CLIN CHEM. 26/8, 1152-1155 (1980)

Muscle Protein Analysis. II. Two-Dimensional Electrophoresis of Normal and Diseased Human Skeletal Muscle

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We used high-resolution two-dimensional electrophoresis to analyze the major proteins of normal and pathological human-muscle samples. The normal human-muscle pattern contains four myosin light chains: three that co-migrate with the myosin light chains from rabbit fast muscle (extensor digitorum longus), and one that co-migrates with the light chain 2 from rabbit slow muscle (soleus). Of seven Duchenne muscular dystrophy samples, four yielded patterns with decreased amounts of actin and myosin relative to normal muscle, while three samples gave patterns comparable to that for normal muscle. Six samples from patients with myotonic dystrophy also gave normal patterns. In nemaline rod myopathy, in contrast, the pattern was deficient in two of the fast-type myosin light chains.

Additional Keyphrases: *ISO-DALT system · pattern for normal muscle and muscle from myotonic dystrophy, nemaline rod myopathy, and Duchenne dystrophy · tissue analysis · contractile and minor proteins of muscle · myosin · light chains*

One-dimensional electrophoresis has been extensively used to compare the proteins found in normal and abnormal human skeletal muscle. Although few differences have been reported in the actin, tropomyosin, or troponin contents of preparations from different muscles, a controversy exists over the number of myosin light chains found. Normal human skeletal muscle has been reported to contain three (*1, 2*) or four (*3*) light chains, but pathological muscles have been found to contain only two (*2, 3*). Recently, two-dimensional electrophoresis of normal human skeletal muscle myofibrils resolved a set of five myosin light chains, three fast type and two slow type (*4*). In the same paper, the slow myosin light chain phenotype was found to predominate in patterns of muscle samples from patients with congenital myopathies. High-resolution two-dimensional electrophoresis separates denatured proteins by isoelectric focusing in the first dimension and sodium dodecyl sulfate (SDS) electrophoresis in the second dimension (*5*). Instrumentation for two-dimensional electrophoretic analysis of multiple samples is available (*6, 7*), and the feasibility of using such a system for the analysis of small muscle-biopsy samples was demonstrated in the first paper of this series (*8*).

In the studies reported here, two-dimensional electrophoresis was used to compare whole homogenates prepared from biopsies of normal and pathological human skeletal muscle. The pathological samples analyzed were obtained from patients with Duchenne muscular dystrophy, myotonic dystrophy, and nemaline rod myopathy.

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Received Jan. 29, 1980; accepted April 4, 1980.

Materials and Methods

Muscle samples. Biopsy samples of normal human skeletal muscle were obtained from the quadriceps, gastrocnemius, or deltoid of three individuals with no histological signs of muscle pathology. Muscle samples from seven patients with Duchenne muscular dystrophy and one patient with nemaline rod myopathy were from biopsies of quadriceps; samples from six myotonic dystrophy patients were from biopsies of quadriceps or gastrocnemius. Diagnosis of all three pathological conditions was confirmed by histological methods. The muscle samples were frozen immediately after removal and stored at −70 °C until prepared for electrophoresis. Rabbit muscle samples (extensor digitorum longus, a fast-twitch muscle, and soleus, a slow-twitch muscle) were homogenized immediately after the tissue was removed from the animal.

Sample preparation for electrophoresis. Muscle samples (100–200 mg) were homogenized in 10 volumes of pyrophosphate relaxation buffer as described by Zak et al. (*9*). For homogenization we used a Polytron on ice. The homogenate was then mixed with an equal volume of the SDS-CHES buffer described previously (*8*), heated for 5 min at 95 °C, and centrifuged for 5 min in a Microfuge (Beckman Instruments, Inc., Fullerton, CA 92634) to remove any insoluble material. Some muscle samples contained enough fat to make the homogenate turbid after heating with SDS-CHES buffer. The fat was removed after centrifugation, however, as the top layer of the supernate. The clear supernatant fluids were then used for two-dimensional electrophoresis.

Two-dimensional electrophoresis. For two-dimensional electrophoresis we used the ISO-DALT system (*6, 7*).

First-dimension isoelectric focusing was done in 40 g/L polyacrylamide gels [6% C (cross-linking)] with 9 mol of urea and 20 g of ampholytes (pH 2–11) per liter for about 11 000 volt-hours.

The second-dimension separation was done in slab gels consisting of a linear gradient (100 to 200 g/L) of polyacrylamide (2.6% C) with use of the SDS-Tris/glycine buffer system previously described (*7*). Staining of gels with Coomassie Brilliant Blue R-250, destaining, and photography were all done as described elsewhere (*7*).

Results

Normal Human Muscle

Figure 1 shows the two-dimensional electrophoretic pattern of normal human quadriceps, with protein spots labeled where their identity is known. This pattern is similar to that of rabbit psoas muscle whole homogenate (*8*), except for the myosin light chains. The light chain designations shown in Figure 1 are based upon the co-electrophoresis of human muscle proteins with the myosin light chains in rabbit fast (extensor digitorium longus) and slow (soleus) muscles (Figure 2). Similar myosin light chain patterns were obtained when normal human deltoid and gastrocnemius muscles were analyzed. The co-existence of myosin light chains characteristic of both fast and slow fibers in human muscle is consistent with reports

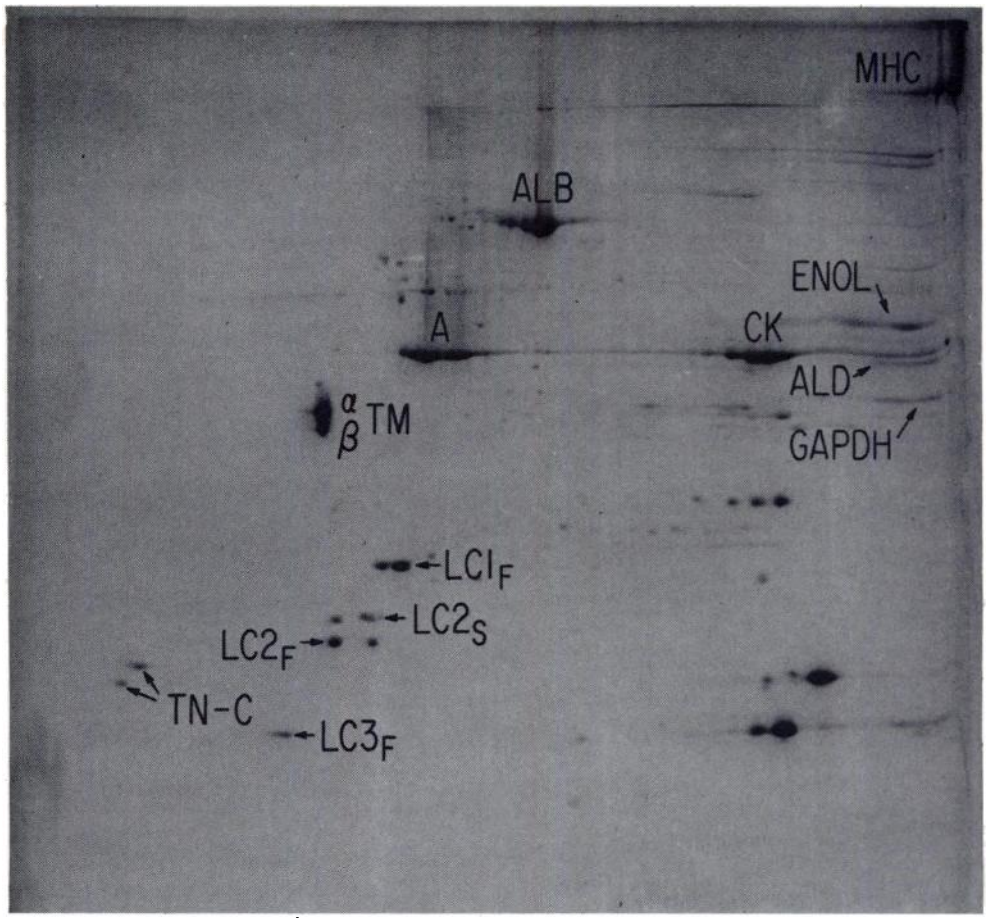

Fig. 1. Two-dimensional electrophoresis of normal human quadriceps whole homogenate

Homogenate was prepared from a biopsy taken from a 7-year-old boy; we applied 150 μg of the protein to the gel for analysis. The gel is oriented with acidic side to the left and basic side to the right. Abbreviations: *MHC*, myosin heavy chain; *ALB*, albumin; *A*, actin; *CK*, creatine kinase; *ENOL*, enolase; *ALD*, aldolase; *TM*, tropomyosin; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *LC*, myosin light chains (*F*, fast muscle; *S*, slow muscle); and *TN-C*, calcium-binding subunits of troponin

that all human muscles studied thus far have a mixed fiber content (*10*).

The human myosin light chain pattern shown in Figure 1 is similar to that of Fardeau et al. (*4*) except that the slow form of myosin light chain 1 is not seen in our patterns. The human slow light chain 1 described (*4*) is of lower molecular weight than that of rabbit (*8*) or rat (*11*) muscle and migrates closely with the fast myosin light chain 1. The two spots in our pattern that co-migrate with the rabbit fast myosin light chain 1 may therefore represent human fast and slow myosin types. Until further chemical analysis confirms this possibility, however, we will rely on the co-migration of human and rabbit proteins for their classification as fast myosin light chain 1. The resolution of four to five light chains with different molecular masses and the further resolution of those proteins into components differing in charge demonstrate the value of the two-dimensional approach. The charge heterogeneity observed in the case of the fast and slow light chain 2 is probably the result of phosphorylation and may indicate deamidation in the other light chains (*8*).

Duchenne and Myotonic Dystrophies

Of the samples from seven patients with Duchenne muscular dystrophy, three produced two-dimensional electrophoretic patterns with no obvious variation from the normal human muscle patterns. A typical myosin light chain pattern from one of these samples is shown in Figure 3*A*. The other four Duchenne samples yielded patterns that contained little or no actin, decreased amounts of myosin heavy chains, and an abundance of low-molecular-mass spots that made it difficult to identify myosin light chains. The apparent deficiency of contractile proteins seen in these samples probably reflects a decrease of myofibrillar components in the muscle tissue, an increase of patient connective tissue, and an increase in the proteolytic activity within the muscle.

Correlation of the clinical status of Duchenne patients with the normal or abnormal two-dimensional electrophoresis patterns will require further analysis of additional samples. All of the samples from six patients with diagnosed myotonic dystrophy had two-dimensional electrophoretic patterns similar to that for normal muscle, including the myosin light chain pattern (Figure 3*B*). The normal two-dimensional electrophoretic profiles of contractile proteins associated with Duchenne and myotonic dystrophy samples confirm studies reported previously in which one-dimensional electrophoresis techniques were used (*12*).

Nemaline Rod Myopathy

A sample from the quadriceps of a patient with nemaline rod myopathy displayed a two-dimensional pattern that differed from that for the normal quadriceps with respect to the myosin light chain pattern (Figure 4*A*). Co-electrophoresis of the nemaline rod myopathy sample with rabbit fast and slow muscle homogenates demonstrated that the two human light chains present are the fast light chain 1 and the slow light chain 2 (Figure 4*B* and *C*). This finding is in agreement with Libera et al. (*2*), who found only two myosin light chains associated with nemaline rod myopathy by one-dimensional electrophoresis. However, those investigators indicated that

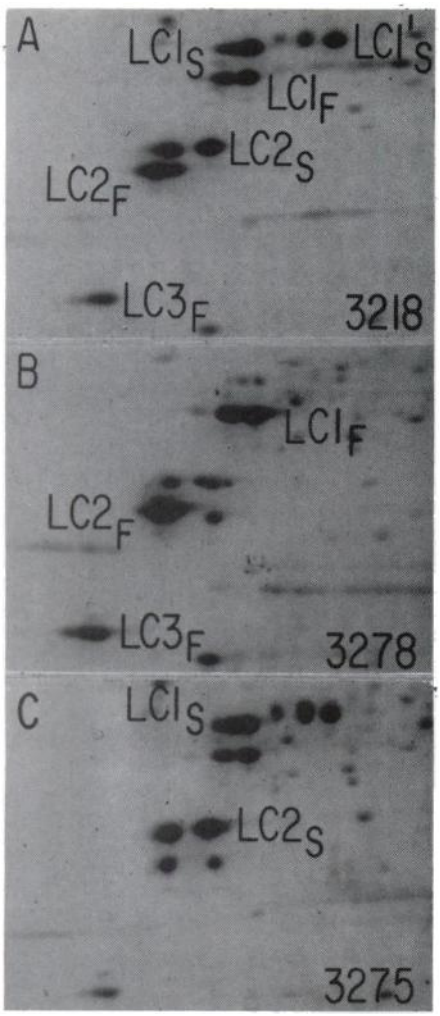

Fig. 2. Myosin light chain portions from rabbit and normal human skeletal muscle two-dimensional patterns

(*A*) rabbit fast muscle with rabbit slow muscle, (*B*) rabbit fast muscle with human quadriceps, and (*C*) rabbit slow muscle with human quadriceps. Light chain (*LC*) designations are the same as those used by Whalen et al. (*11*). Gels were oriented as in Figure 1

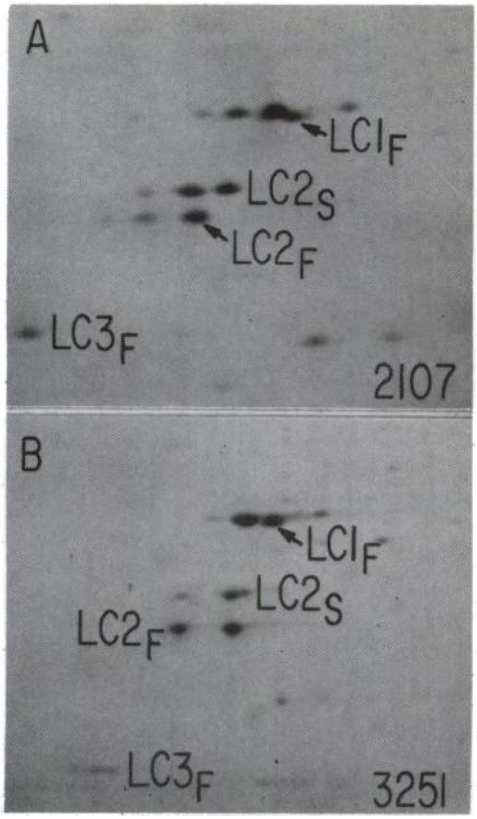

Fig. 3. The myosin light chain portion from pathological human muscle two-dimensional patterns

(*A*) Duchenne muscular dystrophy in a 4-year-old boy, (*B*) myotonic dystrophy in a 38-year-old man. Abbreviations and gel orientations are as in Figure 1

both light chains were of the slow type. Sreter et al. (*3*) also reported that nemaline rod myopathy samples showed two slow light chains when one-dimensional techniques were used, but also found the fast light chain 3. Even at higher protein loadings, we failed to see any trace of the light chain 3. Fardeau et al. (*4*) reported the disappearance of some fast myosin light chains when samples from patients with congenital myopathies were compared by two-dimensional electrophoresis.

The loss of some myosin light chains from muscle affected by nemaline rod myopathy remains to be explained. Little is known about this disorder other than the associated clinical symptoms, the appearance of rod-like inclusions in histological sections of the muscle, and the possibility of autosomal recessive inheritance (*3*). The rod-shaped inclusions may be the result of excessive production and accumulation of Z-band materials (*13*). We now are using two-dimensional electrophoresis to look for increases in proteins that may be associated with the Z-band, such as α-actinin (*13*).

Discussion

Previous electrophoretic studies of normal and pathological muscle have been primarily concerned with analysis of the contractile apparatus, perhaps owing to the abundance of contractile proteins and the wealth of knowledge available about their structure and function. In some diseases such as nemaline rod myopathy this approach has shown some abnormalities. However, in other disorders such as the Duchenne and myotonic dystrophies, no consistent differences can be demonstrated. The results reported here support previous observations and demonstrate the need for studying the other protein components of muscle that, although minor in quantity relative to contractile proteins, may have major roles in maintenance of healthy muscle.

The two-dimensional electrophoretic pattern in Figure 1 shows 100–200 proteins, and about 50 additional components are resolved when non-equilibrium pH gradient electrophoresis (*8, 14*) is used for first-dimension separation (unpublished observation). The problem is to resolve the minor protein components of muscle as well as the major contractile proteins, with use of the small amounts of tissue provided from biopsies. One approach is to develop techniques for the subcellular fractionation of very small muscle samples in order to physically separate the contractile proteins from the other muscle proteins. A second approach is to analyze each muscle sample at different protein concentrations such that in some gels only the contractile proteins are resolved, while in others the contractile proteins are overloaded and minor components are well resolved. We are now using this second approach with individual tissue sections so the two-dimensional electrophoretic patterns can be compared with corresponding histologically stained sections. The development of the silver stain (*15*) for acrylamide gels, more sensitive than Coomassie Brilliant Blue for protein staining, will be useful for detecting minor proteins when either of these two approaches is used. These studies require the capacity to analyze many samples in parallel and are made feasible by the use of the ISO-DALT system (*6, 7*). The quantitation methods required for in-depth comparisons between patterns from hundreds of normal and pathological samples are currently under development (*16*).

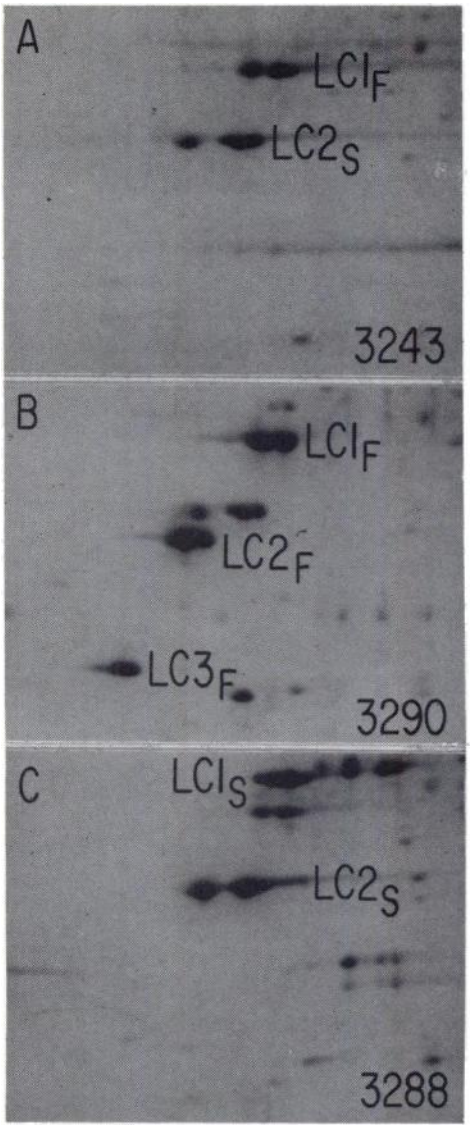

Fig. 4. The myosin light chain patterns from rabbit skeletal muscle and a sample from a patient with nemaline rod myopathy

(A) muscle from a patient (11-year-old girl) with nemaline rod myopathy, *(B)* muscle from a patient with nemaline rod myopathy with rabbit fast muscle, and *(C)* muscle from a patient with nemaline rod myopathy with rabbit slow muscle. Abbreviations and gel orientations are as in Figure 1

Thus, coupled with refined techniques for sample preparation and the means to quantitate major and minor protein components, two-dimensional electrophoresis can be used to unravel disorders that have devastating effects on muscle function with little or no obvious effect on the major proteins of the contractile apparatus.

This work was supported by the U.S. Department of Energy under contract No. W-31-109-ENG-38, the U.S. National Institute of Health under grant No. NS-12172, and the Muscular Dystrophy Association.

Note added in proof: Since these studies, another case of nemaline rod myopathy has been analyzed. A biceps biopsy from a 65-year-old woman showed the same myosin light-chain deficiencies as the case reported in this paper.

References

1. Bailin, G., Myosin and actomyosin from human skeletal muscle. *Biochim. Biophys. Acta* **449,** 310–326 (1976).

2. Libera, L. D., Margreth, A., Mussini, I., et al., Myosin polymorphism in human skeletal muscles. *Muscle Nerve* **1,** 280–291 (1978).

3. Sreter, F. A., Astrom, K., Romahul, F., et al., Characteristics of myosin in nemaline rod myopathy. *J. Neurol. Sci.* **27,** 99–116 (1976).

4. Fardeau, M., Godet-Guillain, J., Tome, F. M. S., et al., Congenital neuromuscular disorders. A critical review. In *Current Topics in Nerve and Muscle Research,* A. J. Aguayo and G. Karpati, Eds., Excerpta Medica Foundation, Amsterdam, 1978, pp 164–177.

5. O'Farrell, P. H., High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **250,** 4007–4021 (1975).

6. Anderson, N. G., and Anderson, N. L., Analytical techniques for cell fractions. XXI. Two-dimensional analysis of serum and tissue proteins: Multiple isoelectric focusing. *Anal. Biochem.* **85,** 331–340 (1978).

7. Anderson, N. L., and Anderson, N. G., Analytical techniques for cell fractions. XXII. Two-dimensional analysis of serum and tissue proteins: Multiple gradient slab-gel electrophoresis. *Anal. Biochem.* **85,** 341–354 (1978).

8. Giometti, C. S., Anderson, N. G., and Anderson, N. L., Muscle protein analysis. I. High-resolution two-dimensional electrophoresis of skeletal muscle proteins for analysis of small biopsy samples. *Clin. Chem.* **25,** 1877–1884 (1979).

9. Zak, R., Etlinger, J., and Fischman, D. A., Studies on the fractionation of skeletal and heart muscle. *Excerpta Med. ICS* **240,** 160–175 (1972).

10. Johnson, M. A., Polgar, J., Weightman, D., and Appleton, D., Data on the distribution of fiber types in thirty-six human muscles: An autopsy study. *J. Neurol. Sci.* **18,** 111–129 (1973).

11. Whalen, R. G., Butler-Brown, G. S., and Gros, F., Identification of a novel form of myosin light chain present in embryonic muscle tissue and cultured muscle cells. *J. Mol. Biol.* **126,** 415–431 (1978).

12. Penn, A. S., Cloak, R. A., and Rowland, L. P., Myosin from normal and dystrophic human muscle. Immunochemical and electrophoretic studies. *Arch. Neurol.* **27,** 159–173 (1972).

13. Kawabuchi, M., and Osame, M., Ultrastructural changes in biopsied muscles of nemaline myopathy. *J. Clin. Electron Microscopy* **9,** 655–656 (1976).

14. O'Farrell, P. Z., Goodman, H. M., and O'Farrell, P. H., High-resolution two-dimensional electrophoresis of basic as well as acid proteins. *Cell* **12,** 1133–1142 (1977).

15. Switzer, III, R. C., Merril, C. R., and Shifrin, S., A highly sensitive silver stain for detecting proteins and peptides in polyacrylamide gels. *Anal. Biochem.* **98,** 231–237 (1979).

16. Taylor, J., Anderson, N. L., Coulter, B. P., et al., Estimation of two-dimensional electrophoretic spot intensities and positions by modeling. In *Proceedings of Electrophoresis '79,* B. Radola, Ed., W. DeGruyter, in press.