Effect of Lithium Bromide Solutions on the Contraction of Glycerinated Muscle Fibers and Muscle Proteins*

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ABSTRACT: The contractile properties of glycerinated muscle fibers and fibers regenerated from its constituent proteins were studied in aqueous LiBr solutions. With the exception of the "actin ghosts," whose behavior was atypical, the results for the other fibrous systems adhered to the conclusions deduced from studies of other similarly constituted systems such as the keratins and collagen. Contraction occurred concomitantly with a loss of ordered structure. The regeneration of the original length and structure, in the absence of a tensile force, occurred when the systems were cross-linked with formaldehyde in the oriented state. Morphological changes occurring during contraction in glycerinated muscle fibers were followed by phase-contrast microscopy. For small amounts of shortening, contrary to that observed for adenosine 5′-triphosphate-induced contraction, a diminution of the A-band width occurs. For large amounts of contraction, the photomicrographs were virtually identical for shortening induced by either adenosine 5′-triphosphate or LiBr.

It has been observed generally that solutions of certain neutral salts such as LiBr, KCNS, or KI, among others, render unstable the ordered structure of polypeptides and proteins (Mandelkern *et al.*, 1962a,b,c). When the macromolecular substance is in fibrous form, the disruption of the ordered structure results in a characteristic contraction. When stable intermolecular cross-links are either initially present or imposed on the ordered state, the contraction is reversible if the salt is removed from the external medium. All the manifestations of a cooperative first-order phase transition are displayed during this process (Flory, 1956; Mandelkern, 1964a,b). In dilute solutions of ordered protein molecules the disruption of the ordered structure manifests itself in abrupt changes in hydrodynamic and optical rotary properties. For a particular system, namely, aqueous collagen solutions, the experimental results (Von Hippel and Wong, 1962, 1963) have been shown to be consistent with the thermodynamic theory of binding of the salt to the peptide group (Mandelkern and Stewart, 1964). The consequence of this binding is a helix-coil transition (Schellman, 1955; Flory, 1957).

Bowen and Laki (1955, 1956) have reported that glycerinated muscle fibers contract in solutions of KI and KCNS. Since the striated muscle fiber system represents a complex structure from the point of view of individual protein constituents and fiber morphology in comparison to the structurally simpler fibrous proteins studied, it is of interest to ascertain whether the aforementioned principles are also valid for this system. We have therefore studied the contractile properties, in aqueous LiBr solutions, of glycerinated muscle fibers and fibers prepared from the constituent proteins. The effect of cross-linking has been investigated and the gross morphological changes were followed by phase-contrast microscopy.

Experimental

Materials. Reagent grade KCl, LiBr, NaBr, formaldehyde, sucrose, and ATP[1] were used without further purification. The aqueous solutions of these reagents were prepared with doubly distilled water. *Psoas* fibers were "glycerinated" for periods up to 5 months at −20° in 50% glycerol–water mixtures, according to Szent-Györgyi (1951).

Myosin A was prepared by a variant of the Szent-Györgyi method and was thrice purified (Szent-Györgyi, 1951). The protein is maximally precipitated at 0.03 M KCl and completely soluble at 0.40 M KCl. The system was held at pH 6.8 between 0 and 4° throughout the preparation. Myosin A prepared in this manner does not superprecipitate under optimal conditions. Myosin B was prepared in a similar manner except that the original extract was made with Weber–Edsall solution (0.6 M KCl made slightly alkaline with $NaCO_3$–$NaHCO_3$). The protein was precipitable in 0.25 M KCl and completely soluble in 0.60 M KCl. "Actin ghosts" were prepared according to the procedures of Hanson and Huxley (1957) except that the fibrils were

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[1] Abbreviation used in this work: ATP, adenosine 5′-triphosphate.

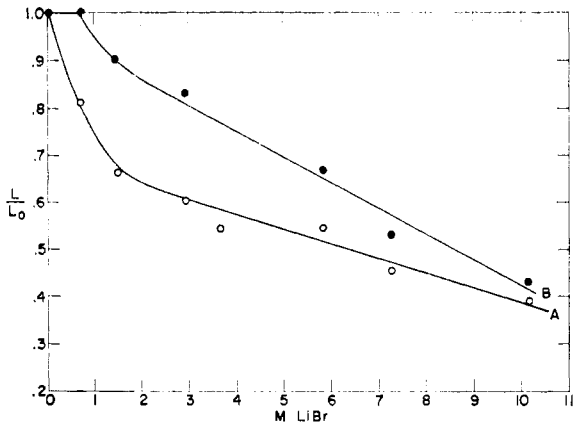

FIGURE 1: Relative length change of glycerinated muscle fibers in aqueous LiBr solutions of different concentrations. Curve A, untreated fibers; curve B, formaldehyde-cross-linked fibers.

not preblended. The extraction of the myosin A took 24 hr, and the residue was stored in 0.1 M KCl, pH 7.0, at 4°.

Regenerated fibers or "threads" were prepared from myosin A and myosin B by extrusion of a solution from a hypodermic syringe into a medium adjusted for maximal precipitation. The myosin B threads could be handled sufficiently well for length measurements. However, the myosin A threads were quite fragile. Hence, rather than transferring them from one solution to another, the fibers were kept *in situ* and the solution around the fibers was changed as desired.

The cross-linking of the fibers was accomplished by immersion in 37% formaldehyde for 1 min. The fibers were then exhaustively washed with water.

Procedures. For the length measurements, the fibers in the solution studied were placed on a glass microscope slide over a metric scale. They were gently straightened with a watchmaker's forceps. The length could be measured to within 0.1 mm by this procedure. For the studies involving the glycerinated *psoas*, thin fibers, *ca.* 200 μ wide, were stripped from the bundle. For experiments in which the fibers were dried the slides were precoated with silicone grease to prevent adhesion of the fiber to the glass slide. The microphotographs were taken with a Zeiss research microscope equipped with phase optics.

Results

Glycerinated Muscle Fibers. The glycerinated muscle fibers shortened in aqueous LiBr solutions at room temperature over the range 0.75–10.15 M. The diminution of length occurred very rapidly. As is illustrated in curve A of Figure 1, the amount of shortening is a function of the salt concentration. A relatively sharp decrease in length occurred up to 1.5 M LiBr. A monotonic decrease in length was observed as the salt concentration was increased further. Wide-angle X-ray diffraction patterns demonstrate that the loss of the characteristic α-keratin-ordered structure occurs concomitantly with contraction (L. Mandelkern and H. Baker, 1964, unpublished results). Neither the original length nor structure is recovered when the fibers are removed from the LiBr solution and immersed in pure water.

In control experiments we have observed that there is no length change when the native fibers are immersed in either concentrated solutions of KCl (5 M) or of sucrose (4 M). Hence the neutral salts, or their ions in aqueous solution, which cause the loss of ordered structure and the concomitant contraction, are specific in their action. The interactions leading to contraction cannot, in these cases, result solely from ionic strength or osmotic effects. On the other hand we have noted that NaBr induces contraction of glycerinated muscle fibers about as well as LiBr. The effectiveness of this salt has also been observed in other systems (Bigelow and Geschwind, 1961). LiBr, therefore, can be considered to be representative of a particular class of salts.

Glycerinated muscle fibers shortened in ATP (pH 7.0, 0.1 M KCl) in the range of 0.001–0.005 M ATP displayed the conventional behavior reported in the past by various investigators (Blum *et al.*, 1957). The fibers contracted in this manner do not reelongate or regenerate their original structure when reimmersed or washed in pure water. The fibers initially shortened in ATP contract further when immersed in 7.25 M LiBr. The final shortening, based on the ratio of final length to initial length, was in the range of 0.33–0.40 and independent of the initial amount contracted and the concentration of ATP. Similar results, for the action of other neutral salts, subsequent to ATP contraction have been reported by Bowen and Laki (1955, 1956). On the other hand, fibers initially contracted in LiBr solutions to lengths less than that expected for ATP solutions do not further shorten when transferred to ATP solutions of optimum concentration for contraction. A minor exception is noted for the initial immersion in dilute LiBr solutions (0.725 M) where an additional 10% contraction is observed after transfer to the ATP solution.

Cross-linked glycerinated muscle fibers did not demonstrate any detectable change in length as a consequence of treatment with formaldehyde. Contraction was observed, however, when the fibers so treated were immersed in aqueous LiBr solution. As is indicated in curve B of Figure 1, the amount of shortening was dependent on the LiBr concentration. However, the functional dependence of the length on the salt concentration differed from that of the untreated fiber. The loss of the wide-angle diffraction pattern again occurred concomitant with shortening (L. Mandelkern and H. Baker, 1964, unpublished results). If, however, the shortened fibers were immersed in pure water, a major portion of the original length was regenerated without the application of any tensile force. Depending on the amount of initial shortening, re-extensions of from 60 to 90% of the initial length were observed. The typical α-keratin wide-angle X-ray pattern again ap-

peared (L. Mandelkern and H. Baker, 1964, unpublished results).

Glycerinated fibers cross-linked with formaldehyde, however, were no longer contractile when immersed in ATP solutions of optimal concentration. If the native fibers were initially contracted in ATP and then cross-linked, a significant further shortening occurred when they were subsequently placed in 7.25 M LiBr solutions. Except for the case of very small initial shortening in ATP, fibers so treated did not detectably regenerate their length when again placed in pure water.

Protein Fibers. LiBr-induced contraction of fibers reconstituted from the soluble proteins extracted from the muscle fibers were also studied. Such fibers, or threads, of myosin B (actomyosin) rapidly dissolved in 7.25 M LiBr solutions. However, if these fibers were treated with formaldehyde in a manner identical with the treatment of glycerinated muscle fibers, the fibers did not dissolve in the LiBr solution. Rather, a contraction to one-third of the original length was observed. When such fibers were returned to pure water, from 90 to 95% of their original length was spontaneously regained, again without the application of a tensile force. The myosin B threads behaved in the conventional manner, in that they contracted in ATP solutions. However, cross-linked threads when exposed to $HCHO_2$ for even as little as 15 seconds would not undergo ATP-induced shortening.

Fibers prepared from myosin A also dissolved quite readily in 7.25 M aqueous LiBr solution. When cross-linked with formaldehyde, the contractile behavior becomes quite similar to that of the glycerinated muscle fibers and the actomyosin threads. In 7.25 M LiBr solutions myosin A threads contracted 55%; after washing with water, about 70% of the length decrease was regained.

As has been described in the experimental portion, glycerinated muscle fibers were treated with specific myosin extractants until the A-bands were no longer visible under phase-contrast microscopy. We term the resulting structures "actin ghosts." Although mechanically frail, the integrity of the fiber structure was sufficient that its contractile properties could be investigated. In contrast to the other fibrous systems studied, aqueous LiBr solutions had no effect on the length of the "actin ghosts" until a critical concentration of 7.25 M LiBr was reached. At this concentration, contraction to about 50% of the original length was observed. High concentrations of LiBr cause about the same amount of shortening. This apparent critical concentration was unaffected by temperature in the range of 25–50%. No increase in length was observed with the return of the contracted fibers to pure water. Treating the "actin ghosts" with formaldehyde did not alter the contractile behavior. Moreover, in contradistinction to the behavior of either cross-linked glycerinated muscle fibers or myosin A or myosin B fibers, the length of the contracted "actin-ghost" fibers again remain unaltered when returned to pure water. We have observed, however, that when the actin fibers are simply air-dried for about 30 minutes and then soaked in pure water prior to immersion in LiBr solutions, their contractile properties become similar to those of the other fibers after formaldehyde treatment. They behave, therefore, as though intermolecular cross-links have been imposed on the system. For example, 50% contraction was observed in 7.25 M LiBr. When contracted fibers were placed in pure water, they re-extended to within 95% of their original length. Qualitatively similar effects were observed with air-dried glycerinated muscle fibers. We have also noted that neither the originally prepared nor dried "actin ghosts" will contract significantly in ATP.

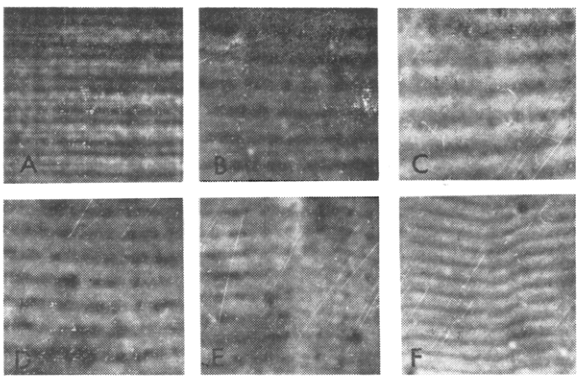

FIGURE 2: Phase-contrast photomicrographs of untreated glycerinated muscle fibers in aqueous LiBr solutions. Magnification 1600×. (A) in pure water; (B) 0.725 M LiBr; (C) 1.45 M LiBr; (D) 2.9 M LiBr; (E) 5.8 M LiBr; (F) 10.15 M LiBr.

Phase-Contrast Microscopy. The morphological changes accompanying the contraction of both the noncross-linked and cross-linked glycerinated muscle fibers in the LiBr solutions were followed by phase-contrast microscopy. The alterations in the morphology can best be described by the changes that occur in the dimensions and appearance of the characteristic banded pattern (Huxley and Hanson, 1960). Typical patterns for noncross-linked fibers immersed in solutions of varying LiBr concentrations are illustrated in Figure 2. The width of the A- and I-bands and the gross amount of contraction under the specified conditions are listed in Table I. Photograph A of Figure 2 for the fiber mounted in pure water is similar in all aspects to photomicrographs previously reported by others (Huxley and Hanson, 1960). The sarcomere width was 2.7–2.8 μ, with the A-band 1.26 μ wide and the I-band 1.47 μ wide. The Z-lines and H-zone were clearly discernible. As the concentration of LiBr in the supernatant fluid progressively increased, the A-band continuously shortened, paralleling the macroscopic dimensional changes. The I-band width remained constant up to 2.9 M LiBr. A slight decrease in the width of this band was noted at 5.8 M and an appreciable diminution of size occurred at the higher salt concentrations. At 2.9 M LiBr the Z-line was just discernible. However, the H-zones disappeared above 2.9 M LiBr. As is indicated in the last column of Table I, the rela-

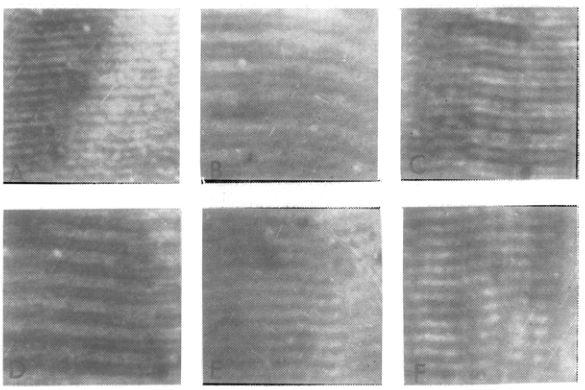

FIGURE 3: Phase-contrast photomicrographs of formaldehyde-treated glycerinated muscle fibers in aqueous LiBr solutions. Magnification 1600×. (A) 0.725 M LiBr; (B) 1.45 M LiBr; (C) 2.9 M LiBr; (D) 5.8 M LiBr; (E) 7.25 M LiBr; (F) 10.15 M LiBr.

TABLE I: Band Dimensions for Untreated Glycerinated Muscle Fibers in Aqueous LiBr Solutions.

LiBr (M)	A-Band (μ)	I-Band (μ)	L/L_0 (measured)
0	1.26	1.47	1.00
0.725	0.84	1.47	0.81
1.45	0.74	1.47	0.66
2.9	0.63	1.16–1.36	0.60
5.8	0.42	1.05–1.26	0.54
10.15	0.35–0.42	0.85	0.39

tive length of the sarcomere (sum of A- and I-band) paralleled the macroscopic observed change in length. The latter quantity is the average value for several different fiber specimens.

Figure 3 presents phase-contrast photomicrographs of cross-linked fibers which were also immersed in solutions of varying LiBr concentration. Pertinent data in regard to the width of the bands and the average extent of contraction for several different samples are given in Table II. The Z-lines and H-zones were resolved for concentrations up to 5.8 M. They cannot be seen at the higher salt concentrations. The photomicrographs for both the noncross-linked and cross-linked fibers were strikingly similar at the high salt concentrations. At the low salt concentrations (up to 2.9 M LiBr), where the amount of contraction is relatively slight, no discrimination could be made between the relative shortening of the two bands. At higher salt concentrations, both bands shortened appreciably.

A-bands of cross-linked fibers contracted in 10.15 M LiBr solutions to about 0.63 μ. Regeneration in pure water resulted in the A-bands' widening to 1.3 μ and in the reappearance of the H-zone. I-bands in 10.15 M LiBr were also about 0.6 μ and when regenerated they swelled to 0.75–0.80 μ. Hence the original dimensions of the I-bands were not regained. They tended to resemble more closely those characteristic of the contracted state.

TABLE II: Band Dimensions for Cross-Linked (Formaldehyde-treated) Glycerinated Muscle Fibers in Aqueous LiBr Solutions.

LiBr (M)	A-Band (μ)	I-Band (μ)	L/L_0
0.00	1.47	1.47	1.0
0.725	1.25	1.26	1.0
1.45	1.47	1.47	0.90
2.9	1.30	1.47	0.83
5.8	1.05	1.05	0.67
10.15	0.63	0.63	0.53

Discussion

From the results reported here we can conclude that, despite their complex morphology, glycerinated muscle fibers behave in the same manner as the other fibrous proteins in respect to the action of LiBr and some other neutral salts. The ordered chain structure is disrupted and, concomitantly, the fibers must shorten (Flory, 1956; Mandelkern, 1964a,b). Since both myosin A and myosin B fibers behave similarly, it is clear that the observed dimensional changes must result from configurational changes induced in the major constituent proteins of the fibers. We can also conclude that myosin A is not configurationally inert but possesses an axially oriented structure which can be disrupted under suitable conditions.

The conclusions previously reached (Mandelkern *et al.*, 1959), in regard to the role of stable intermolecular cross-links in enabling the dimensional changes to occur reversibly and the native structure to be regenerated after contraction in the absence of an applied tensile force, were further generalized and substantiated by the results for the two types of myosin threads and the glycerinated muscle fibers. These conclusions were contrary to the implications of Hoeve's results (Hoeve and Willis, 1963) wherein cross-linked glycerinated muscle fibers, contracted in water–ethylene glycol solutions of KI, showed no tendency to regain their original length and native state when cooled below the contraction temperature. Undoubtedly if the fibers were returned to the liquid mixture, devoid of salt, regeneration would occur.

The fact that fibers first partially contracted by ATP, then cross-linked and subsequently further shortened in LiBr solutions, did not display any length increase when returned to pure water is circumstantial evidence that the ATP-induced contraction was accompanied by some loss of ordered structure. For if the cross-links were imposed on an unaltered, ordered, oriented

structure reversibility would be expected from the foregoing considerations. We have noted that fibers cross-linked prior to shortening in LiBr solutions spontaneously recovered almost their original length when returned to pure water.

The behavior of the fibrous structure that remained after the extraction of the myosin was atypical. The requirement of a critical salt concentration, insensitive to temperature, for contraction and the irreversibility of the process after cross-linking was unique among all the fibrous protein systems studied (Mandelkern *et al.*, 1962a,b,c). It suggested the possibility that the "actin ghosts" were not comprised of axially oriented polypeptide chains as were the other fibrous proteins. This conclusion was consistent with electron micrographs reported for actin fibers (Hanson and Lowy, 1963), especially since in the "ghosts" actin was the predominant protein. On the other hand, it was also possible that the shortening of the "actin ghosts" was due to the well-recognized contraction of the sarcolemma collagen-type protein which was not extracted (Mandelkern *et al.*, 1962c). The reversible contractility observed after air drying the "actin ghosts," although most interesting, must remain at present unexplained.

The decreasing width of the A-bands of contracted noncross-linked fibers, in LiBr solutions, was consistent with the thick filaments, or myosin A, being the prime contractile element in the experiments reported here. No change was observed in the I-band width at low salt concentrations. At the higher salt concentrations, however, an appreciable diminution in the width of this band also occurred. The shortening of the A-band was consistent with the oriented crystalline structure of myosin, the contractile properties of the myosin "threads" in LiBr solutions, and the behavior of fibrous proteins in general. However, for the initial LiBr induced contraction the changes in the banded pattern were opposite to that observed for a similar amount of total sarcomere shortening in ATP solutions (Huxley and Hanson, 1960). In the latter case the width of the A-band appeared to remain essentially constant while the I-band shortened in relation to the macroscopic length changes. If the dimensional changes of the A- and I-bands were direct manifestations of the molecular processes involved in contractility, then it would appear that different fundamental acts were involved with ATP and LiBr. With LiBr configurational changes of the myosin were clearly indicated. However, the distinct possibility still exists that configurational changes of the constituent macromolecules, particularly when only small amounts of shortening are involved, would not necessarily reflect itself in gross morphological changes. We note in this connection that both LiBr and ATP yielded very similar phase-contrast micrographs at the higher degrees of shortening.

The concomitant shortening of the I- and A-bands (as is given in Tables I and II for the higher salt concentrations) is indicative of a continuous protein structure between the Z-lines of a sarcomere as was suggested by Rozsa *et al.* (1950) and by Sjostrand and Anderson-Cedergren (1957). Otherwise the A- and I-bands would separate, leaving a "contraction gap" between them. This gap would then appear instead of the observed sarcomere shortening.

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