A search for protein structural changes accompanying the contractile interaction

(circular dichroism/myosin S-1/tryptophan fluorescence/energy transduction/secondary structure)

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ABSTRACT It appears that small movements (detected hitherto only by fluorescence resonance energy transfer measurements and crosslinking studies) in a region of the myosin S-1 particle may mediate chemomechanical energy transduction in the contractile system. Here we find under conditions of high precision at 10°C and 20°C that ATP binding to S-1 causes small (0.4%) changes in CD signal, $\Delta\varepsilon_{222}$, as do temperature changes in the regime below 16°C. ATP binding perturbs tryptophan residues that we now think are in the mobile region, and we find here that temperature affects tryptophan fluorescence in much the same way that it affects the CD signal, so we believe that the CD signal reports transduction-related movements in S-1. If S-1 is exposed to the range 16–30°C, CD signal falls with temperature; ATP counteracts this fall. Analysis of vacuum-UV CD spectra yields 42% α-helix, 9% antiparallel β-sheet, 7% parallel β-sheet, 14% β-turns, and 29% other structures.

The contractile interaction in a myosin–actin–ATP system is probably a change in spatial relations between the two complexed proteins, without a major change in gross shape of either (1–3), but more subtle protein structural changes must underlie this interaction. Recently evidence has appeared for change in spatial relations (4) and for shape constancy (5) and also for internal movements in the myosin S-1 segment (6–9). With this knowledge we have proposed an S-1 structure and a hypothesis about how it moves in transduction (10, 11). Here we report CD and fluorescence observations on S-1 and consider how these relate to the hypothesis.

Materials and Procedures

α-Chymotrypsin was from Worthington, ATP was Sigma's best grade, and DE-52 (DEAE-cellulose) was from Whatman. Other reagents were the best available. Myosin was from back muscles of rabbits (12); its S-1.A-1 isoform (the S-1 segment of myosin, with the heavier alkali-releasable light chain attached) was prepared with α-chymotrypsin, then purified with DE-52 (13), and dialyzed against 100 mM KCl/1 mM MgCl$_2$ containing 10 mM Mops or potassium phosphate at pH 7.0.

Absorption was measured in a Cary 15 spectrograph; N$_2$ flushing enabled measurements to 180 nm. A Jasco J-40 spectrograph was used for CD measurements near 280 nm and for precision measurements fixed at 222 nm; spectral slit width was 2 nm; data were collected on an IBM-type personal computer (PC). A vacuum-UV spectrograph (14) was used in the 178- to 260-nm region; spectral slit width was 1.6 nm; signal was collected for 30 s at every 0.5 nm and was recorded on the PC. Chosen concentrations and path lengths kept total

absorbance below 1.0. Extinction coefficients are on a per-amide basis. For the 280-nm region S-1 was at 1 mg/ml in Mops; the cell containing 600 μl had a 1-cm path length. For an S-1 M_r of 131,470 [calculated from sequences (15–17)], 1 mg/ml is 7.6 μM. There are 1159 residues (average M_r, 113.5), so if $A_{280}^{0.1\%} = 0.74$ (18), $\varepsilon_{280} = 84$ per amide, and at the absorption maximum, $\varepsilon_{278} = 86$ per amide. For measurements at 222 nm S-1 was 0.25 mg/ml in Mops in a cell of 2000 μl and 2-mm path length. Injection of 10–20 μl of 40 mM ATP caused a small, correctable volume change; samples were mixed by gentle rotation to avoid S-1 aggregation. Data were collected over 1–10 min. For measurements in the amide region S-1 was at 1 mg/ml in potassium phosphate in a cell of 50-μm path length. Using concentrations calculated from 280-nm absorbance, $\varepsilon_{190} = 1.10 \times 10^4$ per amide.

Intrinsic S-1 fluorescence was measured on an SLM 8000 fluorometer, by exciting at 293 nm and recording at 335 nm. Quartz cuvettes, 2 × 10 mm, were held in a thermoregulated cuvette holder. Solution temperature was directly measured by a fine wire thermocouple in the cuvette, reporting to an Omega DP 41 indicator. This same arrangement measured 90° scattering except that 530-nm light was used throughout.

CD Observations

Detecting the effect of adding ATP to S-1 required the highest precision. For this purpose we employed the Jasco J-40 fixed at 222 nm, corrected for dilution, for the slight loss in S-1 resulting from the (rotary) mixing motion and for the CD contribution of the added nucleotide (identical for ATP and the produced ADP). After taking these precautions we found consistently at 10°C and 20°C, but not at 30°C, that addition of ATP increases the (negative) CD signal by an average of 0.4% ($P < 0.01$). This observation can be expressed as a $\Delta\varepsilon_{222}$ of -0.017 per amide or of -19.5 if due to a single residue. If this is interpreted as a change in α-helical content, it means adding two residues, but of course it could arise from change in other secondary structure or from chromophoric side chains.

From 0°C to $\approx$16°C there is a smooth, reversible fall in 222-nm signal with increasing temperature (Fig. 1). At the accuracy of these experiments, ATP addition has no perceptible effect. The behavior of the system at temperatures >16°C is complicated, probably by aggregation (19) and light chain release (20). Now ATP has an effect of a different kind. Without ATP the fall in signal steepens in the 16–30°C range and is only partially reversible. When ATP is added at a lower temperature and the system is brought to a higher temperature, signal also falls with temperature, but not nearly as steeply as without ATP, so the curves with or without ATP diverge. Nevertheless, addition of ATP at a higher temperature, say 30°C, has no measurable effect. Such behavior in

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Abbreviations: PC, personal computer; S-1.A-1, S-1 segment of myosin, with the heavier alkali-releasable light chain attached.
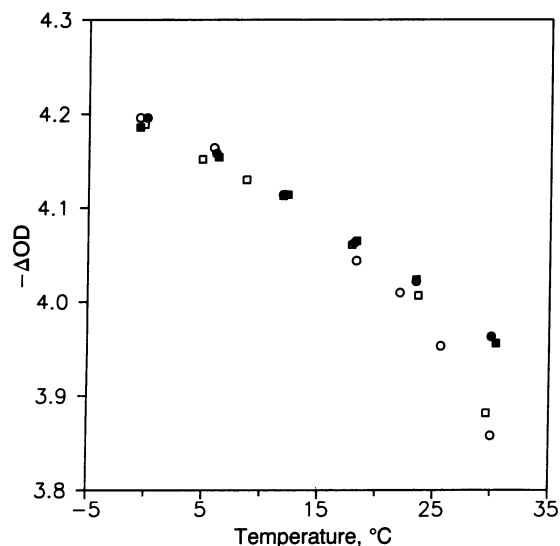
FIG. 1.   Temperature dependence of the 222-nm CD signal from S-1, without (open symbols) and with (filled symbols) ATP in the Mops buffer system.

the high-temperature regime illustrates "substrate protection" (21, 22). Signal loss in this regime can be time dependent. After addition of 1 M sucrose (to inhibit S-1 aggregation and the attendant light scattering) the signals of systems brought to a temperature that is high but <31°C (Fig. 2) stabilize quickly, but at 37°C signal stabilizes only after 3 hr, at about 89% of its 20°C value.

Conventional spectra were also taken. The 250- to 320-nm spectrum (not shown) was like that reported previously (23, 24) and also changed with ATP addition, as expected from earlier work. Within the limits of our experimental error for these studies (±5% for vacuum-UV scans), the 178- to 260-nm spectrum (Fig. 3) is the same at 4°C or 20°C and whether or not ADP or ADP plus vanadate is added. Analysis by variable selection (25), with a basis set of 22 proteins, gives 42% α-helix, 9% antiparallel β-sheet, 7% parallel β-sheet, 14% β-turns, and 29% other structures. Our helicity result is very different from earlier reports (26, 27) but not inexplica-
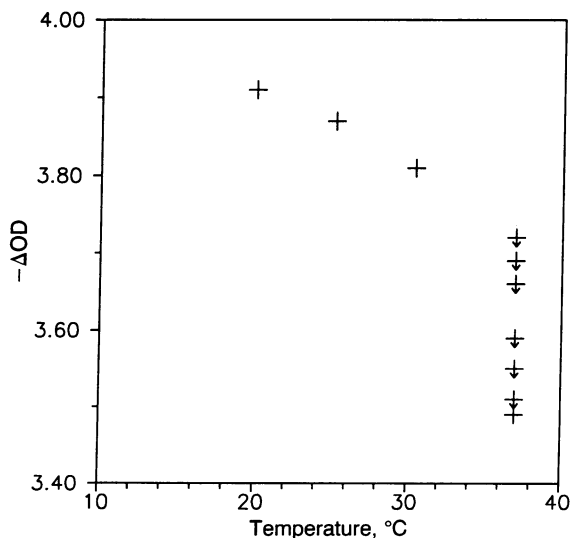

FIG. 2.   Temperature dependence of the 222-nm CD signal from S-1 in the Mops buffer system with 1 M sucrose added. Below 37°C the signal stabilized quickly; at 37°C, however, the signal was time dependent (arrows) and readings were taken at 10, 31, 57, 87, 130, 177, and 192–239 min.
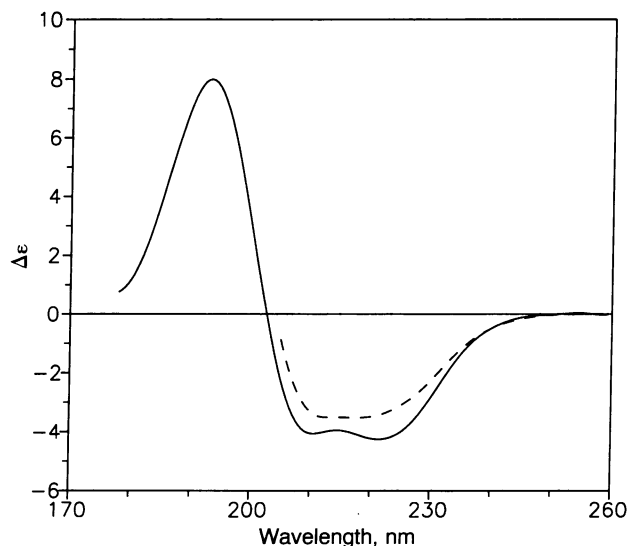

FIG. 3.   CD spectrum of S-1 at 4°C in the potassium phosphate buffer system (solid line); the spectrum was the same at 20°C and also when ADP or ADP plus sodium vanadate was added. However, at 37°C, in the Mops buffer system with 1 M sucrose added, the spectrum was that shown (dashed line).

bly so. The helicity of the *standard* proteins has been revised since that given in refs. 26 and 27 and concerned a differently prepared S-1. We reinforced our result by using its relation to $\Delta\varepsilon_{222}$; our basis set then gives a helicity of 42%. Also, using the helicity of intact myosin (26) and our S-1 molecular weight, we calculate a helicity of 40%.

Fluorescence Observations

According to our structure and hypothesis (11), the ATP-induced functional movements that CD might sense would most likely originate in the strands of a loop that connects the ATP-binding and actin-binding sites of S-1. Believing that the tryptophan residues that report ATP binding (28, 29) reside on one of these strands, we inquired whether temperature affected their fluorescence as it does the CD signal.

Torgerson (30) showed that the emission of the ATP-perturbable tryptophans is not quenched by 0.1 M acrylamide [and we had shown previously that this level of acrylamide does not impair S-1 function (31)]. Accordingly, we studied this emission as a function of temperature (Fig. 4). Clearly, in the low-temperature regime the emission falls reversibly, in a manner strikingly similar to the CD signal. Moreover, this emission-in-the-presence-of-acrylamide accounts for nearly all of the fall with temperature.

Discussion and Conclusions

It has been found here that (in the 0–16°C regime) either temperature changes or ATP binding produces slight, reversible changes in the CD signal from S-1 and in the fluorescence emission from certain of its tryptophans. Earlier (11) a rough structure of S-1 was proposed, and it was suggested that in transduction there is movement in a certain region. Because this region is thought to have mobility, and because we believe it to contain the tryptophan reporters (see below), we think that our present findings also indicate this functional movement.

Attribution of movement was made earlier (11), but the ATP-perturbable tryptophans have never been located. Torgerson (30) classified the five tryptophans of S-1 by emission lifetime and found that the ATP-perturbable and acrylamide-susceptible tryptophans are largely in different classes. Muhlrad and his collaborators (32–35) have found that all of the
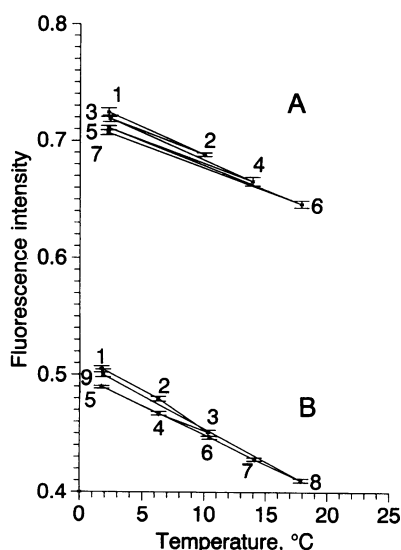
FIG. 4. Temperature dependence of the 335-nm fluorescence emission intensity (arbitrary units) of 2.5 μM S-1 in the Mops buffer system with certain additions. Numbers indicate the order in which readings were taken. Error bars are at 1 SD. (A) The buffer system was set at pH 7.3 and also contained 1 mM dithiothreitol and 1 mM sodium azide. (B) The solution composition was that of A, but an addition of 5 M acrylamide was made bringing the final acrylamide concentration to 200 mM. Data were not corrected for the $\approx$4% dilution or for the acrylamide self-absorption. At the excitation wavelength acrylamide produces an OD of about 0.08.

ATP-perturbable tryptophans reside on the 50-kDa domain of S-1 and that Trp-130, on the 25-kDa domain, is most susceptible to quenching even though it may be near to bound ATP (36). Our structure (11) suggests that of the three tryptophans in 50-kDa, Trp-402 is too far from the region of movement, but Trp-510 is on a moving strand, and Trp-594, on the intersite connection, responds to ATP in fragment experiments (37). We found that a bimane astride the two moving strands (38) resonates with two tryptophans (39). So, we suggest that Trp-510, and Trp-594, on the 50-kDa strand are the ATP-perturbable tryptophans. This may mean that the effect of crosslinking thiols on the 20-kDa domain changes CD but is not felt by the tryptophans on the 50-kDa domain (19, 40).

In inducing slight CD and fluorescence changes at low temperature, and in protecting against large decreases in CD signal at high temperature, ATP seems to play distinct roles, perhaps the former being functional and circumscribed and the latter being purely structural and global. That 16°C partitions the temperature range into distinct regimes supports Levy et al. (41), who suggested that a structural change occurs at this temperature. Our contention that functional movements occur at a 50-kDa–20-kDa interface also seems related to interpretations of differential scanning calorimetry (42). Finally, small but significant ATP-induced 222-nm CD changes probably originate in a circumscribed region while most of the macromolecule remains static. This makes it difficult to detect the changes; otherwise, several highly competent investigations of systems larger than S-1 (43–45) would have noted them.

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