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Inactivation, Subunit Dissociation, Aggregation, and Unfolding of Myosin Subfragment 1 during Guanidine Denaturation†

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ABSTRACT: The effect of guanidine hydrochloride on ATPase activity, gel filtration, turbidity, exposure of thiol groups, far-UV circular dichroism, and the fluorescence emission intensity of myosin subfragment 1 (S-1) was studied under equilibrium conditions. It was found that the denaturation process involves several intermediate states. The enzymatic activity of S-1 is at first lost at very low concentrations of GdnHCl (lower than 0.5 M). At a slightly higher GdnHCl concentration (about 0.5 M), the light chains dissociate and this dissociation is closely followed by the formation of aggregates between the naked heavy chains of S-1 molecules in the guanidine hydrochloride range of concentrations 0.5–1 M. At GdnHCl concentrations above 1 M, aggregates gradually disappear and S-1 loses its secondary and tertiary structures. These phenomena are partly reversible, and ATPase activity is only partially recovered under highly limited conditions. These results are discussed in relation to the nature of myosin subunit assembly. The head fragment of 20 kDa is thus suggested to be implicated in the binding of light chain to heavy chain and in the self-association of free heavy chains.

Muscle contraction originates from the sliding of myosin filaments on actin filaments, the energy for which is supplied by the hydrolysis of ATP by myosin. Conformational changes in the myosin head have been suggested to occur during this hydrolysis and to be mechanically transmitted to the distant actin-binding site (Botts et al., 1989; Mornet et al., 1989), allowing the conversion of chemical energy into the mechanical energy of contraction.

Experiments of protein denaturation–renaturation may help us to understand how a multidomain oligomeric protein, such as the myosin head, folds and how its subunits and domains acquire stability [for a review, see Jaenicke (1987)]. The portion of myosin studied in this work is the myosin head or myosin subfragment 1 (S-1)[1] obtained after chymotryptic proteolysis. It consists of one essential light chain of either 21 or 16.5 kDa and one heavy chain (approximately 95 kDa). The latter is usually considered to comprise three domains, i.e., the consecutive 25-, 50-, and 20-kDa fragments. The effect of guanidine hydrochloride on ATPase activity, gel filtration, turbidity, exposure of thiol groups, far-UV circular dichroism, and the fluorescence emission intensity of myosin subfragment 1 was studied, and the transition curves of these different signals were established as a function of the concentration of the denaturant for the processes of denaturation and renaturation.

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[1] Abbreviations: S-1, myosin subfragment 1; GdnHCl, guanidine hydrochloride; HC, heavy chain; LC, light chain; A1, higher molecular mass alkali (or essential) light chain; A2, lower molecular mass alkali (or essential) light chain; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); TNB, 5-thio-2-nitrobenzoic acid.

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Materials and Methods

Myosin and the myosin subfragment 1 (S-1) from skeletal muscles of adult or newborn rabbits were prepared and characterized as previously described (Bêchet et al., 1979, 1982; Houadjeto, 1989). Guanidine hydrochloride (GdnHCl) was a Pierce product (Sequanal grade); the concentrations of GdnHCl solutions were checked by refractive index measurements (Nozaki, 1972).

For denaturation experiments involving physicochemical measurements, S-1 was incubated for 24 h at 4 °C in an appropriate buffer usually containing 100 mM phosphate (pH 7.5), 1 mM EDTA, 1 mM dithiothreitol, and guanidine hydrochloride at different concentrations. As the denaturant markedly lowers the second ionization pK of phosphate ion (potentiometric titrations reveal a pK decrease of about 0.85 unit at 1 M GdnHCl), it was diluted in 100 mM disodium phosphate before it was added to the study medium. The pH of the study medium was therefore in the range of 6.5–7.5 according to the GdnHCl concentration and, for some experiments, was neutralized to 7.5 before the addition of enzyme. It was verified that variations in pH between 6.5 and 7.5 had no effect on the results. The incubation was followed by fluorescence, gel filtration, or other measurements as indicated below; these assays were carried out with the undiluted sample at 20 °C in the minimal required time.

For renaturation experiments, S-1 (at a sufficiently high concentration, usually 10–20 μM) was denatured in the same buffer as above, in the presence of 4–5 M guanidine hydrochloride. After incubation for 24 h at 4 °C, guanidine hydrochloride was diluted to the desired final concentration and the medium was left again for 24 h at 4 °C before physicochemical studies. Sometimes, dialysis for 24 h at 4 °C was preferred.

Fluorescence measurements were made using a Jobin-Yvon spectrofluorometer, Model JY3 D. The excitation wavelength was 295 nm, and emission spectra were recorded between 310 and 410 nm. Circular dichroism spectra were recorded between 200 and 250 nm using a Jobin-Yvon Mark V dichrograph connected to an Apple 2e microcomputer. In both methods, the S-1 concentration was usually 0.5–1 μM, and in a few experiments giving comparable results, it was 5 μM.

Turbidity was measured with a Cary 118 spectrophotometer by monitoring optical density readings between 600 and 320 nm or, especially for the low protein concentrations, with a Jobin-Yvon spectrofluorometer whose entrance and exit monochromators were both set to 450 or 500 nm. The enzyme concentration was varied between 2 and 30 μM in the same buffer as above.

For gel filtration experiments, 2 mL of S-1 (at 20–50 μM) in the phosphate buffer containing various concentrations of GdnHCl was filtered on a column of Sephacryl S-200 HR (2.5 × 14.5 cm; Pharmacia) previously equilibrated against the same solvent; 2.3-mL fractions were collected with a flow rate of 25 mL/h, and the elution profile was monitored by recording the absorbance of fractions at 280 nm. The denaturant was removed by dialysis and aliquots of absorbing fractions were added to an equal volume of boiling solution containing 0.125 M Tris-HCl (pH 6.8), 5% SDS, 1 mM EDTA, 5 mM dithiothreitol, and 20% glycerol. After 1 min at 100 °C, samples were subjected to polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS), as previously reported (d'Albis et al., 1979). Gels were stained with Coomassie blue R-250 and scanned with a densitometer Vernon PH16 equipped with an integrator. The relative percentage of heavy and light chains was estimated from the staining intensities of bands on gels; for each analyzed fraction, several loads with different amounts were run on gels.

HPLC gel filtration of S-1 was also carried out by using a Beckman setting composed of a 110 B solvent delivery module, a 210 sample injection valve with a 20-μL sample loop, and a 160 absorbance detector. The column employed, a Spherogel-TSK G 3000 SW column (0.75 × 30 cm), was equilibrated with the usual phosphate buffer containing 0.2 M NaCl and guanidine hydrochloride at different concentrations. It was calibrated with a Bio-Rad gel filtration molecular weight standard (number 151-1901). The rate of flow through the column was maintained at 0.5 mL/min, and the absorbance at 280 nm of the effluent was recorded.

The reactivity of thiols in S-1 was measured by monitoring at 412 nm the time course of the reaction of the protein (10 μM) with DTNB (90-fold excess over S-1) in the usual phosphate buffer without dithiothreitol, pH 7.3, at 22 °C. The reaction was followed for about 20 min in a Cary 118 spectrophotometer (at longer times, some of the protein occasionally precipitated), and the molar absorption coefficient at 412 nm was taken as 13 600 M^{-1} cm^{-1} (Ellman, 1959).

For inactivation experiments, S-1 (usually 0.5–1 μM) was incubated for 24 h at 4 °C in an appropriate buffer containing Tris (vide infra) instead of phosphate and guanidine hydrochloride at different concentrations. A small negligible volume of ATP was afterward added to the medium for testing the enzymatic activity. For reactivation experiments, S-1 (at a sufficiently high concentration, usually 10–20 μM) was at first denatured in a Tris buffer containing guanidine hydrochloride at a concentration varying between 0.05 and 1 M or, for a few experiments, at a concentration of 3 or 5 M. After incubation for 24 h at 4 °C, guanidine hydrochloride was diluted to a nearly zero final concentration, and the medium was left again for 24 h at 4 °C before activity studies. Sometimes, dialysis for 24 h at 4 °C was preferred.

The Mg^{2+}-dependent ATPase activity of S-1 (at the concentration of 0.5–1 μM) was determined by two methods which gave equivalent results. In the first method, the fluorescence of S-1 in the presence of ATP was recorded as a function of time (Bêchet et al, 1979), and the catalytic constant of the hydrolysis reaction was calculated from the kinetic curve by using Chance's equation as described by Morita (1967). The buffer used for incubation and activity measurements contained 50 mM Tris-HCl, 50 mM KCl, 1 mM dithiothreitol, 2 mM $MgCl_2$, and guanidine hydrochloride at the appropriate concentration, pH 7.5, at 20 °C; 2.5–5 μM ATP was added at time zero for activity tests. In the second method, aliquots of S-1 solutions containing the same constituents and 25 μM Mg^{2+}ATP (added at time zero) were diluted 100-fold at various times in 200 mM Hepes–NaOH, pH 7.75, at 20 °C. The ATP concentration was then determined by bioluminescent measurements using the luciferase–luciferin system (DeLuca, 1976). The apparatus employed was a Lumac biocounter, M 2010 A, and the reaction was performed with an ATP assay kit (Boehringer).

The amount of Mg^{2+}ATP bound to S-1 was also determined by the addition of pyruvate kinase (0.1 mg/mL) and phosphoenolpyruvate (1 mM) to the medium before bioluminescence was measured with the luciferase–luciferin system (Furukawa et al., 1980). The buffer was as above for Mg^{2+}-ATPase activity studies; S-1 was at the concentration of 12 μM, and ATP was in slight excess.

The K^+-ATPase activity of S-1 (at the concentration of 0.5 μM) was determined by pH-stat measurements with a Radiometer titralab. The buffer used for incubation or kinetic

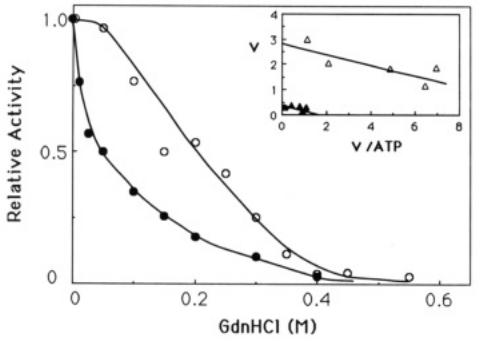


FIGURE 1: Relative changes of the enzymatic activity of myosin subfragment 1 as a function of guanidine hydrochloride concentration. The enzyme (0.5 μM) was incubated for 24 h in the presence of GdnHCl at different concentrations, and its activity was tested in the presence of denaturant. K^+-ATPase activity (●) and Mg^{2+}-ATPase activity (○) are indicated. The inset shows the Eadie plot of the Mg^{2+}-ATPase activity of S-1 in the absence (△) or in the presence (▲) of 0.4 M GdnHCl; the rate V is in arbitrary units. Experimental conditions are as given in Materials and Methods.

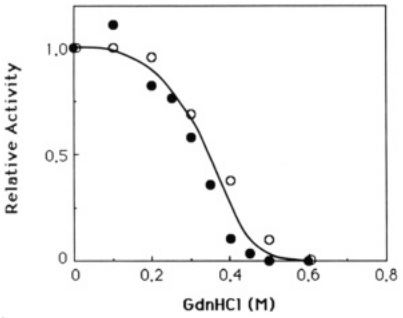


FIGURE 2: Residual enzymatic activity of myosin subfragment 1 after incubation for 24 h in solutions of guanidine hydrochloride at different concentrations as indicated, dilution to nearly zero concentration of GdnHCl, and assay 24 h later in the absence of denaturant. K^+-ATPase activity (●) and Mg^{2+}-ATPase activity (○) are indicated. Experimental conditions are as given in Materials and Methods. The final concentration of S1 was 1.5 μM.

measurements contained 0.5 M KCl, 2.5 mM EDTA, 0.2 mM DTT, 0.01 M Tris-HCl (pH 7.8 at 21 °C), and guanidine hydrochloride at the appropriate concentration. ATP at the final concentration of 1.1 mM was added at time zero for activity measurements.

The transition curves reported in this study were fitted at best to simple- or double-sigmoidal curves with commercial nonlinear regression programs.

RESULTS

The changes in K^+-dependent and Mg^{2+}-dependent ATPase activities of S-1 with guanidine hydrochloride are shown in Figure 1. Transition curves were obtained in a narrow guanidine hydrochloride concentration range (0–0.5 M) with concentrations of about 0.05 and 0.2 M for the half-transitions in the losses of K^+- and Mg^{2+}-dependent ATPase activities, respectively. For the latter activity, the main effect of the denaturant was on the catalytic constant, V_m (Figure 1; inset). At [GdnHCl] ≥ 0.5 M, the enzyme no longer bound Mg^{2+}-ATP, as estimated by the method of Furukawa et al. (1980). It was also found that the shape of the inactivation curve for the Mg^{2+}-ATPase activity changed only to a small extent when the incubation time in guanidine hydrochloride was reduced to zero (that for the K^+-ATPase activity remained the same; data not shown).

After the removal of the denaturant, only a partial reactivation was observed, depending on the concentration of GdnHCL in the incubation mixture. It this concentration was 0.35 M, 50% of the K^+- or Mg^{2+}-ATPase activities was recovered; if it was ≥ 0.5 M, the activity was irreversibly lost (Figure 2). A few attempts to reactivate S-1 under conditions recommended by Jaenicke and Rudolph (1989) for oligomeric proteins proved unsuccessful. The conditions employed included use of denaturant at a low (1 M) or high (3–5 M) concentration in the inactivation medium and removal by quick dilution, low protein concentration (0.15–1 μM) in the reactivation medium, the addition of bovine serum albumin or isolated light chains in excess in the reactivation medium, and incubation of the reactivation mixture for varying periods of time. Reactivation was possible after quick dilution only if the incubation time in the inactivation medium containing GdnHCl at a concentration between 0.5 and 1 M was reduced to a few minutes (in these conditions, the inactivation in the presence of denaturant was practically complete) instead of the usual 24 h of the assay.

The chymotryptic S-1 used in this study consists of one essential light chain of either 21 (light chain A1) or 16.5 kDa (light chain A2) and one heavy chain (approximately 95 kDa). The heavy and light chains of myosin are dissociated by 2 M guanidine hydrochloride (Dreizen & Richards, 1972). Gel filtrations of S-1 in solutions of guanidine hydrochloride at concentrations between 0 and 1 M show that the protein elutes as a main peak and that the elution volume of this peak is lower in the presence of high GdnHCl concentration than in the absence of denaturant (Figure 3A). SDS–PAGE patterns of the main-peak fractions indicate that light chains dissociate from S-1 (Figure 3B). The dissociation of A2 occurs earlier than that of A1, with a half-transition concentration of about 0.3 M as opposed to 0.4 M (Figure 3C).

The decrease of the elution volume of the protein in GdnHCl, as shown in Figure 3A, led us to suspect that aggregation could occur in the concentration range of 0–1 M. Effectively, turbidity measurements indicated that the intensity of light diffracted by S-1 solutions passed through a maximum at about 1 M GdnHCl in the guanidine hydrochloride range

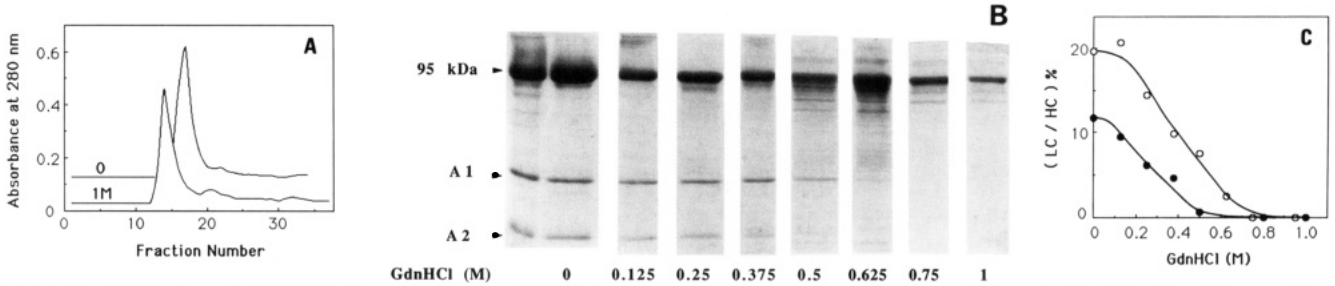


FIGURE 3: (A) Sephacryl S-200 HR chromatography of S-1 in the absence or the presence of 1 M guanidine as indicated. The elution profiles are shifted along the y-axis for the clarity of the figure. (B) SDS–PAGE patterns of the peak fractions obtained after elution of S-1 in the presence of guanidine hydrochloride at the indicated concentrations. The sample in the first lane is an S-1 control before chromatography. (C) Weight percentage of the ratio A1/HC (○) or A2/HC (●) as a function of guanidine hydrochloride concentration. Percentage was estimated as described in Materials and Methods.

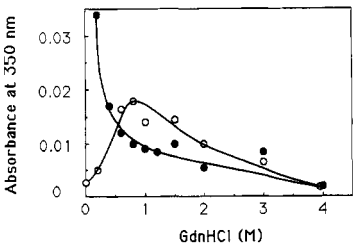


FIGURE 4: Change in absorbance of S-1 at 350 nm as a function of GdnHCl concentration: O, denaturation process; ●, renaturation process. The protein concentration was 9 μM.

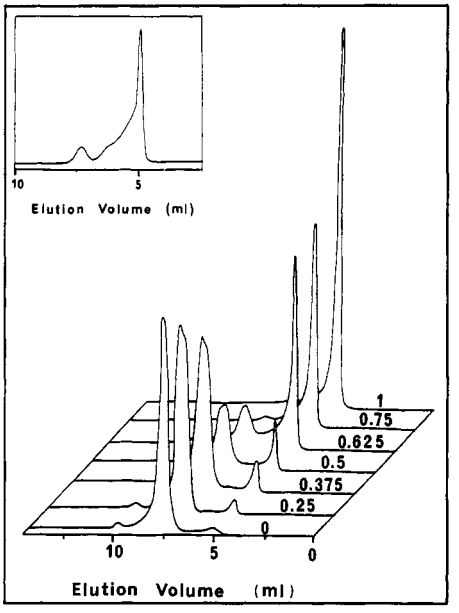


FIGURE 5: Elution profiles of S-1 incubated at the indicated molar concentrations of guanidine hydrochloride, as determined by HPLC gel filtration on a TSK G 3000 SW solumn. A total of 20 μL of S-1 at the concentration of 45 μM was injected into the column. In the inset, S-1 in 1 M GdnHCl was at the concentration of 2.5 μM.

0.25–4 M (Figure 4). This phenomenon was observed at a high (30 μM) or low (2 μM) protein concentration but more markedly at the highest concentrations (data not shown). HPLC experiments by using a TSK G 3000 SW column showed that S-1 incubated in 1 M GdnHCl eluted as a single peak in the void volume of the column with an apparent molecular mass equal to at least 300 kDa (similar results obtained with a Bio-Sil TSK-400 column suggest a value as high as 1000 kDa), while the native enzyme eluted as a single peak with an apparent molecular mass of about 120 kDa. At the half-transition ([GdnHCl] $\approx$ 0.65 M), two separate peaks were observed, corresponding to the native and aggregated enzyme species (Figure 5). Comparable chromatograms were obtained for enzyme concentrations between 17.5 and 45 μM or even for a concentration of 2.5 μM; however, at this lower enzyme concentration, some species having a molecular mass lower than 300 kDa were still present in 1 M GdnHCl (Figure 5, inset).

The chemical reactivity of the thiol groups within S-1 was determined by DTNB titration at different guanidine hydrochloride concentrations. The time courses of TNB release were found to be biphasic (Figure 6A); some of the thiol groups reacted quickly while others reacted slowly. The number of thiol groups that reacted rapidly increased greatly at guanidine hydrochloride concentrations between 0.25 and 0.75 M, inclusive (Figure 6B).

The circular dichroism spectra of S-1 in the native or denatured state are shown between 200 and 250 nm in Figure

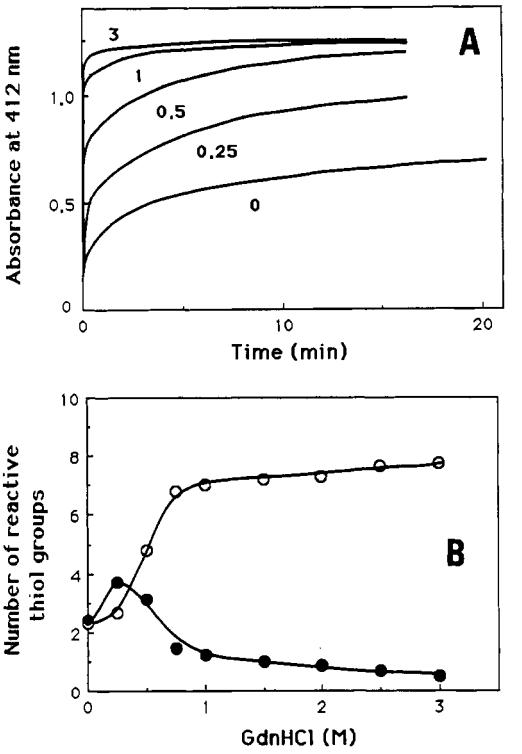


FIGURE 6: (A) Time course of the reaction of myosin subfragment 1 with DTNB at different guanidine hydrochloride concentrations. The numbers under the curves refer to the molar guanidine hydrochloride concentrations used for incubation and titration experiments. Experimental conditions are as in Materials and Methods. (B) Number of rapidly (O) and slowly (●) reacting thiol groups as a function of the guanidine hydrochloride concentration. The experimental curves in (A) were fitted to the five-parameter equation $A_t = A_\infty - A_1e^{-k_1t} - A_2e^{-k_2t}$ by means of a nonlinear regression program (Duggleby, 1984); the parameters A_1 and A_2 are proportional to the number of fast and slow thiol groups, respectively.

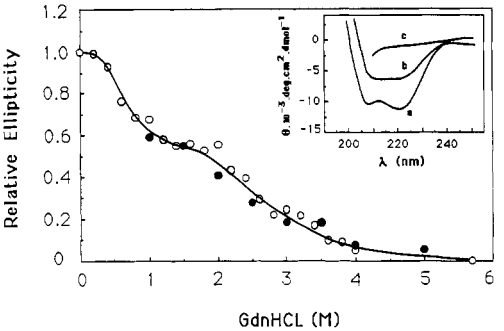


FIGURE 7: Relative changes of the ellipticity of myosin subfragment 1 at 222 nm as a function of guanidine hydrochloride concentration. The relative ellipticity is equal to $(\theta - \theta_{min})/(\theta_{max} - \theta_{min})$, with θ as the ellipticity of S-1 for any guanidine hydrochloride concentration. θ_{max} and θ_{min} are the ellipticities of the native and denatured forms of S-1, respectively: O, denaturation process; ●, renaturation process. The inset shows the circular dichroism spectra of S-1 (1 μM) in the absence (a) or presence of 1.5 M (b) or 4 M (c) guanidine hydrochloride. Experimental conditions are as given in Materials and Methods.

7 (inset, curves a and c). The spectrum of the native form had a double minimum at 222 and 208 nm, while the spectrum of the denatured form was continuous in the studied wavelength range. On the same figure (inset, curve b) is also shown the spectrum of S-1, at an intermediate GdnHCl concentration (1.5 M); at this concentration, the spectrum presented some features of the spectrum of an aggregated protein with a high value of the ellipticity at 250 nm [see Urry (1972)]. The decrease in ellipticity of S-1 at 222 nm as a function of guanidine hydrochloride concentration is reported (Figure 7).

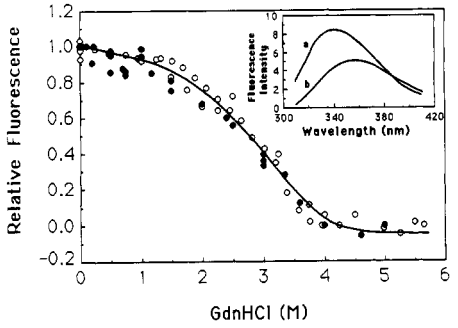


FIGURE 8: Relative changes in the fluorescence emission intensity of myosin subfragment 1 at 336 nm, as a function of guanidine hydrochloride concentration. The relative fluorescence is equal to $(F - F_{min})/(F_{max} - F_{min})$ with F as the fluorescence intensity of S-1 for any guanidine concentration. F_{max} and F_{min} are the fluorescence intensities of the native and denatured forms of S-1, respectively: ○, denaturation process; ●, renaturation process. The inset shows the fluorescence emission spectra of S-1 (1 μM) in the absence (a) or presence (b) of 5 M guanidine hydrochloride. Experimental conditions are as given in Materials and Methods.

The transition curve exhibited two parts, with an intermediate narrow plateau. The midpoints for each part of the curve were 0.65 and 2.8 M, respectively. For the process of renaturation, the transition curve was close to that of denaturation in the range of high guanidine hydrochloride concentrations up to 1 M, but in the low range (below 1 M), the experimental values were not reproducible, probably because the sample aggregated. After the enzyme was incubated in 4 M GdnHCl and the excess denaturant was removed by dilution or dialysis, solutions of S-1 were indeed turbid in the guanidine hydrochloride concentration range 0–1 M (Figure 4).

The emission spectra of S-1 in the native or denatured state are shown in Figure 8 (inset). A red shift of the emission maximum of the intrinsic protein fluorescence spectrum, as well as a decrease in its intensity, was observed in denatured S-1, due to exposure of buried tryptophan residues. The changes in the fluorescence intensity of S-1 at 336 nm as a function of guanidine hydrochloride concentration are reported in Figure 8 (the plot of the change in the maximum emission wavelength as a function of [GdnHCl] has a comparable shape). The transition curve for the process of denaturation was close to that of renaturation; its midpoint was 2.8 M.

Discussion

The progressive addition of guanidine hydrochloride to myosin subfragment 1 leads first to the disappearance of the enzymatic activity, second to the dissociation of light chains and the aggregation of heavy chains, and third to a conformational change of the protein. This succession of events is shown in Figure 9, which gathers the main results of this study.

The enzymatic activity of S-1 is lost at very low concentrations of GdnHCl (lower than 0.5 M). This phenomenon is not due to specific binding of the positively charged guanidinium ion upon the enzyme, since the inactivation curves (particularly for the Mg^{2+}-ATPase activity) are not hyperbolic. In fact, a similar loss of activity has been observed for many enzymes. Tsou (1986) has suggested that the active sites of some enzymes are located in limited and flexible molecular regions. This might be true for the myosin ATP-binding site, which has been postulated to be situated in an interdomain pocket between two opposing parallel α/β domains (Yanagisawa et al., 1987).

At a slightly higher GdnHCl concentration (about 0.5 M), the light chains dissociate as shown by gel filtration. That the light chain A2 dissociates before the light chain A1 is not

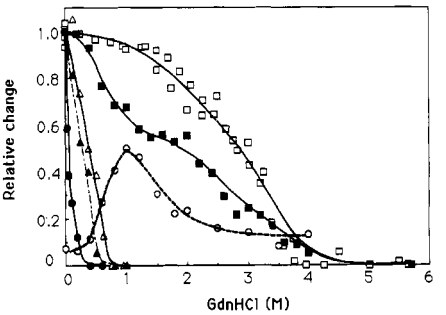
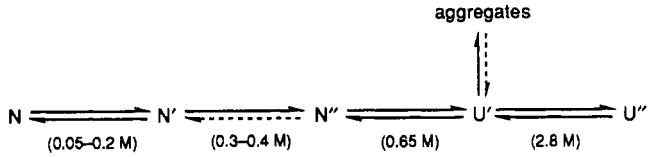


FIGURE 9: Effect of GdnHCl on the K^+-ATPase activity (●), the dissociation of the light chains A1 (△) and A2 (▲), the circular dichroism (■), the turbidity (○), and the fluorescence (□) of myosin subfragment 1. Data for this figure were taken from Figures 1, 3C, 4, 7, and 8. The maximal value of the turbidity is taken as 0.5 rather than 1, for the clarity of the figure.

surprising; Burke and Sivaramakrishnan (1981) have previously suggested, on the basis of light chain exchange experiments, that the interactions of heavy chain with A1 are significantly more stable than those of heavy chain with A2. This dissociation is closely followed by the formation of aggregates between the naked heavy chains of S-1 molecules. This aggregation, which likely results from interdomain interactions between partially unfolded intermediates (Teschner & Garel, 1989), frequently occurs during the denaturation of oligomeric proteins (Jaenicke et al., 1981; Herold & Kirschner, 1990; Liang et al., 1990). Moreover, it is not unexpected for myosin S-1 because of the known presence of sticky patches in the nude heavy chain (Bagshaw, 1980) and the tendency of its isolated peptide fragments to aggregate (Muhlrad et al., 1986; Muhlrad, 1989). Thus, previously hidden hydrophobic surfaces may become exposed, favoring the formation of highly aggregated species. In the range of guanidine hydrochloride concentrations between 0.5 and 1 M, some buried thiols become exposed and the secondary structure of the protein changes.

At GdnHCl concentrations above 1 M, aggregates gradually disappear and S-1 loses its secondary and tertiary structures, as shown by the progressive changes of ellipticity and fluorescence intensity of the protein.

Thus, the transition curves obtained by the different methods used do not occur at the same guanidine hydrochloride concentrations. This result implies that guanidine hydrochloride denaturation of the myosin subfragment 1 involves the appearance of several molecular forms in succession. A plausible scheme of the denaturation–renaturation process is as follows:



in which N and N′ are "folded forms" of the enzyme which are fully active and reversibly inactivated, respectively; N″ is the dissociated form of S-1 with separated heavy and light chains; and U′ and U″ are the consecutive unfolded forms observed by circular dichroism or fluorescence, with the species U′ tending to aggregate. During the refolding process, the irreversible formation of aggregates and the unlikelihood that light chains reassociate with the protein since alternative complexes are forming or have already formed lead to the irreversible inactivation of the enzyme. A close relationship between subunit dissociation and irreversible loss of ATPase activity has also been found in a series of experiments on myosin in concentrated salt solutions at pH 7 and 4 °C

(Dreizen & Richards, 1972, and references therein). In the above scheme, the numbers in parentheses are the guanidine concentrations at which there is an equilibrium or a quasiirreversible transition between two successive states. Whether this scheme actually corresponds to the denaturation of myosin subfragment 1 is being investigated by kinetic studies now in progress.

The chymotryptic S-1 used in this study consists of one essential light chain of either 21 or 16.5 kDa and one heavy chain (approximately 95 kDa) which is usually considered to be formed by three domains, i.e., the consecutive 25-, 50-, and 20-kDa fragments. The 20-kDa fragment is known to bind the light chains in the native protein (Vibert & Cohen, 1988) and is therefore at the interface between the light and heavy chains. The subunit dissociation occurs at a low guanidine hydrochloride concentration, and no marked structural change of these two subunits seems to occur at the point of dissociation, indicating that the interaction between subunits may be mainly of electrostatic origin and not very strong, the interface having a relatively limited size. A limited portion of the 20-kDa fragment has also been recently implicated in the association of cardiac myosin subunits (McNally et al., 1991), and a similar segment (sequence 785–802) might be implicated in the association between skeletal myosin subunits, as deduced from theoretical studies (Bêchet & Houadjeto, 1989).

However, as a consequence of the subunit dissociation, the isolated heavy chains aggregate. This phenomenon is not unexpected, as heavy chain preparations are unstable and the role of light chains may be to stabilize the myosin conformation (Wagner & Giniger, 1981). Protein fluorescence does not change during the aggregation of the isolated heavy chains. Therefore, the main sites of interaction between the heavy chains may involve regions of the protein that carry no tryptophan. These regions might be the 20-kDa fragment, which is at the interface between the light and heavy chains in the native protein (Vibert & Cohen, 1988), or the N-terminal region of the 50-kDa domain, which has been recently described as the most thermolabile part of S-1 (Levitsky et al., 1990).

The thermal denaturation of myosin subfragment 1 has been intensively studied in recent years (Hamai & Konno, 1989, and references therein). As with the denaturation by guanidine hydrochloride, light chains dissociate and naked heavy chains aggregate with heating. All three of the S-1 domains are equally susceptible to tryptic attack at 37 °C, although after cooling down to 25 °C the 50-kDa segment of the thermally denatured S-1 does not refold and remains more susceptible to tryptic attack (Burke et al., 1987). As with guanidine hydrochloride denaturation, the enzymatic activity is irreversibly lost above 35–40 °C.

Finally, the reality of these phenomena for the family of various myosin isoenzymes may be questioned. As verified in a few parallel denaturation–renaturation experiments including activity, circular dichroism, and fluorescence measurements, similar results were observed for S-1 species from skeletal muscles of adult or newborn rabbits. Even though these two isoforms have different primary and secondary structures and catalytic activities (Houadjeto et al., 1990), their unfolding and refolding follow the same pathways, which indicates that their domains are similarly organized and have similar stability.

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