Thermal Transitions of Myosin and Its Helical Fragments. II. Solvent-Induced Variations in Conformational Stability†

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ABSTRACT: The melting behavior of myosin and myosin rod has been studied over the pH range 5.4–7.0, in 0.5 M KCl. Both proteins exhibit almost identical T_m values, which increase from about 37 to 43° as the pH is elevated through the range of study. T_m follows a sigmoidal dependence upon pH, and the inflection point occurs near pH 6.5. The influence of salt concentration on T_m was studied, by the variation of KCl from 0.15 to 2.92 M. With an increasing KCl concentration, both proteins exhibit similar, sigmoidal declines in T_m from about 44 to 34°. Under all conditions of pH and ionic strength studied, melting is accompanied by an absorption of H^+ by the protein. The concentration of the protein, the age of the preparation, and the presence of divalent metal ions fail to exert a significant effect on the T_m values obtained by our methods. The effects of salt concentration and pH on the thermal stability of myosin and myosin rod are discussed in terms of the location of the melting process within the myosin molecule. Myosin is shown to possess several of the requisite features for energy transduction *via* a proton-coupling mechanism. These features provide a framework for investigating some aspects of the molecular basis of muscle contraction within the context of the sliding filament model.

In the search for a molecular basis for muscle contraction, a great deal of interest has centered on the conformation of the myosin molecule. A number of investigators have studied the conformation of myosin in the presence of physiological species such as salts, metal ions, and nucleotides (Morita and Yagi, 1966; Gratzer and Lowey, 1969; Godfrey and Harrington, 1970). These studies have all shown that below 25°, the conformation of rabbit-muscle myosin is stable and insensitive to the solvent environment. Recent studies, however, suggest that the conformation of the rod portion of rabbit-muscle myosin is much less stable near physiological temperature (Burke *et al.*, 1973; Jacobson and Henderson, 1973). The discovery of this marginal stability suggested to us that the conformation of the molecule might become sensitive to the solvent environment at temperatures approaching 37°. Thus, in this paper we describe the influence of solvent composition on the conformation of myosin at elevated temperatures.

Direct observation of conformation-related parameters (*e.g.*, ORD, intrinsic viscosity, sedimentation coefficient) is prevented under many circumstances by heat-induced coagulation of myosin (see Goodno and Swenson, 1975). We have thus chosen to study the conformational *transition* of myosin as a measure of its structural stability, rather than its static conformation under a particular set of solvent conditions. In the preceding paper we have shown that changes in pH are a reliable observable for following conformational transitions of myosin and its fragments. Since these transitions can be viewed as the melting of regions of crystalline structure within the protein, we have chosen the "melting temperature," denoted by T_m, as a convenient indicator of conformational stability. The effects of solvent parameters, especially pH and ionic strength, on the T_m values of myosin and its rod moiety have been investigated and some of myosin's requisite features for energy transduction are presented.

Methods

Protein isolations, analyses, and the application of the pH melting method with appropriate controls are described in the preceding paper.

Protocols for Specific Types of Melting Experiments. VARIATION OF T_m WITH PH. Stock solutions were prepared from fresh protein preparations or preparations stored by the previously described methods. Solutions were dialyzed against several changes of 0.5 M KCl and centrifuged to remove aggregates. Immediately before use, the stock solution was volumetrically diluted with 0.5 M KCl to give a final volume of 5.0 ml having the appropriate protein concentration. The pH was adjusted to the desired initial value with 0.1 N HCl or 0.1 N NaOH, and the experiment was begun. T_m was surveyed at several pH values between 5.5 and 7.0 at protein concentrations between 0.4 and 2.0 mg/ml. A systematic study of T_m in the pH range 4–8 was done on one batch of myosin and one of myosin rod, at the same concentrations used in the surveys.

VARIATION OF T_m WITH IONIC STRENGTH. Myosin and myosin rod were melted in KCl solutions ranging from 0.15 to 2.9 M. Various concentrations of KCl were obtained by volumetric dilution of protein stock solutions (in 0.5 M KCl) with either 4.0 M KCl or with distilled H_2O. Protein concentrations were 1.68 mg/ml for myosin and 0.60 mg/ml for myosin rod. All experiments in this series were initiated at pH 6.00 ± 0.01.

VARIATION IN T_m WITH PROTEIN CONCENTRATION. A concentrated stock solution of myosin in 0.5 M KCl (9.3 mg/ml) was diluted with 0.5 M KCl to various concentrations from 0.09 mg/ml to 9.3 mg/ml. All experiments were initiated at pH 6.50 ± 0.05, with melting observed by the pH method.

MYOSIN ATPASE ACTIVITY DURING MELTING. Aliquots for assay of myosin ATPase activity were taken at

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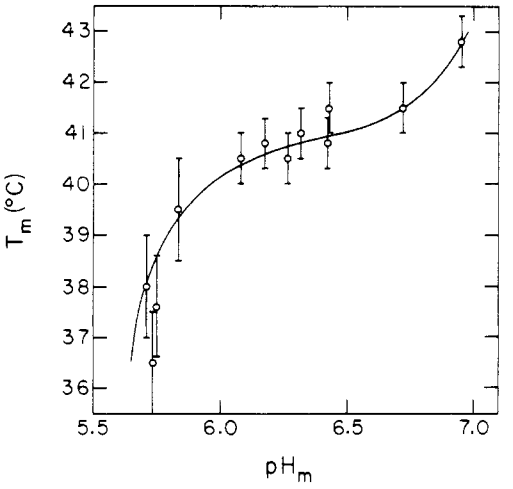

FIGURE 1: Dependence of the myosin T_m on the value of pH_m. Conditions: myosin (1.85 mg/ml) in 0.5 M KCl.

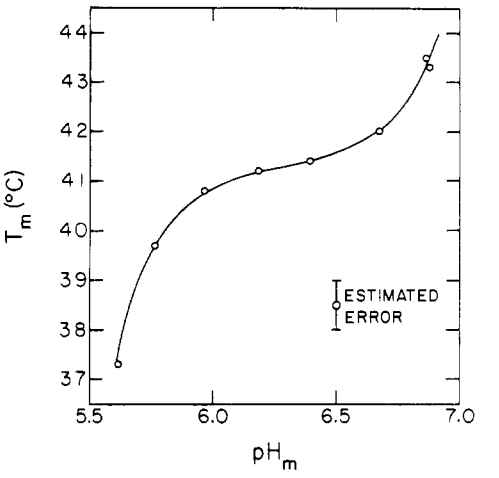

FIGURE 2: Dependence of T_m on pH_m for myosin rod. Conditions: myosin rod (0.45 mg/ml) in 0.5 M KCl.

various temperatures to determine the effect of heating on activity. All samples were kept on ice until the conclusion of the melting experiment and then were assayed at 23° by the usual ATPase assay.

SURVEY OF THE EFFECTS OF POTENTIAL PERTURBANTS ON T_m. Melting experiments were performed on myosin and myosin rod in the presence of a number of perturbants. The following reagent grade salts were used: $CaCl_2$, $MgCl_2$, disodium nitrilotriacetate (NTA),[1] and disodium ethylenediaminetetraacetate (EDTA). The dipotassium salt of ATP was obtained from P-L, and the tetrasodium salt of adenylyl imidodiphosphate (AMPPNP) was obtained in 90% purity from ICN. All salts and nucleotides were used without further purification. Experiments with myosin were performed at a protein concentration of 1.85 mg/ml, and those with rod were done at 0.45 mg/ml. The general procedure consisted of addition of the protein in 0.5 M KCl to a well-stirred solution of the perturbant in the pH cell. Nucleotide solutions were neutralized with 1 N KOH, and the final sample volume was 5.0 ml. Melting was carried out as previously described.

Protein Titrations. Potentiometric titrations of myosin and myosin rod were performed by the continuous method, employing the same apparatus which was used for the melting experiments.

Results

T_m as a Function of the Solution Environment. Effect of pH. Melting of myosin and myosin rod was observed between pH 5.4 and 7.0 by the pH method. The melting curves of both proteins were qualitatively very similar over the whole accessible range. T_m values obtained from these curves could be compared with the ones which Jacobson and Henderson (1973) have obtained for myosin and which Burke *et al.* (1973) have obtained for myosin rod. Allowing for slight differences in pH and ionic strength, our T_m values are in close agreement. Although Jacobson and Henderson use a pH of 7.4, they are apparently referring to the initial pH rather than the pH at the melting point (pH_m). In Tris buffer their pH_m value must be considerably below the reported value of 7.4. The agreement between our values is striking since Jacobson and Henderson determined T_m by viscosity studies and Burke *et al.* determined it by viscosity and ORD.

The shape of our myosin melting curve (see preceding paper) is quite similar to the one which Jacobson and Henderson obtained by viscometry. Our myosin rod curve is similar to the one Burke *et al.* obtained by viscometry, which shows a single transition near 44°. By the pH method, however, we observe no apparent transition at higher temperature resembling the one Burke *et al.* found at 53° by ORD. In studies on light meromyosin, however, we were able to reproduce their light meromyosin melting curves by the pH method.

The dependence of the T_m of myosin upon pH_m (the pH of melting) is shown in Figure 1; an almost identical curve obtained for myosin rod is shown in Figure 2. Both show an unmistakable decrease in T_m with decreasing pH_m. It is apparent that a sigmoid curve gives a much better fit than a monotonic one and that both curves have inflections near pH 6.5.

The value of pH_m was found to be remarkably linear as a function of pH_i (the initial pH of the melting experiment). This can be related to varying base-line shifts with temperature, which can be explained in terms of heats of ionization of the various classes of titratable groups on the protein (Wyman, 1939). Since this observation does not give information on the characteristics of the conformational transition of interest, it will not be dealt with in further detail.

While the shift in pH from pH_i to pH_m varies systematically with pH_m, no systematic variation appears to hold for the pH shift (ΔpH) across the entire cooperative region of the melting curve—in fact, within experimental error ΔpH is constant over the range of pH_m values studied. If a single class of residues absorbed the protons which are taken up during melting, ΔpH would certainly decrease as that class was titrated. Since this does not occur, we propose two possible explanations: (1) the class of residues which absorbs the protons is not titrated in the pH 5.4–7.0 region; (2) no particular class of residues absorbs the protons. Rather, the absorption is the net result of small changes in the pK values of several classes of residues. Since melting is not observed outside the pH 5.4–7.0 range, the first possibility appears not to be valid. Thus, the proton absorption probably arises from small adjustments in the pK values of several classes of residues.

[1] Abbreviations used are: NTA, disodium nitrilotriacetate; EDTA, disodium ethylenediaminetetraacetate; AMPPNP, tetrasodium adenylyl imidodiphosphate.

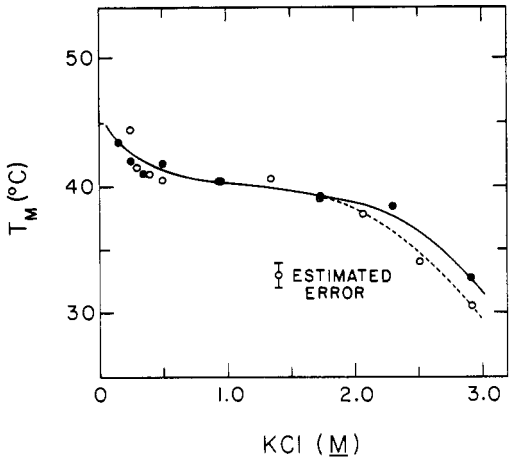

FIGURE 3: Dependence of T_m upon KCl concentration. All T_m values were obtained with a pH_i value of 6.00 ± 0.02. (●) Myosin (1.68 mg/ml); (○) myosin rod (0.60 mg/ml).

EFFECT OF IONIC STRENGTH. Since the pH study suggested the importance of the net charge on the protein during melting, the effect of ionic strength was also examined. Figure 3 demonstrates that T_m values for both myosin and myosin rod undergo a decrease of about 10° as KCl concentration is raised from 0.15 to 2.9 M. Just as we found in the pH study, the curves for the two proteins are remarkably parallel. No difficulty was encountered in obtaining sharp melting curves at high ionic strengths. However, below 0.25 M, the curves broadened; and below 0.15 M no melting point could be located at all. At ionic strengths below about 0.2 M, myosin and rod formed filamentous aggregates, even before melting. Although two-phase systems often give anomalous pH readings (Linnet, 1970) these readings generally arise from incomplete stirring or from the impermeability of one of the phases to protons. In our experiments the suspensions were well stirred, and the aggregated phase was gel-like, making it essentially no different from the bulk solution in its permeability to protons.

The lack of apparent melting at low ionic strength appears to be due to the nature of the protein filaments rather than experimental difficulties. Incorporation of the protein molecule into the filament may perturb its structure in such a way that uptake of protons can no longer accompany melting. Josephs and Harrington (1968) have shown that simple polymerization of myosin into filaments results in an absorption of protons. The ionizable groups which absorb these protons may be the same ones which absorb protons during melting. If absorption has already occurred before melting, the melting process will be "silent" when pH is used as the observable. It is interesting that Pepe (1967) has proposed that a structural change in the rod portion of myosin is necessary for filament formation. While the existence of this change is far from certain, one might speculate that it involves the same region of the molecule which produces the pH change upon melting.

The ΔpH value for myosin rod shows no systematic variation with ionic strength. This situation is similar to the one encountered in the pH study and suggests that ΔpH is independent of both pH_m and ionic strength. This conclusion further suggests that the ionizable residues whose pK's are altered upon melting are not the ones whose ionization is affected by KCl concentration.

EFFECT OF MYOSIN CONCENTRATION. Since myosin tends to associate to form low n-mers, even in the presence of 0.5 M KCl (Godfrey and Harrington, 1970b), it was of interest to determine whether T_m depended upon the amount of n-mer present. The T_m of myosin was studied in 0.5 M KCl over a 50-fold range of protein concentration. For optimal sharpness of melting, the studies were done at a pH_m of 6.5. Figure 4 shows that T_m remains constant as the protein concentration is varied. Using the association constant obtained by Godfrey and Harrington at neutral pH (1970b), we have estimated that our concentration range encompasses fractions of n-mer ranging from about 50 to 80%. If T_m of the monomer and n-mer were different, we would have expected some variation in T_m over this range. Since no variation was observed, we conclude that T_m is the same for monomer and n-mer.

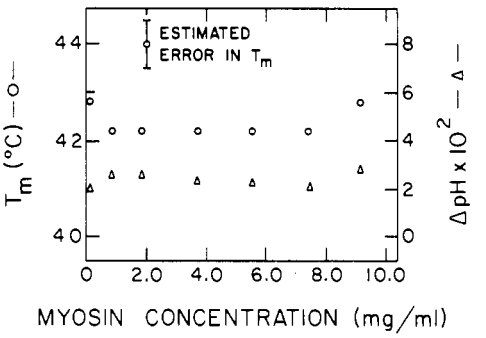

FIGURE 4: Effect of myosin concentration on T_m and ΔpH. Experiments were performed at a pH_i value of 6.50 ± 0.05 in 0.5 M KCl.

Table I: Effects of Added Solutes on T_m.[a]

Protein	Added Solute	pH_m	T_m	Control T_m
Myosin rod	8.4 mM $CaCl_2$	6.0	40.7	40.8
Myosin rod	12 mM $MgCl_2$	6.0	41.0	40.8
Myosin rod	1.6 mM NTA	6.4	41.0	41.4
Myosin	12 mM $MgCl_2$	6.0	41.5	40.5
Myosin	0.15 M KCl and 1 mM $CaCl_2$	6.6	44.0[b]	42.3[b]
Myosin	1.6 mM NTA	6.4	41.0	41.0
Myosin	0.6 mM MgAMPPNP			41.0

[a] Unless otherwise noted, all additions were made to protein samples in 0.5 M KCl. Controls were done at the same pH and KCl concentration. [b] Value is the average of the results of three experiments.

The constancy of ΔpH with myosin concentration is at first somewhat surprising (see Figure 4). It appears, however, that ΔpH remains constant because changes in Δn_{H^+} due to the conformational transition are exactly balanced by increasing buffer capacity as the protein concentration is raised.

EFFECTS OF VARIOUS SOLUTES. The perturbation of T_m by various solutes of physiological interest was investigated, and the results are collected in Table I. Removal of divalent metals was effected by addition of the chelator disodium nitrilotriacetate. (Our untreated myosin preparations ordinarily contained 2 mol of Ca^{2+}/mol of protein.) Concentrations of the divalent metals were deliberately kept low to avoid significant changes in the overall ionic strength. Studies in the presence of ATP were frustrated by the proton production accompanying hydrolysis. Studies

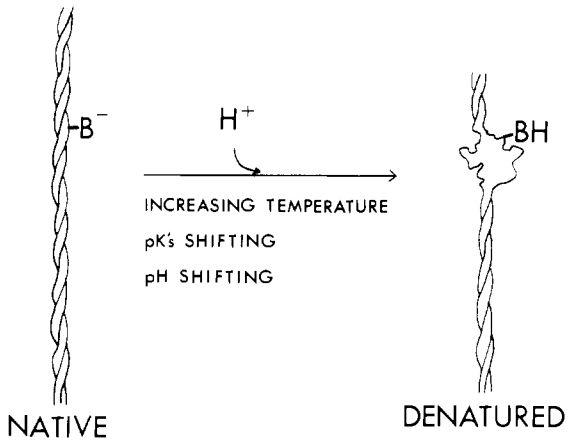

FIGURE 5: Schematic diagram showing relationship between conformational equilibrium, melting, and proton absorption in the rod portion of myosin.

with AMPPNP were unfortunately obscured by its buffer capacity.

REVERSIBILITY OF MELTING. The reversibility of melting was checked for myosin and myosin rod at several pH values. We found no conditions under which myosin could be melted in a reversible manner. Myosin rod, however, underwent annealing and was found to remelt at its original T_m value. Regardless of the number of times the protein had been melted, the pH of the cooled solution was found to be several tenths of a unit above the initial pH. Even if the pH was readjusted, the original melting curve could not be exactly retraced. We noted that greater deviations from the original curve were obtained in cases where large increases in turbidity accompanied the first melting. It was found that adjustment of the pH to about 7.5 with 0.05 N KOH resulted in clarification of the solution. If the clarified solution was then readjusted to the original pH and remelted, the original melting curve was retraced. These observations suggest that melting of the rod is an intrinsically reversible process which is accompanied by aggregation of the melted molecules. Reversible denaturation has also been reported for light meromyosin (Biró *et al.*, 1972; Szilágyi *et al.*, 1972); reversible melting appears to be a general feature of the helical fragments of myosin.

ATPASE ACTIVITY DURING MELTING. Aliquots of myosin solution were taken at various times during a melting experiment and assayed under uniform conditions (see Methods). In agreement with the results of Jacobson and Henderson (1973) for myosin, virtually all ATPase activity is lost by the time T_m is reached.

ERROR ANALYSIS. The reliability of our measured T_m values was evaluated on the basis of a number of duplicate experiments. These experiments showed a precision of ±0.5° over the pH and ionic strength range surveyed, except at the low extremes of pH and ionic strength, where precision fell to ±1.5°. This decline in precision was traceable to an increased width of the melting transition, which introduced greater uncertainty into the assignment of T_m. In spite of the decrease in precision, the trend in T_m with a particular variable (*e.g.*, pH) was readily discerned.

QUANTITATION OF PROTON ABSORPTION. In the survey studies of T_m, melting was always found to occur with an increase in pH. It was of considerable interest to determine the number of protons which are absorbed by the protein upon melting. Two experimental approaches were tried to quantitate these protons. First, melting was carried out in a pH-Stat in the hope that proton absorption could be measured directly during melting. This method proved too insensitive for the small pH changes involved. Second, identical samples of myosin rod were titrated through their pH_m values (predicted on the basis of Figure 2) at several elevated temperatures. This approach proved similarly insensitive. While these findings were disappointing, they demonstrated the utility of the pH method for observing conformational transitions which occur with very small changes in proton binding.

We were nevertheless able to use the pH shift across the melting transition to obtain an estimate of the proton absorption. A typical pH_m value of 6.5 was selected, and the buffer capacities of myosin and myosin rod were calculated at that pH from our titration data and those of Mihályi (1951). A typical value of 0.02 was chosen for the pH shift across the melting transition. On this basis, we found that myosin and myosin rod each absorb about 2 H^+ per molecule upon melting at pH 6.5.

Discussion

Nature of the Melting Process. Although results obtained by the pH method are not very amenable to theoretical analysis, they can be used to devise a qualitative model which accounts for the melting process in myosin. Since pH and salt concentration are the only variables which we have found to influence T_m, the emphasis will be on explaining these effects. While only myosin will be referred to, it should be appreciated that the same discussion applies to myosin rod. It has been shown that when the concentration of either H^+ or KCl is increased, the T_m of myosin decreases. The salt effect occurs at a concentration which is much too high to be regarded as a bulk ionic strength effect, so KCl must be presumed to exert its influence by specific binding, as does H^+. Lewis and Saroff (1957) have shown that myosin, indeed, binds K^+. We may conclude that the binding of K^+ has roughly the same effect as the binding of H^+, although at much higher concentration. If we consider melting to be a simple two-state process, we can write

$$N + mH^+ + nK^+ \rightleftharpoons DH_m^+K_n^+$$

where N and D represent the native and denatured forms of the protein, respectively. It is apparent that an increase in either proton concentration or salt concentration will shift the conformational equilibrium to the right (Figure 5). Since equalization of the concentrations of N and D occurs at a lower temperature, T_m will be decreased. Thus, this provides that the binding of H^+ or K^+ leads to a structural destabilization within myosin.

One might question why ion binding should displace the conformational equilibrium of myosin. Binding alters the charge on the molecule, thereby disturbing the balance between the electrostatic forces and the other noncovalent interactions which maintain the structure. With the balance of forces already disturbed by ion binding, less thermal energy is required to loosen the structure, and T_m becomes correspondingly lower. Numerous such examples can be found in which ion binding produces melting of proteins at room temperature.

Collagen, another fibrous protein, responds to salts and low pH in much the same way as does myosin. The results of Lennox (1949) indicate that the addition of KCl up to a concentration of 0.5 M results in a 5° decrease in the shrinkage temperature (T_s) of sheep-skin collagen. In the

same study, T_s was observed to fall from 68 to 50° as the pH was lowered from 4.0 to 3.0. Hayashi and Nagai (1973) have shown that the melting temperature of soluble collagen declines as pH is decreased from neutrality, but the decline is much less dramatic than that observed by Lennox. Neither of these studies gives any indication that the T_m of collagen varies sigmoidally with pH in the manner of the myosin T_m. The T_m of myosin, however, has been studied at intervals of 0.1 pH unit, while the collagen T_m has been studied only at intervals of 1.0 to 0.5 pH unit. It is possible that sigmoidal behavior in the collagen studies may have been missed because of the large pH interval between measurements.

The most striking feature in the pH dependence of the myosin T_m is the fact that an inflection point occurs near pH 6.5. This finding suggests that the charges on certain histidine residues may play an important part in the regulation of myosin structural stability. Our finding that increasing [KCl] alters T_m in qualitatively the same way as lowering the pH has important bearing on this suggestion. Lewis and Saroff (1957) found that both H^+ and K^+ bind to histidine sites on myosin. Thus, our studies of T_m *vs.* [KCl] further support the hypothesis that alterations in the charges at certain histidine sites might be important in regulating the structural stability of myosin. This hypothesis, however, does not necessarily imply that histidines are the residues which account for the proton absorption that accompanies the thermal transitions. In fact, the lack of a systematic variation of ΔpH with pH_m suggests that histidines are not specifically involved in the proton absorption. It appears entirely possible that certain histidines might act as "trigger groups" which regulate the protein unfolding process without directly participating in it.

Our comparative studies on the pH dependence of T_m values of myosin, myosin rod, and light meromyosin enable us to speculate further about the location of the protein unfolding process. The T_m values of all three molecules are practically identical, and these values undergo a parallel decline with decreasing pH. This observation strongly suggests that the observed thermal transitions occur in a portion of structure which is shared by all three. Burke *et al.* (1973) have proposed that this portion consists of the "hinge region" of myosin, and our results support this hypothesis.

Biological Significance of the Melting of Myosin. MYOSIN MELTING AND ENERGY TRANSDUCTION. One implicit objective of the present study was to examine the suitability of the isolated myosin molecule for participation in energy transduction (Harrington, 1971). While our studies have been performed at ionic strengths higher than physiological, they show that myosin has the following requisite features for energy transduction by a proton-coupling mechanism: (1) marginal stability of conformation near physiological temperature; (2) a region of the molecule whose structural stability is sensitive to the binding of protons in the physiological pH range; (3) a phase transition which occurs with the absorption of protons in the physiological pH range. Since we have seen that the transition occurs in the helical, rod-like portion of the molecule, we may conclude that it is a helix → coil transition. In fact, the similarity of our melting curves to those of Burke *et al.* (1973) suggests that we have both observed the *same* helix → coil transition. Flory (1956) has pointed out that contraction is a natural consequence of such an order-to-disorder transition, and it is known that helix → coil transitions can occur quite rapidly, with relaxation times on the order of 10^{-6}–10^{-7} sec (Barksdale and Stuehr, 1972).

ADDITIONAL ROLES FOR THE MELTING OF MYOSIN. A number of important roles in addition to energy transduction can be visualized for melting of the hinge region of myosin. Huxley (1969), Harrington (1971), and others have proposed that this portion of the molecule acts as a hinge to allow the heavy meromyosin portion of a myosin molecule in the thick filament to swing out to attach to an actin molecule in the thin filament. Since muscle contraction is accompanied by cyclic attachment and release of cross-bridges, the conformational changes which produce attachment must be reversible. We have shown that melting of myosin rod in the vicinity of the hinge region is, indeed, a reversible process. We have also shown that the stability of this region is regulated by the binding of cations. We might even speculate that the binding of protons or other cations could act as a switch which controls the hinging of the cross-bridge. Viewed in another way, melting of the hinge region, which is equivalent to a helix → coil transition, might easily furnish elasticity, thereby allowing the cross-bridge to sustain jerking or wrenching motions without detachment. The work of Huxley and Simmons (1971) suggests that the cross-bridge actually has such elastic properties. From yet another perspective, melting transitions in the hinge region of myosin may play an important part in determining the structure of the thick filament. Pepe (1967) has proposed that myosin molecules are packed into the thick filament in such a way that they are deformed near the hinge region. In preliminary experiments our failure to observe melting of myosin filaments lends support to this idea and suggests that at least partial melting of the hinge region may accompany filament formation.

Additional Information Available. Additional data were submitted for the examination of the reviewers and are available from the authors on request.

Acknowledgments

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Conformational Flexibility of Angiotensin II. A Carbon-13 Spin–Lattice Relaxation Study†

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ABSTRACT: Carbon-13 spin–lattice relaxation times (T_1) have been determined for the carbons in the octapeptide hormone [5-isoleucine]-angiotensin II in aqueous solution. Two possible models for molecular motion are considered: isotropic overall motion of the hormone with internal motion of some residues and anisotropic overall molecular motion. The data are interpreted in detail using the former model. The α carbons of the peptide backbone are all equally restricted in their motion. The correlation time for overall molecular reorientation, calculated from an average T_1 value of 95 msec for the α carbons in the peptide backbone, is *ca.* 5×10^{-10} sec. The carbons in the side chains are more mobile than those in the peptide backbone, with the exception of the side chain of the Tyr residue which does not undergo rapid segmental motion. We propose that [5-isoleucine]-angiotensin II has a restricted backbone conformation and that the α carbons of the N- and C-terminal residues are constrained to nearly the same extent as the remaining α carbons in the peptide backbone. Chemical shift data indicate that the Pro residue adopts the trans conformation about the His–Pro bond and that the imidazole ring of His has a strong preference for the N^{τ}-H tautomer.

The conformations of linear peptide hormones have been more difficult to elucidate than those of cyclic peptides of similar size due to the larger number of degrees of freedom of the peptide backbone. The conformations of angiotensin II, Asp-Arg-Val-Tyr-Val(or Ile)-His-Pro-Phe, a natural pressor agent, and its synthetic analog angiotensinamide II, Asn-Arg-Val-Tyr-Val(or Ile)-His-Pro-Phe,[1] have been extensively investigated by thin film dialysis (Craig *et al.,* 1964; Franze de Fernandez *et al.,* 1968), gel filtration (Ferreira *et al.,* 1969), ultracentrifugation (Paiva *et al.,* 1963), hydrogen–deuterium exchange (Paiva *et al.,* 1963), hydrogen–tritium exchange (Printz *et al.,* 1972b), infrared and Raman spectroscopy (Fermandjian *et al.,* 1972a,b), circular dichroism (Fermandjian *et al.,* 1972a,b), and ^{1}H nuclear magnetic resonance spectroscopy (^{1}H nmr) (Vine *et al.,* 1973; Glickson *et al.,* 1973; Marshall *et al.,* 1973; Weinkam and Jorgensen, 1971; Bleich *et al.,* 1973b). A number of conformations have been proposed for angiotensinamide II such as the random coil (Paiva *et al.,* 1963), the antiparallel β-pleated sheet (Fermandjian *et al.,* 1972a,b), the α-helix (Smeby *et al.,* 1962), the γ-turn (Printz *et al.,* 1972a; Bleich *et al.,* 1973a), as well as other conformations peculiar to this peptide (Weinkam and Jorgensen, 1971). Fermandjian and coworkers (1972a,b) have stressed the point that the conformation of angiotensamide II varies greatly depending on experimental conditions. A recent ^{1}H nmr study by Glickson *et al.* (1973) revealed that angiotensin either exists in a unique conformation, different from the α-helix, the β-turn, and γ-turn models, or is in rapid equilibrium ($>10^3$ sec^{-1}) between various conformations. ^{1}H nmr spectra also reveal conformational transitions associated with titration of the α-amino and/or imidazole group as well as with titration of the phenol group in tyrosine. The exact nature of the conformational transitions has not been elucidated (Glickson *et al.,* 1973).

Zimmer *et al.* (1972) have investigated the structure of [5-valine]-angiotensinamide II in aqueous medium using ^{13}C nmr spectroscopy. ^{13}C resonances were identified on the basis of the titration behavior of specific peaks, on the chemical shifts of resonances in the constituent amino acids, as well as by comparison with the spectrum of the C-terminal tetrapeptide. All the pK values obtained for the titratable groups in [5-valine]-angiotensinamide II were determined by following the pH dependence of the ^{13}C chemical shifts. The ^{13}C spectra did not give any evidence of conformational rearrangements as a function of changes in pH similar to those observed in the ^{1}H nmr spectra.

The present ^{13}C nmr studies were undertaken in order to measure the spin–lattice relaxation times (T_1) of the backbone and side-chain carbons in [5-isoleucine]-angiotensin II and the antagonist [5-isoleucine,8-leucine]-angiotensin II.

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[1] The nomenclature used in this paper follows the Recommendations of the IUPAC-IUB Commission on symbols for amino acid derivatives and peptides ((1972), *J. Biol. Chem. 247,* 977).