strained lattice induced by twisting of lamellar ribbons during their growth it implies that there is still a significant amount of twisting at this high degree of stretching. As suggested in Figure 9 this is probably rather localized, however.

Conclusions

The regularly banded structure around the diameter of the cylindrical asymmetric spherulites is considered to arise from a high degree of cooperative lamellar twisting, which in turn suggests a regular packing and specific orientation of the fibrillar bundles. The latter were found to run parallel to the long axis of the cylindrical spherulites.

The deformation of the cylindrical spherulites under the influence of a tensile stress normal to their long axis is explained in terms of a transformation from a spherulitic to a fiberlike structure. From X-ray diffraction and microscopy our experimental results suggest that upon sample stretching, bundles of lamellar fibrils are initially spread to a certain extent and are pulled out from the "spherulitic" aggregates. The bundles then tilt with their long axis toward the direction of stretch and untwist to an extent dependent on the degree of stretching. Soon thereafter reorientation into a new fiberlike structure occurs with the unit cell c axis preferentially oriented in the draw direction as evidenced by the X-ray diffraction results.

The small amount of a crystalline component which is different from orthorhombic and which is present in our transcrystalline polyethylene has been established to belong to a pseudomonoclinic system. The unit cell parameters for this crystalline component have been derived as shown in Table I of this paper. The presence of the pseudomonoclinic crystalline component is common to all the forms of crystalline polyethylene we have investigated and is likely to be a feature common to all polyethylenes. A possible mode of formation of this component has been suggested in terms of a somewhat different packing of the folded polymer chains in a strained lattice array.

Acknowledgments. The authors gratefully acknowledge H. M. Zupko for providing the sample of transcrystalline polyethylene and L. S. Frishkopf and Mrs. H. S. Hanes for providing sectioned specimens of this sample.

The Effect of Salts and of Adenosine 5′-Triphosphate on the Shortening of Glycerinated Muscle Fibers[1]

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ABSTRACT: Studies of the length–temperature–composition relations for a glycerinated muscle fiber (rabbit psoas) in various salt solutions and in ATP are reported. Despite the morphological complexities inherent to this fibrous system, complete shortening is shown to be a consequence of a cooperative structural transition similar to that observed with the other fibrous proteins. The results obtained with the salt solutions help establish the transition temperature in the absence of added monomeric species, *i.e.*, in pure water. Under these conditions, the transition occurs at an elevated temperature. It is, however, found that this transition temperature is lowered monotonically with the addition of increasing amounts of ATP. Complex transition temperature–composition relations are observed when the supernatant solution contains either Mg^{2+} or Ca^{2+} in conjunction with ATP.

It has been established[2] that the fibrous proteins such as collagen, elastoidin, and the α and β keratins undergo a cooperative structural transition at a characteristic temperature which depends on the nature and composition of the medium in which they are immersed. This transformation involves the disruption of a highly axially oriented ordered structure into one which is disordered. All the general manifestations of a first-order phase transition are displayed and a large diminution in length usually accompanies the process. This latter observation is a consequence of the major conformational differences of the constituent macromolecules in the two states. A variety of reagents, known to be effective in disrupting the ordered structure of dilute protein and polypeptide solutions, also induces the transformation in the macroscopic fibrous systems.[2b]

The muscle fiber system, which has a characteristic α-keratin-like wide-angle X-ray diffraction pattern, is morphologically and chemically more complex than the other protein fibers studied. It contains at least two major protein constituents, and the unique morphology of striated muscle has been described in detail.[3] Specific to this fibrous system, shortening can be induced by the addition of ATP[4] and the catalytic hydrolysis of this species is effected during the process. Although major structural and compositional differences between muscle and the other fibrous proteins are recognized, it has been

(1) This research was supported in part by a grant from the U. S. Public Health Service, GM 10614, and a contract with the Division of Biology and Medicine, Atomic Energy Commission.

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(3) H. E. Huxley and J. Hanson in "Structure and Function of Muscle," Vol. 1, G. H. Bourne, Ed., Academic Press, New York, N. Y., 1960, p 183.

(4) Abbreviation used in this work: ATP, adenosine 5′-triphosphate.

reported that reagents such as KI, KCNS, and LiBr can induce shortening in both systems.[4–6] In the present report, we examine in detail the structural transformation of glycerinated muscle fibers (rabbit psoas) giving particular emphasis to the influence of different salts of ATP and of certain salts in combination with ATP on the transition temperature.

Experimental Section

The muscle fiber samples that were used in this work were glycerinated rabbit psoas prepared according to the method described by Szent-Gyorgyi.[7] Bundles of fibers about 10 cm long were tied to sticks at their resting length and stored in 50% glycerol solution at −10° until used. The glycerinated muscle fibers were maintained in this condition for at least 1 month before use. About 1 or 2 hr before a fiber bundle was used in an experiment, it was transferred to a 25% glycerol solution at −10°. Just prior to use, the sample was transferred from the 25% glycerol solution to pure deionized water at 0° and washed several times. Fibers about 0.5 mm thick were teased from the bundle and used for the subsequent measurements. A typical fiber length was about 50 mm. The fibers were kept in pure water for a short time prior to their being involved in the experimental observations. However, if it was necessary to keep the fibers in water for more than 2 hours they were discarded. Thus, a fresh fiber preparation was used for each determination.

The length–temperature relations for the glycerinated muscle fibers immersed in a specified medium were determined in the following manner. Each experimental point was the result of the average length of two or more fibers. Usually three to five fibers were involved in each determination. The reference length L_0 was determined at room temperature with the fiber immersed in pure water. The lengths were determined using a graduated metric scale and could be measured to within 0.5 mm.[6] Each fiber was immersed in the liquid medium at a specified temperature for about 0.5 hr and the length characteristic of that temperature determined. The lengths in most experiments were measured in the medium after the sample was returned to room temperature. Preliminary experiments showed that the length change between the elevated temperature and room temperature was within the observational error. The resulting plot then consisted of points determined for a set of fibers at each temperature. The temperature interval was usually about 5° but in special cases of interest this interval was reduced to 1–2°. The procedure adopted here for the glycerinated muscle fibers differs from that previously used for collagen (elastoidin)[8] and keratin.[9,10] In these latter experiments the length–temperature measurements, for a given fiber, were determined in a continuous heating experiment. If this procedure was utilized for the glycerinated muscle fibers, highly irreproducible and erratic results are obtained. This can be attributed to the degradation of the fibers because of the length of time that they have to be maintained at elevated temperatures. The fiber–liquid system was immersed in constant-temperature baths maintained at ±0.2° over the range from room temperature to 90°.

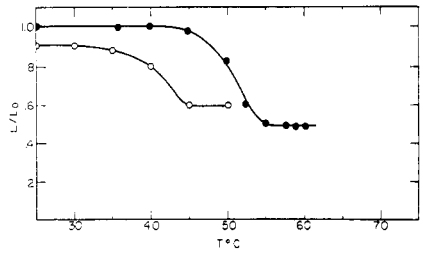

Figure 1. Plot of relative length change, L/L_0, as a function of temperature for glycerinated rabbit psoas immersed in 0.1 *M* KI (●) and 0.5 *M* LiBr (○).

The salts used in this work, KCL, LiBr, $MgCl_2$, and $CaCl_2$, were reagent grade and used without further purification. Stock solutions in water were prepared and the concentrations determined by Mohr titrations. The particular concentrations used were prepared by dilution of the stock solution whose concentration was periodically checked. Adenosine triphosphate, disodium, 99% pure, ATP, was obtained from Nutritional Biochemicals Corp. A stock solution of 0.01 *M* was prepared each week and stored refrigerated at 0°. The required ATP solutions were prepared by dilution of the stock solution and were kept at 0° until ready for use. Solutions more than 1 week old were discarded. In one class of experiments, the ATP solutions were buffered at pH 7 with Tris-maleate, 0.025 *M*. In another set of experiments, the ATP solutions were unbuffered but the pH was adjusted to 7 with either HCl or NaOH. ATP, purified by passage through a Chelex column, following the procedure of Gergely and Seidel[11] was also utilized in selected experiments. The solution which did not involve ATP were not buffered.

In a specific set of experiments the fibers were treated according to the modification proposed by Watnabe, *et al.*[12] This procedure consists of a 2-day pretreatment at 0° with a 50% glycerol solution containing 1 m*M* sodium EDTA at pH 7 followed by 1 day washing at 0° with 50% glycerol not containing any EDTA. The fibers were then stored at −10° in a 50% glycerol solution.

Wide angle X-ray diffraction patterns were obtained by procedures that have been previously described.[9] Patterns were obtained with the native fiber and with selected fibers after the completion of the length–temperature measurements. Single fibers were used in these experiments using a micro-camera with a pinhole collimating system. Nickel-filtered Cu radiation was employed and the X-ray generator tube was operated at 20 kV and 30 mA. The incident X-ray beam was positioned normal to the macroscopic fiber axis, the fiber-to-film distance was 15 mm, and exposure times were in the range of 24–48 hr.

Results and Discussion

Length–Temperature–Composition Relations in Salt Solutions. The first set of experiments to be described was undertaken to ascertain whether the glycerinated muscle fibers displayed the same shortening properties in certain aqueous salt solutions that are universal to the other fibrous proteins.[1,2] Previous reports in the literature indicated that isothermal shortening is observed at room temperature in LiBr[6] and in KI and KCNS solutions.[4,5] Length–temperature relations were obtained as a function of the salt concentration in the aqueous solution. A typical set of length–temperature curves that are obtained is illustrated in Figure 1

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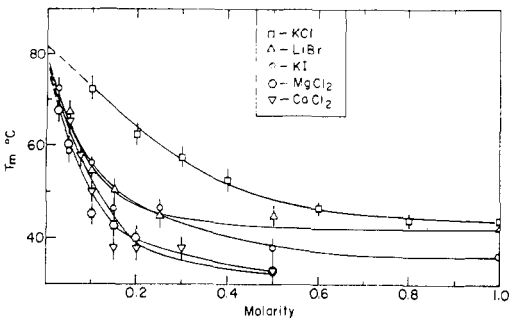

Figure 2. Plot of transition temperature T_m for glycerinated rabbit psoas as a function of molarity of salt solution for salts indicated in diagram.

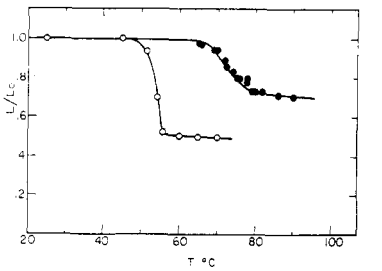

Figure 3. Plot of relative length change, L/L_0, as a function of temperature for glycerinated rabbit psoas immersed in water, pH 7: unbuffered (●); Tris-maleate buffer (○).

for a KI and an LiBr solution. Here the ratio of the length L, in the medium at temperature T, to L_0 is plotted as a function of the temperature. The data, which are typical of a variety of reagents and compositions, clearly indicate that the shortening process is restricted to a relatively narrow temperature interval. The temperature at which the shortening is complete is well defined and on either side of the shortening interval the linear expansion coefficients are normal. These results have all the characteristics of a cooperative process. The transition temperature for a given composition has been taken to be at the termination of the shortening.

For the glycerinated muscle fibers the transition region is not quite as sharp as has been observed for elastoidin.[8,13] This difference can be attributed to the more complex structure and composition of the muscle fiber system, the more complex morphology when compared with elastoidin and the keratins, and the possibility of a nonuniform geometric cross section. All these factors lead to a broader transition region.[14] The diffuseness of the transition leads to an uncertainty of about 2–3° in the assigned transition temperature. The amount of shortening depends on the nature of the salt and its concentration. It was found generally that the amount of shortening decreases with decreasing salt concentration, indicating that the swelling in the transformed state influences the final length to some extent. A comparison of the wide-angle X-ray diffraction patterns in the native state and after the completion of the transformation shows that a major structural change has occurred. The shortening is accompanied by the loss of the 5.2-Å meridional reflection and the intense equatorial spacing at 9.9 Å which are typical of the original fiber.[15]

For the glycerinated muscle fibers reelongation did not occur either on cooling or on reimmersing in pure water. The failure of the shortened fiber to reelongate can be attributed to the lack of a sufficient number of permanent intermolecular covalent cross-linkages which survive the process.[16] The amino acid analysis does not give any basis to expect the presence of such units. It is known, however, that when intermolecular cross-links are artifically introduced, reversibility can be attained.[17]

The behavior just described has been observed in aqueous solutions of KCl, KI, LiBr, $MgCl_2$, and $CaCl_2$. With these reagents it can be concluded that the shortening process in the glycerinated muscle fiber is similar to that of other fibrous proteins and involves a cooperative structural change from a highly axially oriented ordered state to a state of disorder.

From data of the type illustrated in Figure 1, the transition temperature, T_m, was determined as a function of the concentration of each of the salts in the supernatant medium. A summary of the results is given in Figure 2 as a plot of the transition temperature against concentration. It is of interest to note here that the EDTA-treated fibers, subject to the same experimental conditions, give results virtually identical with those obtained with the untreated fibers. The data for each of the salts delineate a unique curve. Each curve is characterized by a relatively rapid decrease of the transition temperature with concentration for the initial amounts of added salt followed by a much slower rate of decline as the salt concentration is increased. From among those studied here, $MgCl_2$ and $CaCl_2$ are most effective salts (on a molarity basis) in lowering the transition followed by KI and LiBr. KCl results in the smallest depression of the transition temperature. These results are qualitatively similar to those previously reported for elastoidin.[8] The salts are ordered in the same manner. The only major difference is that for each of the salts a much greater depression in T_m is observed in the muscle system.

The previous work with elastoidin suggests the possibility that an extrapolation can be made of the transition temperature data for each of the salts to a common temperature corresponding to the transition temperature in pure water. The extrapolation of the data in Figure 2 leads to the conclusion that the transition temperature characteristic of the shortening of glycerinated muscle fiber (rabbit psoas) in pure water should be in the range of 80–85°. This temperature would thus correspond to the occurrence of the cooperative structural transition in the absence of any added species. Following the suggestion of this extrapolation, direct experiments were carried out to study the shortening process in deionized water at pH 7. The resulting length–temperature diagrams are shown in Figure 3. In these particular experiments a large number of fibers were

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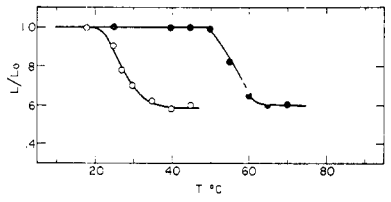

Figure 4. Plot of relative length change, L/L_0, as a function of temperature for glycerinated rabbit psoas immersed in ATP solutions: ●, buffered, 0.1 *M* KCl, 5 × 10^{-4} *M* Ca^{2+}, 0.4 m*M* ATP; ○, unbuffered, 0.1 *M* KCl, 0.4 m*M* ATP.

used at each temperature and the plotted points are the average values. For the unbuffered case, where the pH is adjusted to 7.0, a characteristic length–temperature curve is observed with the termination of the cooperative shortening process occurring at 80 ± 2°. This directly determined transition temperature is thus in excellent accord with the value extrapolated from the experiments involving salt. The transition, in this case, takes place over a 15° temperature interval with about 30% shortening occurring. For the same kind of experiment performed in the Tris-maleate buffer medium, the transition temperature is reduced to 55–60° with the shortening process being much sharper with respect to temperature. The reason for the different behavior found in the buffered and unbuffered supernatant solutions is not readily explicable at present. However, the major conclusion can be made that a well-defined transition can be observed in water without the necessity of adding specific reagents.

The direct experimental observation of a transition temperature at 80° in pure water supports the validity of the extrapolation described. The X-ray diffraction patterns, after shortening in pure water, also demonstrate a complete loss of ordered structure. In earlier work, Varga[18] has shown that the major portion of the shortening of glycerinated rabbit psoas in water occurs in the range of 50–75° which is in good agreement with present results. More recently, Aronson[19] has studied the change in sarcomere length as a function of temperature. His results can be interpreted to represent a cooperative process with a transition temperature in the vicinity of 80°. For glycerinated fibrils of the indirect flight muscle of *Drosophila melanogaster* a transition temperature of about 50° can also be estimated from his data.[19]

Shortening in ATP Solution. With the establishment of a cooperative structural transition for the glycerinated muscle fiber in pure water, it is a matter of interest as to whether ATP has any effect on this transition. Some typical examples of the length–temperature relations obtained when ATP is an integral part of the supernatant solution are illustrated in Figure 4. The characteristics of these plots are similar to those shown in Figure 1 for the salt solutions. A readily discernible transition temperature, defining the termination of the shortening, exists. Hence the transformation in these fibers can also be induced by ATP. Since the hydrolysis of ATP in the absence of enzyme is perceptible at temperatures above 60°, our experiments have not exceeded this temperature in order to avoid any possible complication due to the thermal splitting of ATP.

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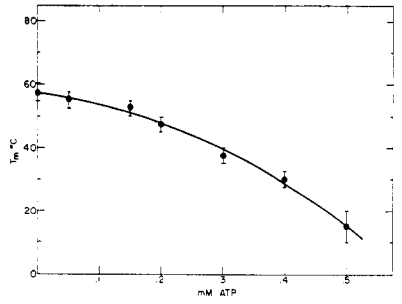

Figure 5. Plot of transition temperature T_m as a function of ATP concentration for buffered solution pH 7 containing 0.1 *M* KCl.

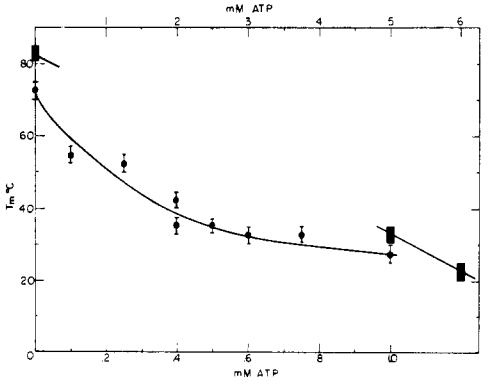

Figure 6. Plot of transition temperature T_m as a function of ATP concentration for unbuffered solution pH 7: ●, with 0.1 *M* KCl, lower axis; ■, without added KCl, upper axis.

From data of the type illustrated in Figure 4, the relationship between the transformation temperature and the ATP concentration could be determined. Several different variations in the composition of the supernatant were studied. These include buffer at pH 7, buffer with added KCl, unbuffered solution at pH 7 and unbuffered solution, pH 7 with added KCl. The results of these experiments are summarized in Figures 5 and 6. Figure 5 gives the results for the buffered system in 0.1 *M* KCl. Under these conditions, in the absence of ATP, the directly determined transformation temperature is in the range 55–60°. The transition temperature is monotonically reduced as the ATP concentration in the supernatant solution is increased. The transition is decreased to below room temperature when 0.5 m*M* ATP is present in the supernatant under the specified conditions. In these experiments the shortened fibers do not reelongate or regain their original structure upon reimmersion in pure water. The wide-angle X-ray pattern again indicate the loss of the ordered structure upon completion of the shortening process. Since we are primarily concerned with the relation between the transformation temperature and the ATP concentration the observations are made through the complete shortening range and into the shortened state.

The ATP concentration required for cooperative shortening at room temperature corresponds to the reduction of the transition temperature below room temperature. This concentration is comparable under similar conditions to that which has been required for

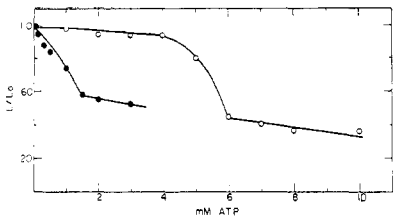

Figure 7. Relative length change as a function of ATP concentration at 25°; unbuffered solution pH 7: ●, with 0.1 *M* KCl; ○, without added KCl.

shortening to take place at room temperature in glycerinated rabbit psoas in other investigations.[20-23] In the present work, we arrive at this concentration in a natural way starting with a transformation temperature at an elevated temperature as a base point. This transition temperature, which has been established from studies with other reagents, is very similar in character to that found in the other fibrous proteins. We can conclude, therefore, that the ATP-induced shortening at room temperature is a consequence of the same type of structural transformation.

A similar set of results is presented in Figure 6 for the relations between the transition temperature and ATP concentration when the supernatant is unbuffered. In one example shown 0.1 *M* KCl is present and in the other it is absent. The results are qualitatively similar to those in Figure 5 in that there is a systematic and monotonic decrease of the transition temperature with increasing ATP concentration. For the unbuffered case, however, the transition temperatures, in the absence of ATP, are different depending on the composition of the supernatant. Without KCl the transition temperature is 80–85°, while it is lowered to 70–75° by the addition of 0.1 *M* KCl. In contrast, as has been noted above, the transition temperature for the buffered case, with 0.1 *M* KCl, is 55–60°. The ATP concentration required to reduce the transition temperature to room temperature is dependent on the nature of the supernatant. For the unbuffered system containing 0.1 *M* KCl, 1 m*M* ATP is required. This concentration is approximately twice as much as is needed in the comparable buffered situation. Similarly, in the absence of buffer and of added KCl, about 6 m*M* ATP is required to induce shortening at room temperature. These observations at room temperature are thus a direct result of the different values for the transition temperature in the dissimilar supernatants and the different rates of depression with added ATP. This latter factor is clearly affected by the presence of KCl. The fact that increased amounts of ATP are required to induce shortening at room temperature, as the supernatant composition changes in the manner indicated, has been known previously.[20,23] The data reported here offer a formal explanation of these observations. We have also found that essentially the same results are obtained if Chelex treated ATP is used.

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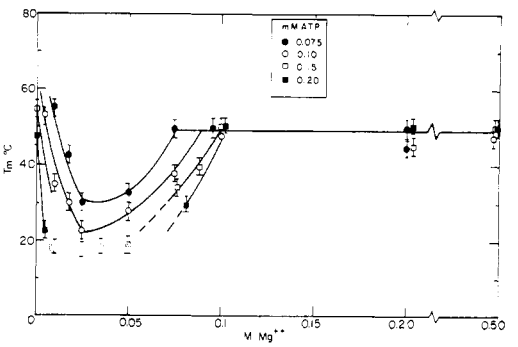

Figure 8. Plot of transition temperature T_m as a function of Mg^{2+} concentration for buffered solution, pH 7, 0.1 *M* KCl, and indicated ATP concentrations.

The data summarized in Figures 5 and 6 suggest that the transformation could be carried out isothermally by varying the ATP concentration.[17,20] Plots of the relative change in length at room temperature as a function of ATP concentration is given in Figure 7 for the unbuffered system with and without KCl. Shortening occurs over a relatively narrow range of ATP concentration indicative of a cooperative process. As is anticipated, higher ATP concentrations are required when KCl is absent. Thus, the dimensional control that can be achieved at room temperature, by varying the ATP concentration, is a direct consequence of the transformation that occurs at an elevated temperature in a different compositional environment.

Mg–ATP, Ca–ATP Shortening. It is known that Mg^{2+} and Ca^{2+} ions, in conjunction with ATP, influence the isothermal shortening process.[20,22] In the context of the present work, it is of interest to ascertain whether the dimensional properties in such systems bear any relation to changes in the transition temperature. We have, therefore, studied the dependence of the transition temperature on composition when the supernatant contains ATP, 0.1 *M* KCl and either Mg^{2+} or Ca^{2+}. For each individual experiment in this series, the length–temperature curves are very similar to the cases previously cited so that the transformation temperature is well defined. The results for the Mg–ATP combinations are summarized in Figures 8 and 9 for the buffered solution, pH 7, containing 0.1 *M* KCl. Each curve represents the transition temperature for a solution con-

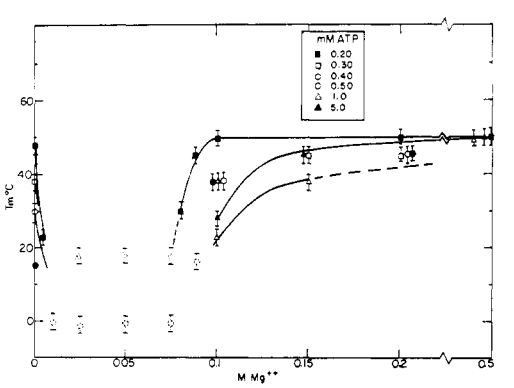

Figure 9. Plot of transition temperature T_m as a function of Mg^{2+} concentration for buffered solution, pH 7, 0.1 *M* KCl, and indicated ATP concentrations.

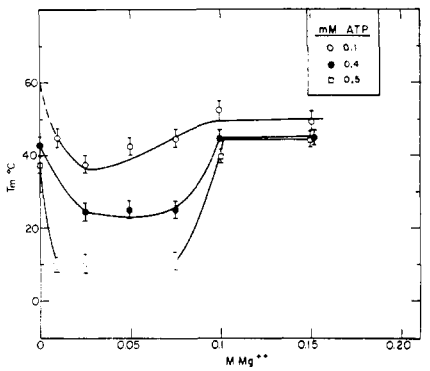

Figure 10. Plot of transition temperature T_m as function of Mg^{2+} concentration for unbuffered solution, pH 7, 0.1 *M* KCl, and indicated ATP concentration.

taining a fixed concentration of ATP with varying amounts of Mg^{2+}. The points on the vertical axis then represent the transformation temperature for a given ATP concentration in the absence of Mg^{2+}. The presence of this additional component in the supernatant fluid results in a transformation temperature–concentration diagram which contains some unusual features. As is indicated in the figures, at all ATP concentrations the initial addition of Mg^{2+} results in a sharp reduction in the transformation temperature. As the Mg^{2+} concentration is increased to 0.025 *M* this temperature decreases by approximately 40° for the lowest ATP concentrations used. For the higher levels of ATP, the transformation temperatures are reduced well below room temperature. In these latter cases, the exact values could not have been obtained with great reliability so that the dashed symbols represent estimated upper limits for T_m. The reduction in the transition temperature in this system is much greater than would be anticipated if only Mg^{2+} were present. From Figure 2, for example, 0.025 *M* Mg^{2+} only causes a 10° depression in this temperature. Another interesting feature of the data is the existence of a minimum in the transition temperature in the range 0.02–0.05 *M*, Mg^{2+}. This minimum appears to be essentially independent of the ATP concentration. With a further increase in Mg^{2+} concentration, the transformation temperature increases and then levels off at about 50°. This effect is strikingly illustrated in Figure 9 when 5m*M* is present in the supernatant. In the absence of Mg^{2+}, shortening occurs at room temperature as a consequence of the transformation temperature being reduced well below this temperature of observation. However, with the addition of 0.1 M Mg^{2+} the transformation temperature is raised to slightly above room temperature. A qualitatively similar behavior is also found with the unbuffered supernatant, as is illustrated in Figure 10. Although the quantitative temperature–concentration relations are altered, the major features described for the buffered system are still present. The behavior described contrasts sharply with the situation when ATP is absent since either Mg^{2+} or Ca^{2+} by themselves causes a large monotonic depression in the transition temperature over a similar concentration range.

Based on the temperature–composition relations of Figures 8–10, predictions can be made with respect to isothermal length changes as the concentration of Mg^{2+}

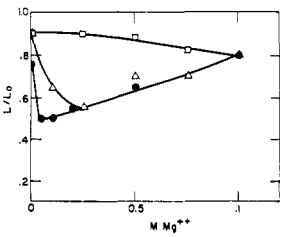

Figure 11. Plot of relative change of length L/L_0 at 25° as a function of Mg^{2+} concentration. Buffered pH 7, 0.1 *M* KCl: □, 0.05 m*M* ATP; ▲, 0.075 m*M* ATP; ●, 0.15 m*M* ATP.

is varied at a fixed ATP concentration. The conclusions reached will necessarily depend crucially on the ATP concentration and the temperature selected for study. Of primary importance is the relationship between the temperature so chosen and the transformation temperature at the given composition. For example, if the transformation temperature is never reduced below the temperature fixed for observation no significant changes in length are expected and the length typical of the relaxed or native state should be observed for the whole range of Mg^{2+} concentration. On the other hand, if the ATP concentration is so chosen that the temperature of observation is always above the transformation temperature, then a length characteristic of the shortened state would be observed. Alternatively, according to the data in Figures 8–10, it is possible to choose an observation temperature and ATP concentration so that as Mg^{2+} is added the transformation temperature is initially lowered below the observation temperature and then as the Mg^{2+} concentration is increased further, the transition temperature will be above the observation temperature. It is then expected that initially the characteristic length of the native state will be observed followed by a cooperative shortening with the addition of Mg^{2+}; as the concentration of this species is increased, the original length should be observed.

The expectations described above are fulfilled in direct length measurements carried out isothermally at 25°. Typical results are illustrated in Figure 11. According to Figure 8, for 0.05 m*M* ATP, the transformation temperature is never reduced below 25°. Therefore, as is indicated in Figure 11, virtually no change in length is observed over the complete range of Mg^{2+} concentration. However, for ATP concentrations of either 0.075 m*M* ATP or 0.15 m*M* ATP, the transformation temperature can be reduced below room temperature with the initial addition of Mg^{2+}, and then raised above it. Hence as is also shown in Figure 11, the corresponding changes in length are observed. A minimum in the length occurs at about 0.02 *M* Mg^{2+} and with further increase in Mg^{2+} concentration the amount of shortening is reduced. These independent measurements are thus completely consistent with the conclusions drawn from Figures 8–10. Since the length–composition coefficient, at constant temperature, is directly related to the force–composition coefficient, with a change in sign, complementary results must occur in isometric experiments where the force is measured at a fixed lentgh. The results described above are in accord with the experiments on isothermal shortening and

tension development reported by Bowen and Martin.[22] We can conclude, therefore, that the specfiic effect of Mg^{2+} in either altering dimensions or in developing tension is dependent on the ATP concentration (as well as other species in the supernatant fluid) and is directly related to the location of the transformation temperature relative to the temperature of observation.

Similar types of experiments have been carried out with Ca^{2+}–ATP combinations. The results in this case are distinctly different from those found for the Mg^{2+}–ATP system. For the entire range of ATP studied, 0.075–5 m*M*, and for the same range of divalent salt concentrations, 5×10^{-3} to 5×10^{-1} *M*, no decrease in T_m is observed upon the addition of $CaCl_2$. In fact, in many cases this temperature is increased. For example, in the buffered system with 0.1 *M* KCl and 0.2 m*M* ATP, the transformation temperature is 45–50°. The addition of $CaCl_2$ raises the transformation temperature to 57–62°. This observation is in striking contrast to the results with Mg^{2+} previously discussed where is was shown that this temperature is easily reduced below room temperature upon similar conditions. It is also found that when the initial transition temperature is below room temperature, it is rapidly elevated with the addition of $CaCl_2$. Thus, it is clear that for comparable salt concentrations the initial rapid depression of the transformation temperature is not observed in the Ca^{2+}–ATP system. Therefore, for isothermal experiments conducted at room temperature in this composition range the relaxed length would be observed for all Ca^{2+} concentrations if the transformation temperature was initially above room temperature. However, if the transformation temperature was below room temperature, then no shortening would be observed with Ca^{2+} addition. These expected changes in dimensions and the accompanying changes in tension development have been reported.[22]

In considering the data for the Ca^{2+}–ATP system that is reported here, it is important to note the restricted range of salt concentration that is involved. In this connection, Seidel and Gergely[11,24] have reported that, in isometric experiments, when highly purified reagents are used a large increase in tension develops in the range 10^{-6} to 10^{-5} *M* Ca^{2+}. Consequently, under comparable concentration conditions shortening should be observed when no force is applied. In the context of the present work, it is then expected that in plots similar to those of Figures 8–10 a minimum would be observed. However, this would occur at Ca^{2+} concentrations several orders of magnitude less than has been observed for Mg^{2+}. The direct experimental verification of this prediction has not as yet been achieved.

Conclusion

The experimental results described above are essentially thermodynamic in nature. Consequently a molecular mechanism with regard to the shortening process and the influence of added species on the transformation temperature cannot be developed solely from this point of view. However, certain general conclusions can be made and more quantitative approaches outlined. Despite the complexities in protein composition and morphology that are well recognized and characteristic of the striated muscle fiber system,[3] the transformation is very similar to that undergone by the other fibrous proteins having a much simpler structure. Hence by analogy it can be concluded that a cooperative structural change from an axially oriented state to a disordered one occurs. This conclusion is substantiated by the concomitant changes that take place in the wide-angle X-ray diffraction patterns.

The expected effects of composition and concentration on the transition temperature can be examined through the formalism of classical thermodynamics.[25] For a three-component system, composed of a major solvent species (1), an added monomeric species (2), and a macromolecular species (p) held at a fixed concentration, Peller[26] has shown that

$$\frac{d\,T_m}{d\,\ln a_2} = \frac{-RT_m^2\Delta\alpha_{2,p}}{\Delta\bar{H}} \tag{1}$$

Here T_m represents the transition temperature, a_2 is the thermodynamic activity of species 2 and $\Delta\bar{H}$ is the partial enthalpy change in the macromolecular species in transforming from the ordered to disordered state. The parameter $\alpha_{2,p}$ is defined as

$$\alpha_{2,p} \equiv \left(\frac{\partial m_2}{\partial m_p}\right)_{\mu_2} = \left(\frac{\partial \mu_p}{\partial \mu_2}\right)_p \tag{2}$$

and represents the interaction between species 2 and the macromolecule in a given state. $\Delta\alpha_{2,p}$ represents the difference in this interaction between the disordered and ordered states and is termed the preferential interaction. In the special case where the interaction is restricted to one phase the conventional binding equation results.[26,27] Equation 1 is the three-component analog of the usual expression for the melting point depression or boiling point elevation of a species. It states that in order for the transformation temperature to change when a new species is added $\Delta\alpha_{2,p}$ must have a nonzero value, *i.e.*, there must be a net preferential interaction. The direction of the change in the transformation temperature will depend on the signs of $\Delta\alpha_{2,p}$ and $\Delta\bar{H}$.

An extension of this development to a system of many components yields

$$\left(\frac{\partial T_m}{\partial \ln a_2}\right)_{m_{i,p}} = \frac{-RT_m^2}{\Delta\bar{H}}\left[\Delta\alpha_{2,p} + \sum_{i=3}^{r}\alpha_{i,2}\Delta\alpha_{i,p}\right] \tag{3}$$

where there are now $(r - 1)$ added monomeric species in addition to the major solvent and macromolecular species. The change in the transition temperature with the added species (2) now depends not only on $\Delta\alpha_{2,p}$ but on the preferential interaction of the other species with the macromolecule, $\Delta\alpha_{i,p}$, and on the interaction of the monomeric species with each other $\alpha_{i,2}$. The activity of species 2 is for the specified composition.

The only purpose for the introduction of eq 1 and 3 into the present discussion is to illustrate and emphasize

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(25) Since in certain of the experiments ATP hydrolysis occurs concomitant with the shortening, in a strict sense of the term one is dealing with a steady state rather than thermodynamic equilibrium.

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the many different factors that can influence the transformation temperature–concentration relation. For a three-component system $\Delta\alpha_{2,p}$, $\Delta\bar{H}$ and a_2 need to be independently known to be able to analyze quantitatively the results. When more species are present not only do additional preferential interactions with the polymer need to be taken into account but the interaction of the monomeric species with each other can also play an influential role. It is clear from eq 3 that, depending on the sign and magnitude of these interaction parameters, a wide spectrum in temperature–composition relations can be accommodated which could include monotonic increases, monotonic decreases, and the existence of extremals. Therefore, it is not unexpected that a supernatant solution composed of ATP and salts displays a complex and unusual behavior. An explanation of the observations that for a supernatant solution containing Mg^{2+} and ATP there is a minima in the transition temperature as a function of salt concentration while the Ca^{2+}–ATP systems behave quite differently must be sought in the nature of the interaction coefficients. It is premature to attempt a quantitative analysis of these data without this information. However, it is important to note in this connection that both Ca^{2+} and Mg^{2+} form complexes with ATP and the equilibrium constants are approximately the same for both species.[28,29] On the other hand Hammes and Levison[30] have reported that the forward rate constant for the complexing reaction is at least a hundredfold greater for Ca^{2+} than for Mg^{2+}. There are, therefore, features in the aqueous chemistry of these ions which are different and which could reflect themselves in differences in the activity coefficient and interaction coefficients that are involved.

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Studies on Aqueous Solutions of Sodium Poly-L-glutamates. Determinations of Mean Activity Coefficient, Osmotic Coefficient, Transference Number, and Partial Molal Volume

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ABSTRACT: The mean activity coefficient of sodium poly-L-glutamate, NaPGA, in the two-component system water–NaPGA was investigated by the isopiestic vapor pressure and/or emf measurements. The transference and related measurements were also carried out for the determination of the net valency and the transference number of PGA ions. The degrees of neutralization of PGA with NaOH were 0.3, 0.6, and 1.0, and the solution temperature studied was between 25 and 50°. The fraction of free gegenions and the transference number of PGA ions were concentration insensitive, and dependent on the degree of neutralization and solution temperature, being 0.6~0.95 and 0.2~0.4, respectively. The osmotic coefficient was between 0.5 and 1.3, varying with the degree of neutralization, polymer concentration, and temperature, and increased sharply by the helix–coil transition induced by rising temperature. The mean activity coefficient decreased with concentration at low concentrations; the decreasing tendency was more remarkable for the helical structure than for the coiled state. The two coefficients indicated the strong water structure-forming tendency of the PGA ions. The partial molal volume was further measured for various salts of PGA. The individual molal volume of PGA ions was calculated to be 85.7 ml/monomol; the electrostrictional hydration effect and the hydrophobic structural effect were −7.7 and −11.7 ml/monomol, respectively. From this, it is concluded that the monomer unit of PGA is hydrated electrostrictionally by three water molecules.

Poly-L-glutamic acid, a simple model compound for proteins, has been investigated by using various experimental techniques. Most of them are concerned with the change in physicochemical properties associated with the helix–coil transition.[1-5] However, the mean activity coefficient, which is one of the most fundamental thermodynamic quantities, has not been measured. The mean activity coefficients of several synthetic polyelectrolytes have been investigated by emf measurements of a concentration cell with transference, and isopiestic vapor pressure measurements by us and Dolar, *et al.*[6-14] In the present paper, we report the activity, transference, and density data for sodium salts of poly-L-glutamic acid, NaPGA, at various degrees of neutralization and temperatures.

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Experimental Section

Materials. A Koch-Light Laboratories sample of a sodium salt of poly-L-glutamic acid (NaPGA) (mol wt

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