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DISCUSSION

Session Chairman: Frederic Richards *Scribe*: Andrew W. Fulmer

LEHRER: This very interesting temperature study allows us to compare the molecule in the crystal to the molecule in solution and to find out what temperature is doing in both cases. I'd like to refer to our data (1978, *J. Mol. Biol.* **118**:209 and 1978, *J. Biol. Chem.* **253**:3757), for localized chain separation and localized instability in the cys-190 region in the middle of the carboxyl half of the molecule, and then pose a question.

In circular dichroism melting profiles, we find a different melting profile for tropomyosin in which the sulfhydryls are kept reduced without a disulfide crosslink between them. Woods earlier showed a general loosening or monotonic decrease in the low temperature region up to ~40°C or below 1.0 M GuHCl. The unfolding profiles are qualitatively similar whether temperature or GuHCl is used as the denaturant. If a disulfide crosslink is introduced between the two sulfhydryl groups —if one were not careful to keep it reduced or, on purpose, by air oxidation or by a reaction

with DTNB (1975, *PNAS* **72**:3327)—there would be a completely different unfolding profile. The expected stability of the S-S crosslink is seen at high temperatures or high guanidine and an increased instability appears in the low temperature or low GuHCl region. In fact, it resembles a cooperative pretransition. The evidence indicates there's a local unfolding at physiological temperatures in the region of Cys-190. This unfolding becomes more cooperative if the molecule contains a disulfide link.

My question is, in what state is the tropomyosin in these crystal studies? I suggest that by studying these two forms of tropomyosin in the crystal one could more clearly correlate in the temperature effects in the crystal with what is known to occur in solution.

PHILLIPS: A very good point. The straightforward answer is that although we start with reduced tropomyosin we have not checked to see what the state of the sulfhydryl groups is after x-ray analysis. We plan to do this by simply running the crystals on a gel after the x-ray pattern is taken. I mentioned that our electron density maps show some deviation from a coiled-coiled structure in the C-terminal half, the region where these sulfhydryl groups are contained.

SYKES: Dr. Edwards and I, using NMR to observe tropomyosin, find it hard to keep those sulfhydryl groups reduced. Local unfolding goes back to his 153 and extends out to affect his 274. There is a big change.

KORETZ: In light of the current controversy on how tropomyosin might prevent cross-bridge binding, could you discuss your 14 quasi-equivalent sites and differential flexibility of the tropomyosin molecule?

PHILLIPS: You're referring to the controversy raised by Seymour and O'Brien (1980, *Nature* **283**:680)?

KORETZ: Yes. Specifically, does tropomyosin sterically block the crossbridges or not?

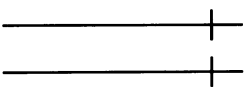
PHILLIPS: The model reviewed in the text has been referred to as the "steric blocking model." Tropomyosin is thought to lie in a position such that it can sterically block myosin heads from attaching to actin. Some results now indicate tropomyosin is on the opposite side of the actin groove relative to the side that the myosin heads attach to, so it couldn't possibly interfere with the binding of myosin to actin. I don't know if I believe those results yet. Peter Vibert of our laboratory supports the original choice of side of the actin groove for the location of tropomyosin. I think it is a question to be resolved.

KORETZ: You must have speculated about this, though.

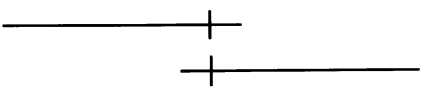
PHILLIPS: We see tropomyosin and its seven equivalent interacting regions making and breaking bonds either in the on- or off-state. We see perhaps one tropomyosin molecule, one end of it may be in the on, one end in the off. The range of motions that we see in the crystal is consistent with that type of flexibility on the thin filament. So the steric blocking model with its rigid gears and cogs moving tropomyosin from one position to the other is much too simple a view for the process.

SANDER: Just a factual question. Is it unambiguously clear from your crystal structure that the two helices are parallel in tropomyosin?

PHILLIPS: No, but taken with the chemical evidence, which is very strong, I don't think they can be antiparallel. Cross linking experiments indicate that the sulfhydryl groups in the molecule align, so that the two possibilities for the structure are like this (parallel)



or like this (antiparallel)



If it were antiparallel we would expect to see two areas where molecules overlap and indeed we see one area where molecules seem to form an end-to-end joint rather than a staggered structure.

HENDRICKSON: Back to the motion problem. Is it possible to put the crystals in a more viscous medium, for example, glucose, and ...

PHILLIPS: ...to get damping or something like that? That's possible. I have been trying to put them in ethylene glycol for another purpose, to take pictures at lower temperature. That's a good point. Glucose at the same temperature might be an interesting experiment.

HENDRICKSON: I think that the result is not completely obvious. It could be that what you have is a set of disorders rather than an actual motion.

PHILLIPS: Right. We would argue that if it is a set of disorders then you must of course go from one conformation to the other, whether or not we're actually looking at a harmonic vibration or just a set of conformational substates. We don't really have to distinguish to make inferences about the role of tropomyosin in muscle.

F. RICHARDS: You said you were trying to put in glycol, implying that it does not go easily. Are the experiments difficult?

PHILLIPS: The crystals, being 95% solvent, are very labile. They're very easy to disorder both chemically and mechanically.

F. RICHARDS: I have an anonymous referee's question here. "Did you ever attempt to follow the process of disordering of the long arm of tropomyosin in a more quantitative way? It seems to me that by scanning the photographs and plotting the total (or average) intensity of the spike due to scattering from the long arm vs temperature (using the other spike for calibration), the process could be described in a less subjective way, pointing out more accurately the transition temperature. Since at physiological temperatures the long arm will be much more disordered than the short arm, this could have potential implications for the mechanism of action. Could you comment on this point?"

PHILLIPS: We do have pictures at 5 different temperatures. I did try to plot them as a function of temperature, taking the ratio of some of the key intensities in each of the spikes. It was a fairly continuous function. I don't think the accuracy of the data allows the identification of an inflection point or a transition temperature, or of anything of that nature.

F. RICHARDS: I hope all of you are as impressed as I am by this comparison between the fiber people, who are clearly defined as a class, and the single crystal types. The fiber types have minimal data; when even this starts to disappear but reappears as diffuse scattering (which most of us throw away as background), they start to extract information from it. They've done such a successful job that they have now convinced the single crystal people that it is worth looking at the same thing. That's very impressive.