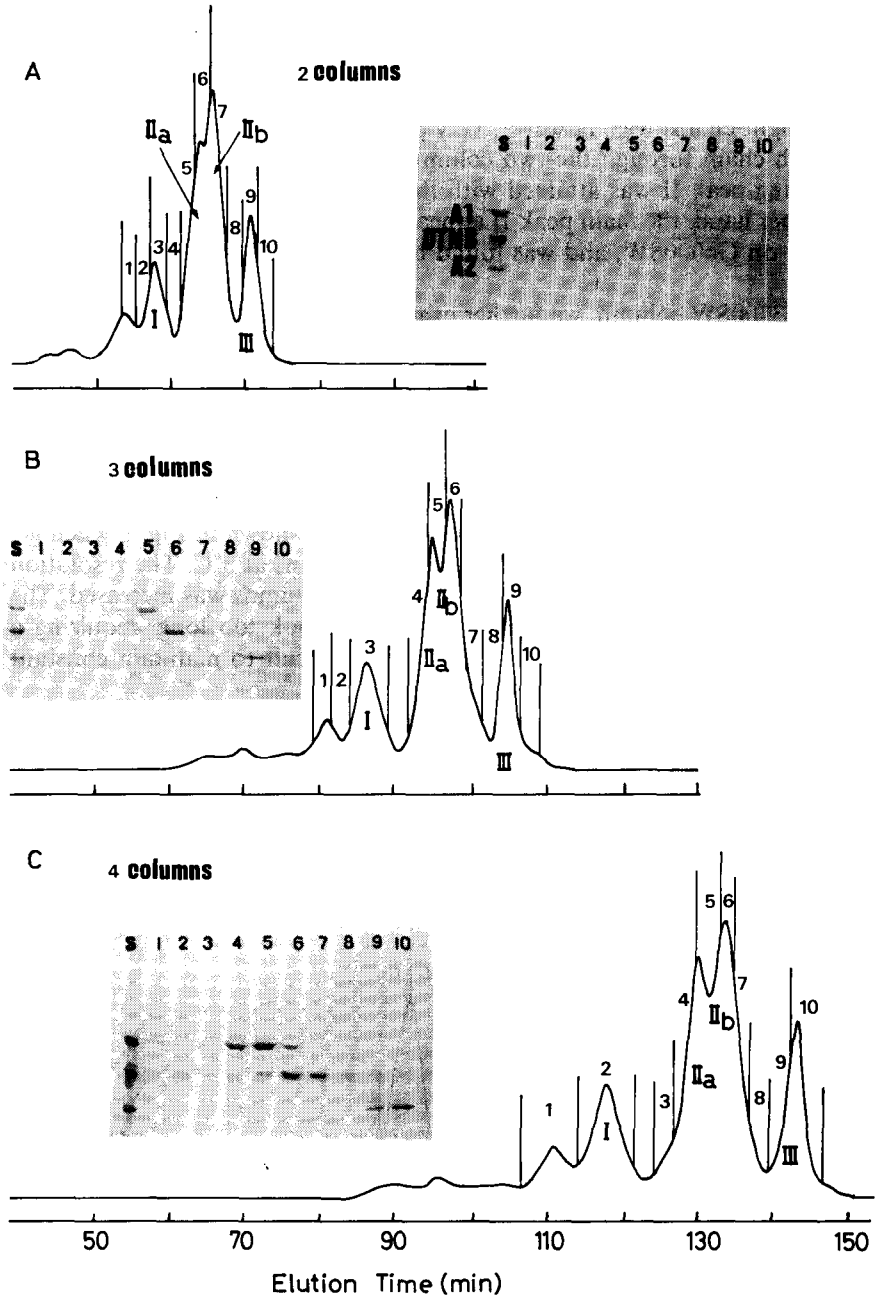

Fig. 3. Elution profiles of rabbit skeletal myosin light chains obtained by gel filtration on (A) two, (B) three and (C) four successive columns of G3000SW. Eluent as in Fig. 1. Photographs show SDS gel electrophoretic patterns of the applied sample and selected fractions.

G2000SW column or at about 35 min on the G3000SW column contained A_1 and DTNB light chains, as shown electrophoretically. On the leading edge, each main peak showed a slight shoulder which consisted of A_1 light chain almost exclusively, while the trailing edge consisted mainly of DTNB light chain. The separation between A_1 and DTNB light chains was, however, very poor. Peak III was composed exclusively of A_2 ligth chain through the two columns. A better resolution of this peak from the preceding peak II was attained with the G3000SW column (Fig. 1B).

On the other hand, the main peak II appeared at around 47 min after injection when gel filtered on G4000SW, and was found to contain all light chains, as shown in Fig. 1C.

Using a G3000SW column, we further examined the influence of column temperature (3, 14 and 25°C) on the resolution of the three myosin light chains. The results obtained (Fig. 2) showed that the best separation between peaks II (or IIb) and III was achieved at 3°C. The separation between peaks IIa and IIb was clearly poorer at 3°C than at 14 or 25°C. However, light chains may be denatured at the latter temperatures. Thus we made further attempts to separate peaks IIa and IIb on a G3000SW column system at 3°C.

Rabbit myosin light chains gave the elution profiles shown in Fig. 3 when gel filtered on two, three and four successive G3000SW columns at 3°C. The resolution of peaks IIa and IIb was improved as the number of the columns was increased. The gel filtration on the four successive columns, however, took too long, requiring a high pressure of 110 kg/cm^2, which was much more difficult to maintain constant than the pressure required with fewer columns.

It was concluded from these results that the best separation of A_1, DTNB and A_2 light chains of rabbit myosin is attained by gel filtration on three successive columns (180 cm) of G3000SW at 3°C.

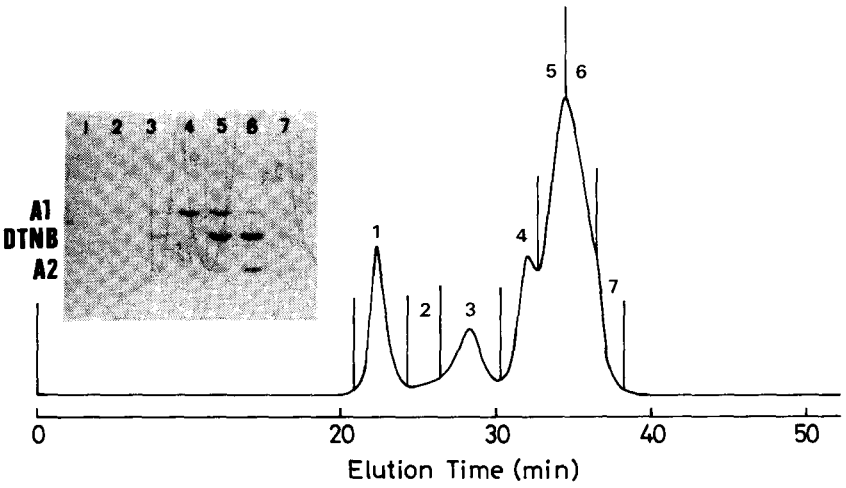

Fig. 4. Elution profile of rabbit skeletal myosin light chains obtained by G3000SW gel filtration in 6 *M* Gdn-HCl at room temperature. Photograph shows SDS gel electrophoretic patterns of selected fractions.

DISCUSSION

Gel filtration on the three successive G3000SW columns separated A_1, DTNB and A_2 light chains almost satisfactorily (Fig. 3B). Only 0.6 mg of the whole light chains was loaded here, and it took about 100 min to complete the gel filtration. Similar results were obtained when up to 2 mg of the same sample was loaded (data not shown). This means that it will take only 3–4 days to purify such light chains from 30 mg of rabbit skeletal muscle myosin by the above technique. Klotz *et al.*[8] reported that it took 9 days to attain a corresponding light-chain separation from 30–50 mg of rabbit skeletal myosin by gel filtration on three successive columns of Biogel A-1.5 m (240 cm × 12 mm I.D.). Their experiments were performed at room temperature with 6 *M* guanidine hydrochloride (Gdn-HCl), resulting in poor resolution of DTNB and A_2 light chains. For comparison, high-speed gel filtration of rabbit myosin light chains was carried out on a G3000SW column at room temperature, using 6 *M* Gdn-HCl[12]. The resolution of the light chains was not satisfactory, as shown in Fig. 4. Also, the use of Gdn-HCl was accompanied by corrosion of the stainless-steel column and tubes of the high-performance liquid chromatograph.

Further improvements to our chromatographic technique are in progress.

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