The Optical Rotatory Dispersion of Myosin A

1. EFFECT OF INORGANIC SALT*

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Many reports have been published on the effect of inorganic salts, especially LiBr, on the secondary structure of protein and synthetic polypeptide. Harrington and Schellman (1) worked with solutions of silk fibroin, serum albumin, chupein, and ribonuclease and deduced that LiBr increases the stability of intramolecular hydrogen bonds. Perlmann (2) observed the change in dispersion constant, $\lambda_c$, and the decrease in enzymic activity of pepsin in LiBr solution. Mandelkern and Roberts (3) have recently reported that the thermodynamic stability of the $\alpha$-helix in ribonuclease decreases with increase in concentration of LiBr. Haly and Snaith (4) observed that the $\alpha$-helix in wool fiber keratin is destroyed in a narrow range of concentration of LiBr. Furthermore, Laki and Bowen (5) reported shortening of glycerol-washed muscle and myosin B fibers on adding KI or KSCN, which suggests that the secondary structure of muscle structural protein changes on the addition of these salts.

The changes in the molecular structure of myosin A and in its enzymic activity caused by salt, however, have not been reported yet. Therefore, we have made a detailed investigation of the effect of inorganic salts, such as KCl, LiBr, LiCl, KSCN, and KI, on adenosine triphosphatase (ATPase) activity, optical rotatory power, and viscosity of myosin A and have obtained the following results. (a) The $\alpha$-helical content and reduced viscosity of myosin A decrease sharply and reversibly in a narrow range of concentration of LiBr, LiCl, KI, or KSCN. The effect of temperature and concentration of LiBr on the helical content obeys the Schellman equation (6). (b) ATPase activity decreases irreversibly in a range of low concentration of these salts, although the helical content and reduced viscosity remain almost unaffected. (c) In KCl solution, however, the helical content and reduced viscosity are almost unchanged, and ATPase activity is decreased reversibly by increasing the concentration.

EXPERIMENTAL PROCEDURE

Myosin A solutions were prepared by the method described previously (7). The solutions were clarified by centrifugation at $10^4 \times g$ for 2 hours before use.

Crystalline sodium salt of ATP was the product of Sigma Chemical Company.

KCl, LiCl, LiBr, KI, and KSCN were commercial products of the best reagent grade available. The salt composition of the myosin A solution was changed by adding a constant volume of a salt solution of constant concentration into a constant volume of 0.6 M KCl solution of myosin A. The concentrations of KCl and the salt added are described in “Results.”

The optical rotatory dispersion of the myosin A solution was measured usually at pH 7.0 by means of a model 2008-50 photoelectric spectropolarimeter with an oscillating polarizer prism (O. C. Rudolph and Sons) in the wave length range from 3200 to 5500 A. A xenon compact arc lamp was used as the source for continuous spectra. The concentration of myosin A was approximately 5 mg per ml, and the angle of rotation was from $-0.15^\circ$ to $-1.50^\circ$. The polarimeter tube was 10 cm in length. Its temperature usually was maintained at $20 \pm 0.2^\circ$ by circulating water from an ultrathermostat (Haak, Germany) through the jacket surrounding the tube. The results were expressed in terms of the equation of Moffitt and Yang (8),

$$[m'] = \frac{3}{n^2 + 2} \frac{M_o}{[a]} = \frac{a \lambda \lambda^2}{\lambda^2 - \lambda^2} + \frac{b \lambda^4}{(\lambda^2 - \lambda^2)^2}$$

where $[m']$ is the so-called effective residue rotation at any wave length, $\lambda$, this being the observed specific rotation, $[a]$, corrected for the effects of the refractive index, $n$, which was measured by an Abbe-type refractometer, and the average molecular weight of the single residue, $M_o$, which was calculated to be 117 (9). The adjustable parameter, $\lambda_0$, was taken as constant and equal to 2140 A. Excess right-handed helical content was estimated by dividing the $-b_9$ term by 580 (10), since the $a_9$ term varies not only with the helical content but also with the change of the medium. Unless otherwise stated, the rotatory powers were measured 10 to 60 minutes after the salt composition was changed, since the rotations in various salt solutions, except for KSCN, were independent of incubation time.

ATPase activity was measured at $20^\circ$ and pH 7.0 (10 mM Tris-maleate buffer) in the presence of 0.5 mM ATP, 7 mM CaCl$_2$, and monovalent salts. The reaction was stopped by adding triehloroacetic acid at measured intervals of time, and P$_i$ liberated was determined by the Martin-Doty method (11). The activity was expressed as the value relative to that in 0.6 M KCl, except for the case of LiCl. The color development of P$_i$ by the method of Martin and Doty was interfered with in the presence of KCl or KSCN. Therefore, the standard curves for P$_i$ measurement were made for each medium, but P$_i$ was not determinable in KSCN of concentration higher than 1 M by this method.

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The sulfhydryl groups in myosin A were titrated with p-chloromercuribenzoate by the spectrophotometric procedure of Boyer (12) in the presence of KCl and LiCl. In 3 M LiBr, cysteine was not titrated by the Boyer method, probably because of binding of Br⁻ with the reagent.

The viscosity was measured at 20 ± 0.05° by the use of an improved Ostwald-Fenske-type viscometer in which the average velocity gradient, (G), was in the range from 100 to 150 sec⁻¹. The protein concentration was approximately 1 mg per ml. These conditions were experimentally reasonable, since in this range of (G) and at this protein concentration or below, reduced viscosity, ηvp/c, was independent of protein concentration and (G) (13).

Sedimentation runs were made in a Spinco model E ultracentrifuge, operated at 56,100 r.p.m., with a bar angle of 70°, and at pH 7.0 and 25 ± 0.2°. The apparent sedimentation coefficients were calculated from plots of the logarithm of the distance versus time. Usual corrections for solvent viscosity and buoyancy were made in calculating s20, w (14). The partial specific volume of myosin A was assumed to be 0.728 (15).

RESULTS

Effect of Salt—Fig. 1 shows the dependence on KCl concentration of the excess right-handed helical content, reduced viscosity, and ATPase activity of myosin A. As shown in this figure, the helical content of this preparation in 0.6 M KCl was 58% and was almost independent of KCl concentration in the range from 0.6 M to 5.1 M. The ηvp/c decreased slightly with increase in KCl concentration. ATPase activity decreased, however, in the low concentration range of KCl. The activities in 1.7 M and 4 M KCl were 50 and 12% of that in 0.6 M KCl, respectively. The decrease in ATPase activity was completely reversible; for example, when myosin A was transferred to 0.6 M KCl solution after being incubated in 3 M KCl solution for 5 minutes, the activity completely returned to the control.

In Fig. 2 are shown the Moffitt-Yang plots of rotatory dispersion data of myosin A in various concentrations of LiBr. Below 2.1 M, the −b₀ term remained almost unchanged, but the −a₀ term increased with increasing LiBr concentration. The sharp increase in the −a₀ term (from 107 to 334) and the sharp decrease in the −b₀ term were observed at 2.45 M LiBr. In Fig. 3, the helical content of myosin A obtained from the −b₀ term, ηvp/c (100 ml per g), and ATPase activity were plotted as functions of LiBr concentration. A dramatic decrease in ηvp/c (from 2.22 to 0.50) and in helical content (from 60 to 8%) oc-
Fig. 4. Irreversible change of ATPase activity in LiBr. Ca++, 7 mM, 0.5 mM ATP, 0.06 M KCl, 0.36 mg of protein per ml, pH 7.0, 20°. ○ and △ show activities measured after incubation with LiBr for 5 and 20 minutes, respectively. ● and ▲ show activities measured in 0.6 M KCl after incubation with each concentration of LiBr for 5 and 20 minutes, respectively.

Fig. 5. Dependence of reduced viscosity and ATPase activity on LiCl concentration. pH 7.0, 20°. □, Reduced viscosity, 1 mg of protein per ml, 0.1 M KCl; ○, ATPase activity expressed as value relative to that in 0.6 M LiCl, 0.25 mg of protein per ml. ■, Reduced viscosity measured in 2.68 M LiCl after 5 minutes of incubation in 4.82 M LiCl. ○, ●, and X show ATPase activity measured in 0.6 M LiCl after incubation in 2.45, 3.6, and 4.8 M LiCl, respectively.

curred at 2.45 M LiBr, as seen in this figure. The decrease in ηvp/c and in helical content were apparently reversible; both of them increased reversibly with decreasing LiBr concentration. In the ultracentrifugal pattern of myosin A in 3.0 M LiBr solution, two major peaks were revealed; their sedimentation coefficients, s20, w at 6.03 mg per ml were 6.04 and 6.97 S. After this sample was dialyzed against 0.6 M KCl for 16 hours, one main peak was revealed; its s20, w at 6.2 mg per ml was 5.72 S. These values of s20, w are larger than the 3.49 S obtained for original myosin A in 0.6 M KCl and at 6.93 mg per ml.

ATPase activity decreases sharply at low concentrations of LiBr (it was completely inhibited by 2 M LiBr), where the helical content and ηvp/c remained unchanged. The presence of 1 to 10 mM cysteine did not change the decreasing effect of LiBr on the activity. The decrease in ATPase activity caused by LiBr was partially reversible; the activity decreased gradually with the incubation of myosin A in LiBr solution. As shown in Fig. 4 (dashed lines), when myosin A was incubated in 1.5 M LiBr solution for 5 and 20 minutes and then transferred into 0.6 M KCl, 18.5% and 2% of the control activities, respectively, were recovered. By incubation of myosin A in 2.5 M LiBr for 5 minutes, followed by dialysis against 0.6 M KCl solution for 16 hours at 0°, 8% of the control activity was recovered, although the activity remained lost when myosin A was transferred from 2.5 M LiBr directly into 0.6 M KCl.

The ηvp/c decreased reversibly from 2.3 to 0.5 (100 ml per g) at 3.3 M LiCl, and ATPase activity was completely lost at 3 M (Fig. 5). The decrease in the activity was completely reversible, partially reversible, and completely irreversible, respectively, at 2.4, 3.6, and 4.8 M. In the presence of 0.6 M KCl or 3 M LiCl, the amounts of sulfhydryl groups of 3- to 5-day-old myosin A preparations were 7.6 to 8.0 moles per 10^6 g.

Fig. 6 shows the Moffitt-Yang plots of myosin A in various concentrations of KI. Up to 1 M KI, only the −α0 term increased, and above 2 M KI both the −α0 and the β0 terms increased with increasing KI. In Fig. 7, the helical content, ηvp/c, and ATPase activity were plotted as functions of KI concentration. A fairly sharp decrease in the helical content and ηvp/c occurred at 2.8 M KI, the former decreasing from 88.1 to 28% at 3.3 M and the latter, from 2.12 to 0.14 (100 ml per g) at 3.6 M KI. These changes were found to be completely reversible.

On the other hand, ATPase activity was reduced to 40% of the control even in 0.3 M KI and was completely lost in 1.5 M KI. The reversibility of ATPase activity in KI was very similar to that in LiBr. The activity depended on the incubation time; for example, after incubation in 1 M KI for 5 and 20 minutes, the activities decreased to about 5% and below 1% of that in 0.6 M KCl, respectively.

Fig. 8 shows the Moffitt-Yang plots of myosin A in various concentrations of KI. KCI, 0.17 M, 3.3 mg of protein per ml, pH 7.0, 20°. Concentration of KI: ○, 1; ●, 2; △, 2.6; ▲, 3.3 M. ○, 0.6 M KCl.
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Icx
(A)
6C
CONCENTRATION OF KI (M)

FIG. 7. Dependence of helical content, reduced viscosity, and ATPase activity on KI concentration. pH 7.0, 20°C. A, Helical content, 3.3 mg of protein per ml, 0.15 M KCl; □, reduced viscosity, 0.99 mg of protein per ml, 0.125 M KCl; ○, ATPase activity, 0.2 mg of protein per ml, 0.05 M KCl. △, ■, and ● show helical content, reduced viscosity, and ATPase activity in 0.6 M KCl, respectively.

CONCENTRATION OF KSCN (M)

FIG. 8. Moffitt-Yang plots of dispersion data in various concentrations of KSCN. KCl, 0.17 M, 4.72 mg of protein per ml, pH 7.0, 20°C. Concentration of KSCN: 0, 0.6; □, 1; △, 4; ●, 5.33 M. Concentrations of KSCN solution (0.5 to 5.1 M). As seen in the figure, the \(-a_0\) term increased and the \(b_0\) term decreased with increase in concentration of KSCN, but the increase in the \(-a_0\) term by KSCN (from 188 to 248) was much smaller than that observed in other salts. In the presence of KSCN, the helical content and \(\eta_m/c\) changed gradually with time, especially at concentrations of KSCN lower than 2 M, although they remained constant in the presence of other salts. For example, \(\eta_m/c\) increased from 2.6 to 3.5 during 50 minutes in 1 M KSCN and from 2.02 to 2.5 during 40 minutes in 2 M KSCN. The values of helical content and \(\eta_m/c\), as shown in Fig. 9, are those measured 10 to 30 minutes after the addition of KSCN. The decrease in helical content and in \(\eta_m/c\) caused by KSCN showed no sharp transition concentration. The helical content was decreased from 60.5 to 16% by 5.3 M KSCN and \(\eta_m/c\), from 2.26 to 0.07 by 4.8 M KSCN. KSCN inhibited ATPase very powerfully; the activity decreased to 10% of the control even in 0.6 M KSCN. The ATPase activity recovered was 46% of the control when myosin A was transferred into 0.6 M KCl after being incubated with 0.6 M KSCN for 5 minutes; i.e. the inhibition of ATPase by KSCN was partially reversible.

Influence of Temperature—Fig. 10 shows the temperature dependence of the rotatory power of myosin A at 4900 Å, \(\chi_{4900}\), in various concentrations of LiBr and at pH 10.5. Measurements
were performed from low to high temperature after equilibration to the required temperature. In this figure, the change in $\chi_{200}$ is expressed as the value relative to the maximal change by changing from a sufficiently low temperature to a sufficiently high one. The temperatures in these curves for the half change in $x_{4900}$ of the maximum are taken as transition temperatures, $T_{tr}$. As shown in Fig. 11, a plot of $1/T_{tr}$ against concentration of LiBr gives a straight line, which satisfies the Schellman equation (6).

The helical content and $\eta_{sp}/c$ of myosin A in KCl solution remained almost unaffected over the range of KCl concentration from 0.6 to 5.1 M. In the case of LiBr, LiCl, KI, and KSCN, the helical content and $\eta_{sp}/c$ of the maximum were 2.45, 3.3, 2.8, and 4 M, respectively. The sedimentation coefficients of the major components of myosin A (at 0.93 mg per ml) in 3.5 M LiBr were 6.04 and 6.97 S, which were much larger than that of the control, 3.49 S. It may be reasonable, therefore, to conclude that inorganic salt induces the transition of myosin A from a fibrous long molecule of helical structure to a globular molecule of random coil.

On the other hand, ATPase activity decreased sharply in a low concentration range of salt where the helical content and $\eta_{sp}/c$ remained almost unaffected. In the case of LiBr, LiCl, KI, and KSCN, the activity decreased almost irreversibly, whereas in KCl it decreased reversibly. Thus, the effect of salt can be classified as follows: (a) the reversible decrease in ATPase may be caused simply by increase in ionic strength, as in the case of KCl, and (b) in the case of other salts, the irreversible decrease is caused by the conformational change in the myosin A molecule. The fact that the irreversible decrease in ATPase activity caused by various salts occurs in a range of low salt concentration, where the helical content almost remained constant, may be interpreted by assuming a local and irreversible melting by salt of the $\alpha$-helical structure around the active site or by assuming a nonhelical structure of the active site of ATPase. In the case of myosin A-ATPase, the former assumption seems to be preferable to the latter, since the heat, alkaline, or acid inactivation of ATPase is accompanied by a decrease of a few per cent in the helical content. However, the possibility that the change in the conformation of some other nonhelical region of the molecule by salt induces the conformational change in the active site still remains.

The relationship between the concentration of the denaturating reagent and the transition temperature, $T_{tr}$, from helix to random coil may be written (6, 16) as

$$
\frac{1}{T_{tr}} = \frac{n - 1}{n - 4} \Delta S_{rel}^{0} + \frac{P R K c}{(n - 4) \Delta H_{rel}^{0}}
$$

where $\Delta H_{rel}^{0}$ and $\Delta S_{rel}^{0}$ denote the standard enthalpy and entropy changes from the unfolding of backbone chain; $P$, the number of the binding site of the reagent; $K$, the binding constant of the reagent to the binding site; and $n$, the number of amino acid residues in helix per 1 molecule. When the molecular weight, the helical content, and the average residue weight of myosin A are taken, respectively, as 6 $\times$ 10^5 (7), 50%, and 117 (9), $n$ is given as 3064. Because of the absence of S-S linkages in myosin A and because our experiments were performed at pH 10.5, the terms due to the existence of a cross-linkage and to the rupture of the side chain hydrogen bond have been neglected. It was assumed that $Kc < 1$. As described in "Results," the plot, $1/T_{tr}$ versus concentration of LiBr (c), gives a straight line. If the value of $\Delta H_{rel}^{0}$ is taken as 1.5 kcal mole$^{-1}$ (6), the observed plot, $1/T_{tr}$ versus c, gives 4.68 entropy units and 166 kcal mole$^{-1}$, respectively, as $\Delta S_{rel}^{0}$ and $PK$, and $P$ must be much larger than 664. This value of $\Delta S_{rel}^{0}$ is in good agreement with the value estimated by Schellman (6) for rupture of the hydrogen bond of polypeptide chains in general.

**SUMMARY**

The helical content and the reduced viscosity, $\eta_{sp}/c$, of myosin A remained almost unaffected in the concentration range from 0.6 to 5.1 M KCl. In LiBr, LiCl, KI, and KSCN the helical content and $\eta_{sp}/c$ decreased sharply and reversibly in a narrow range of salt concentration: when LiBr, KI, and KSCN were added the helical content decreased from 60 to 8, 28, and 16%, respectively, and when LiBr, LiCl, KI, and KSCN were added $\eta_{sp}/c$ decreased from 2.2 to 0.52, 0.5, 0.14, and 0.07 (100 ml per g), respectively. The concentrations of LiBr, LiCl, KI, and KSCN required for half changes in the helical content and $\eta_{sp}/c$ of the maxima were 2.45, 3.3, 2.8, and 4 M, respectively. The sedimentation coefficients of the major peaks in 3.5 M LiBr were found to be 6.04 and 6.97 S, much larger than that of the control. The plot of the reciprocal of transition temperature against the concentration of LiBr gave a straight line, and a value of 4.68 entropy units was obtained as the standard entropy change from the unfolding of backbone chain when 1.5 kcal mole$^{-1}$ was adopted as the standard enthalpy change according to Schellman. Therefore, it was reasonably concluded that inorganic salt induces the transition of myosin A from a fibrous long molecule of helical structure to a compact globular molecule of random coil. On the other hand, the adenosine triphosphatase activity of myosin A decreased in a low range of salt concentration where the helical content and $\eta_{sp}/c$ were almost unaffected. In LiBr, LiCl, KI, and KSCN, adenosine triphosphatase activity of myosin A decreased in a low range of salt concentration where the helical content and $\eta_{sp}/c$ were almost unaffected.
triphasphatase activity decreased irreversibly, whereas it decreased reversibly in KCl.

REFERENCES
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