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A STUDY OF THE DYNAMIC PROPERTIES OF ACTOMYOSIN SYSTEMS BY QUASI-ELASTIC LIGHT SCATTERING

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SUMMARY

A laser light source and a digital autocorrelator were employed in the study of the molecular dynamics of acto-heavy meromyosin during the splitting of ATP. Low protein concentrations were used, so that molecular and not gel properties were evident. The addition of Mg^{2+} to acto-heavy meromyosin solutions in the presence of ATP caused a marked widening of the spectrum at high scattering angles. No such change was observed when chemically inactivated heavy meromyosin was used, when actin was cross-linked or when the proteins were in a high ionic strength solution. The data can be interpreted in terms of pronounced change in flexibility of acto-heavy meromyosin induced by active mechanochemical coupling.

INTRODUCTION

The interaction of actin and myosin which is responsible for many biological motile phenomena involves in all probability conformation changes of the protein species. Since quasi-elastic light scattering has proved to be a powerful tool in the study of conformational changes and intramolecular modes of motion of macromolecules [1] as well as in the analysis of the diffusional characteristics of various particles [2], this method appears to be suitable for the investigation of the mechanochemical processes in actomyosin systems at the molecular level. As we have recently demonstrated [3–7], the interaction of the molecularly dispersed myosin fragment heavy meromyosin or of its subfragment-1 (S-1) with actin in the presence of MgATP is capable of inducing contraction in myofibrils and muscle fibers in which the filamentous myosin has been inactivated, thus disclosing mechanochemical energy transformation. It was, therefore, interesting to follow changes in the dynamic properties of the water-soluble acto-heavy meromyosin, associated with ATP splitting, using the laser light scattering technique. This method has recently been applied to acto-heavy meromyosin by two groups of investigators [8–12] who followed changes in flexibility of F-actin, alone and in combination with heavy meromyosin or heavy

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meromyosin S-1, both in the presence and in the absence of ATP. Since relatively high protein concentrations have been employed by both groups, interparticle interactions and gel formation might have been the dominant effects observed. In order to avoid these effects, which may mask changes occurring in freely moving particles, we chose to use low protein concentrations.

Mg^{2+} is believed to be necessary for the mechanochemical interaction of actin, myosin and ATP. We found that the addition of Mg^{2+} to acto-heavy meromyosin in a solution containing ATP induced a marked widening of the spectrum at high scattering angles. No such widening was observed under conditions which did not permit mechanochemical coupling, i.e. when the corresponding actomyosin would not superprecipitate: when cross-linked actin [13] or heavy meromyosin which had been chemically modified so as to abolish its actin-activated ATPase activity [3, 14] were utilized or when a high ionic strength was employed. The effect induced by Mg^{2+} in the presence of ATP may thus be associated with mechanochemical coupling.

A preliminary account of this work was presented earlier [15].

EXPERIMENTAL PROCEDURE

Homodyne spectra of all samples were obtained using a laser-autocorrelator light beating apparatus described by Alon and Hochberg [16]. An on-line minicomputer (DCC – 116) was used to fit a single exponent of the form $Ae^{-\tau T}$ to the experimental points by the method of least squares. Since the observed spectrum was quite complex, depending on the amount of polydispersity of the actin and actoheavy meromyosin molecules and on the various intramolecular modes of vibration, no finer fitting was tried. However, any change in the gross features of the spectrum could be easily detected by the corresponding change in the single exponent fitted to the data. Thus our results and interpretation are more of a qualitative nature. The method of Koppel [17], which is commonly used in this type of experiments, has been shown [18] not to be an improvement on direct least squares fitting when the comulants are not known with high precision, and to result in such a case in substantial error propagation. Therefore it was not used in our data analysis. Since the power spectrum and autocorrelation function are a Fourier transform pair, we shall refer freely to an increase in τ as spectral widening and vice versa.

Different protein preparations and even different samples taken from the same preparation gave results differing from each other by as much as 25 %. This is a well known phenomenon (cf. ref. 12), probably due to variations in polydispersity of the different samples. Thus, we could not take the average of different samples to obtain the experimental error. The time available for experiments on a given sample was limited by denaturation of the proteins (all experiments were run at room temperature). Whenever it was possible to repeat experiments on the same sample (usually two experiments for each data point), errors were computed in the usual way. In all other cases, errors calculated by the theory of Jakeman et al. [19] were used.

Dust discrimination program was used to reject any data collected when dust particle crossed the laser beam. The system was checked with a monodisperse solution of polystyrene latex spheres for scattering angles between 30° and 130°.

Actin and heavy meromyosin were prepared from the back muscle of rabbits

according to Lehrer and Kerwar [20] and Lowey and Cohen [21], respectively. The ATPase [22] activity of heavy meromyosin (in 125 mM KCl, 5 mM ATP) was 0.056 μmol P_i/mg heavy meromyosin per min in the presence of 0.2 mM EDTA and decreased to 0.03 unit when 5 mM $MgCl_2$ was added. The activity of acto-heavy meromyosin (0.13 mg/ml actin, 0.7 mg/ml heavy meromyosin) under the same conditions was 0.038 unit in the presence of either Mg^{2+} or EDTA; at 0.6 M KCl no ATPase activity could be detected. The Mg^{2+}-ATPase of heavy meromyosin could be activated by actin by a factor of 7–10 (from 0.03 to 0.2–0.3 μmol P_i/mg heavy meromyosin per min) at 50 mM KCl and at a 10–20 : 1 weight ratio of actin to heavy meromyosin. The enzymatic activity of heavy meromyosin was practically abolished by treatment with *N*-ethylmaleimide (MalNEt) [23]. Heavy meromyosin was trinitrophenylated by trinitrobenzene sulfonate (N_3BzSO_3) as described earlier [3, 14]. The Mg^{2+}-activated ATPase activity of the N_3BzSO_3-heavy meromyosin was 0.074 μmol P_i/mg per min and was not further enhanced by actin. Actin was covalently cross-linked by glutaraldehyde according to Gadasi et al. [13]. The Mg^{2+}-activated ATPase activity of heavy meromyosin (0.7 mg/ml) in the presence of cross-linked actin (0.13 mg/ml) was 0.033 unit. Actin and heavy meromyosin were cleaned by centrifugation at 18 000 $\times$ *g* and, after mixing, at 3 000 $\times$ *g* immediately before use. The weight ratio actin: heavy meromyosin in acto-heavy meromyosin was always 1 : 5. Care was taken that the ATP level did not drop below 0.5 mM during the course of an experiment. All experiments were carried out at room temperature (approx. 20 °C).

RESULTS

Acto-heavy meromyosin at low ionic strength

The autocorrelation function of light scattered by acto-heavy meromyosin in the presence of ATP, or ATP plus Mg^{2+}, at nearly physiological ionic strength and pH was fitted by a single exponent (Fig. 1). The decay constant, τ, of this exponent was plotted in Fig. 2 against $\sin^2 \theta/2$, both in the presence and in the absence of Mg^{2+}. As can be seen, the addition of Mg^{2+} caused an increase in the decay constant, the value of which diminished with decreasing scattering angle. It should be noticed that the ATPase activity of acto-heavy meromyosin under these conditions was not affected by complexing Mg^{2+} with EDTA (see Experimental Procedure). This may not be surprising in view of the relatively high ionic strength employed which should give rise to EDTA activation of the ATPase activity of heavy meromyosin and to a low Mg^{2+}-activated ATPase activity (cf. ref. 24).

The observed effect of Mg^{2+} could be attributed to further dissociation of the acto-heavy meromyosin complex. The intensity of light scattered by acto-heavy meromyosin is much larger than that of either actin or heavy meromyosin, and therefore dissociation should lead to an increase in the relative intensity of light scattered by heavy meromyosin molecules as compared to the combined scattering of actin and acto-heavy meromyosin. Since the value of τ for heavy meromyosin ($\approx$ 7000 s^{-1} at 90°) is much larger than that of either actin or acto-heavy meromyosin (Table I), the cross-beating would result in an increase in the τ of the single exponent fit to the data, as observed experimentally. In order to check this possibility, the variation of τ as a function of the ratio of heavy meromyosin to actin concentration was checked: Table II presents the data for increasing actin concentration at constant

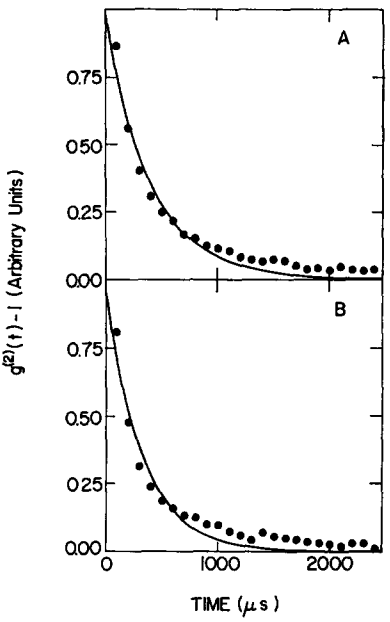

Fig. 1. Autocorrelation functions for acto-heavy meromyosin. (a) 0.1 mg/ml actin, 0.5 mg/ml heavy meromyosin, 100 mM KCl, 25 mM phosphate buffer pH 7.0, 0.1 mM EDTA, 5 mM ATP. (b) as in a plus 5 mM $MgCl_2$. Scattering angle 90°.

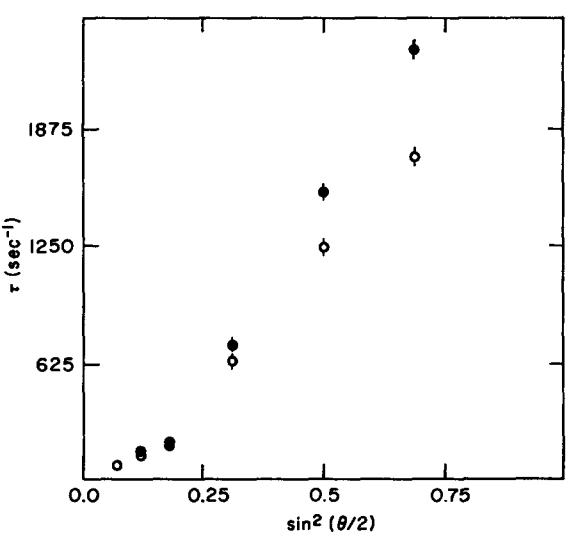

Fig. 2. The decay constant τ of the single exponent fit to the autocorrelation data plotted as a function of $\sin^2 \theta/2$. Open circles: 0.1 mg/ml actin, 0.5 mg/ml heavy meromyosin, 100 mM KCl, 25 mM phosphate buffer pH 7.0, 0.1 mM EDTA, 5 mM ATP. Filled circles: 5 mM $MgCl_2$ added to the above solution. The vertical bars represent S.E. of measurement.

TABLE I

THE DECAY CONSTANTS τ (s^{-1}) OF THE SINGLE EXPONENT FIT TO THE AUTO-CORRELATION FUNCTION

Conditions were the same as in Fig. 2. The theoretically computed error for τ was 10 % for all cases.

Scattering angle	Normal actin		Cross-linked actin	
	90°	118.8°	90°	118.8°
Actin	800	1080	1080	1460
Acto-heavy meromyosin	620	1030	1600	2100
Acto-heavy meromyosin+ATP	840	1680	1630	2300
Acto-heavy meromyosin+ATP+Mg^{2+}	1040	1980	1630	2100

TABLE II

THE VALUES OF τ AND OF THE INTENSITY FOR VARIOUS ACTIN CONCENTRATIONS

0.5 mg/ml heavy meromyosin, medium as in Fig. 2. The theoretically computed error for τ was 10 % for all cases.

Actin concentration (mg/ml)	τ (s^{-1})	Intensity (±1 %) (counts/s)
0.1	1210	795
0.15	1204	944
0.20	1145	1066
0.25	1104	1196

TABLE III

THE VALUES OF τ AND OF THE INTENSITY: A, WITHOUT Mg^{2+}; B, WITH Mg^{2+}

Conditions as in Fig. 2. The theoretically computed error for τ was 10 % for all cases.

Angle	A		B	
	τ(s^{-1})	Intensity (± 1%) (counts/s)	τ(s^{-1})	Intensity (± 1%) (counts/s)
60°	651	3913	732	3395
90°	1261	3156	1561	2452
120°	1746	3056	2331	2559

heavy meromyosin. The values for both τ and intensity are presented. A change of 50 % in intensity is followed by an 8 % change in τ. Since neither the dissociation constant of acto-heavy meromyosin in the presence of ATP (no Mg^{2+} added) nor the degree of polydispersity of heavy meromyosin in solution are known, an exact numerical calculation of τ is not possible. However, qualitatively it is clear that the observed τ is not dependent on the concentration of dissociated heavy meromyosin. The 8 % change in τ may be due to both a stronger intermolecular interaction arising from higher concentration (leading to increased viscosity and smaller diffusion coefficient) and to a smaller activity per actin decreasing the change in intramolecular modes of movement.

Fraser et al. [12] measured the spectral width for acto-heavy meromyosin at various heavy meromyosin actin ratios, under conditions of very low ATPase activity (at 0.5 °C). At large scattering angles, where the effect we found was most pronounced, they actually found a decrease in linewidth with increased heavy meromyosin: actin ratio.

Table III presents the values of τ and the scattered light intensity for three different scattering angles. It can be seen that the addition of Mg^{2+} to a solution of acto-heavy meromyosin in the presence of ATP results in a decrease in intensity which is angle independent, while the change in τ is 10 % at 60° and 35 % at 120°. If the change observed in τ upon addition of Mg^{2+} was caused by dissociation, that is by increased concentration of uncomplexed heavy meromyosin, the relative change in τ would be more likely the same as that in the intensity i.e. angle independent.

Thus, it seems very unlikely that the observed effect is related to dissociation of the complex by Mg^{2+}.

The spectrum of light scattered by a dilute solution of macromolecules can generally be fitted by a sum of exponents [1]:

$$\langle I(t)\, I(t+\tau_c)\rangle = \sum^{n} A_n(x, t) \exp(-DK^2+\lambda_n)\, \tau_c \tag{1}$$

$$x = K\langle l\rangle_e \tag{2}$$

where λ_n represents intramolecular modes of motion. Thus, the change in spectrum by Mg^{2+} could be due to the appearance of new intramolecular modes of motion of acto-heavy meromyosin and/or actin and heavy meromyosin. In order to find out which of the protein species has been affected, the following experiments were carried out:

(i) The effect of Mg^{2+}, as well as of the MgATP complex on the spectrum of heavy meromyosin was studied. The autocorrelation functions are shown in Fig. 2. It is evident that Mg^{2+} affect the autocorrelation function of heavy meromyosin (Fig. 3a) to such an extent that its shape is no longer exponential and the best fit gives a decay time which is much longer than that of heavy meromyosin alone (Fig. 3b). Upon adding ATP, the effect disappears (Fig. 3c). These observations can be ascribed to aggregation of heavy meromyosin molecules by Mg^{2+} which is reversed when ATP is added.

(ii) The effect of Mg^{2+}, ATP and MgATP on the spectrum of the light scattered from a dilute F-actin solution was investigated and shown to be negligible.

(III) It was ascertained that the addition of Mg^{2+} to acto-heavy meromyosin in the absence of ATP did not change the autocorrelation function of scattered light. Subsequent addition of ATP gave results similar to those obtained when the addition of ATP preceded that of Mg^{2+}.

Since MgATP seems to affect neither heavy meromyosin nor actin separately, and any effect Mg^{2+} may have on heavy meromyosin is abolished in the presence of ATP, it appears that the observed change can be ascribed only to the influence of MgATP on the dynamics of the acto-heavy meromyosin complex.

Actin complexed with chemically modified heavy meromyosin

In an attempt to see whether the effect of MgATP on acto-heavy meromyosin was associated with the actin-activated Mg^{2+} ATPase of heavy meromyosin, we

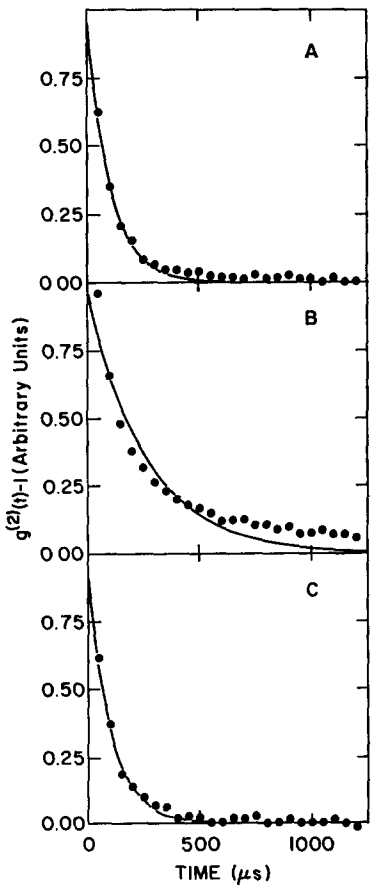

Fig. 3. Autocorrelation functions for heavy meromyosin (a) 2.0 mg/ml heavy meromyosin, 100 mM KCl, 25 mM phosphate buffer, pH 7.0, 0.1 mM EDTA. (b) as in a plus 5 mM $MgCl_2$ added. (c) as in b plus 5 mM MgATP added. Scattering angle 90°.

substituted heavy meromyosin by an equal concentration of MalNEt-poisoned heavy meromyosin (MalNEt heavy meromyosin). The addition of Mg^{2+} to acto-MalNEt heavy meromyosin in the presence of ATP did not cause any change in spectrum at 90°.

Of special interest were experiments in which N_3BzSO_3-heavy meromyosin was used instead of native heavy meromyosin. Mg^{2+} activates the ATPase of this modified heavy meromyosin to such an extent that the addition of actin does not cause any further increase in activity [25]. The spectrum of the light scattered by acto-N_3BzSO_3-heavy meromyosin in the presence of ATP and Mg^{2+} again did not differ from that observed in the absence of the divalent cation.

Acto-heavy meromyosin at high ionic strength

Fig. 4 shows the angular dependence of the decay constant for light scattered by acto-heavy meromyosin at high ionic strength where the actin-activated ATPase

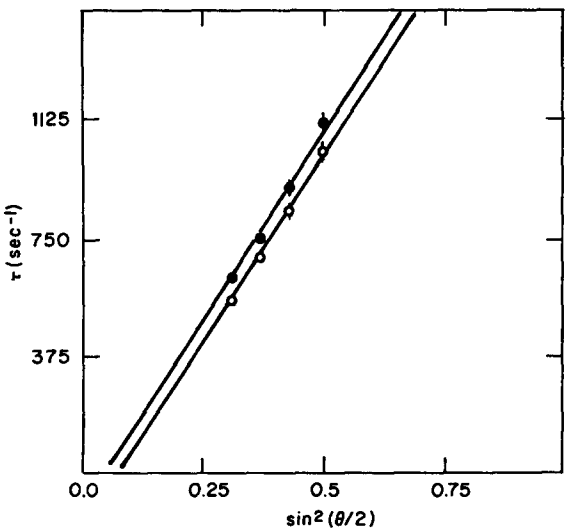

Fig. 4. The decay constant τ of the single exponent fit to the autocorrelation data plotted as a function of $\sin^2 \theta/2$. Open circles: 0.1 mg/ml actin, 0.5 mg/ml heavy meromyosin, 0.6 M KCl, 25 mM phosphate buffer pH 7.0, 0.1 mM EDTA, 5 mM ATP. Filled circles: 5 mM $MgCl_2$ added to the above solution. The vertical bars represent S.E. of measurement.

activity of heavy meromyosin is greatly reduced (see Experimental Procedure). Contrary to the case of low ionic strength (Fig. 1), the increase caused by the addition of Mg^{2+} to an ATP-containing solution of acto- heavy meromyosin was independent of the scattering angle. The straight line fits computed for both sets of experimental points (i.e. with and without Mg^{2+}) have equal slopes while the value of the intercept with the ordinate increases when Mg^{2+} is added. Both intercepts are negative, possibly reflecting on the polydispersity of the scattering elements (cf. ref. 26). The parallel shift of the curve induced by Mg^{2+} may reflect a shift in the equilibrium state of the reaction:

Actin + heavy meromyosin $\rightleftarrows$ acto-heavy meromyosin (cf. ref. 27).

Heavy meromyosin complexed with cross-linked actin

In order to test the possibility that the observed widening of the spectrum was due to partial breakdown of the actin filaments we used covalently cross-linked F-actin. Cross-linking of F-actin was found not to impair activation of the ATPase activity of myosin, but to abolish superprecipitation [13].

Table I lists the decay constants of the single exponent fits to the autocorrelation data. Since the decay constant is proportional to the diffusion coefficient it appears from the table that the chain length of cross-linked actin was smaller than that of ordinary actin, in agreement with electron microscopic observations [13]. The decay constant of the heavy meromyosin-cross-linked actin complex differed markedly from that of the cross-linked actin, in contrast to the case of ordinary acto-heavy meromyosin in comparison with normal actin.

The addition of ATP and Mg^{2+} to the heavy meromyosin-cross-linked actin complex caused little change in the decay constant (Table I).

DISCUSSION

The homodyne spectra of acto-heavy meromyosin in the presence of ATP was found to be strongly affected by Mg^{2+} at large scattering angles. As shown above, it is unlikely that the effect is due to increased dissociation. No effect could be detected under conditions at which no ATP splitting by acto-heavy meromyosin could take place e.g. when the heavy meromyosin component was poisoned by MalNEt or at high ionic strength when the actin activation of the Mg^{2+}-ATPase activity is greatly diminished or when N_3BzSO_3-heavy meromyosin, which does not appear to be activated by actin, was utilized. It thus appears that the Mg^{2+}-induced change in spectra is associated with actin activation of the Mg^{2+} ATPase activity of heavy meromyosin. However, cross-linked actin which is capable of activating heavy meromyosin does not give the effect. This is perhaps related to the fact that this actin does not exhibit superprecipitation in combination with myosin. If we consider superprecipitation to be a manifestation of mechanochemical coupling then we may conclude that actin activation, even though necessary, is not sufficient for the exhibition of the effect of Mg^{2+} on the spectra and that the effect observed reflects the existence of mechanochemical coupling.

Since the initial slope in the plot of the decay constant vs. $\sin^2 \theta/2$ is a measure of the translational diffusion coefficient of the scattering elements and as this slope is hardly affected by the addition of Mg^{2+} (Fig. 2) it is unlikely that Mg^{2+} influences the diffusional characteristics of the proteins. The effect on light scattering might therefore be linked with changes in the flexibility of the actin filaments. This is supported by the results for heavy meromyosin complexed with cross-linked actin which has been shown to be highly rigid [13]. Recalling that such actin is incapable of giving rise to superprecipitation and in view of the fact that heavy meromyosin appears to be potent mechanochemically [3–7, 14], such changes in flexibility of the actin filaments might be essential for mechanochemical transduction and movement in actomyosin systems.

The measurement of ATPase activities is indispensible in any work on actomyosin systems. Since the exhibition of ATPase activity is by itself not sufficient for mechanochemical performance which is the most important feature of these systems, it is desirable to have at one's disposal a simple and rapid method for the detection of mechanochemical coupling in acto-heavy meromyosin systems, in analogy with the use of superprecipitation as an indicator for coupling in the case of actomyosin. Laser light scattering seems to be suitable for this purpose.

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