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DISCUSSION

Session Chairperson: Margaret A. Titus
Scribe: Laura Romberg

DEAN ASTUMIAN: It seems that there are fairly long periods of time where no stepping is taking place, but you still have a pretty significant fluctuation. Has anyone done an analysis where you parcel those second period of time into about 10 ms and analyze the variance in there as a function of time?

CHRIS COPPIN: No.

ASTUMIAN: Because basically what you'd hope to get out of that is the answer to the question of whether you're having ATP binding and then being hydrolyzed and released without making a productive step. With the idea being that when ATP is on, the variance might be different than when ATP is off.

COPPIN: You're talking about a fluctuation like this?

ASTUMIAN: Yes, I'm talking about things like right there, around 1.5 s, for example. It looks to me like there are periods of time which are very constrained, and then less constrained in their motion.

COPPIN: We do see things like this that look like a little dip. We don't see systematic changes or rapid changes in the RMS noise in the trace.

ASTUMIAN: And so you've actually looked and done some nice. . . .

COPPIN: Qualitatively.

ASTUMIAN: OK. Because it would probably take some fairly heavy statistical analysis of the data, but you would be expecting a bimodal distribution.

COPPIN: Yes. Thank you. That's a good idea.

VINCENZO LOMBARDI: When you make your stepping length, and you record stepping force, did you try to see if there is a linear relationship between the size of the length step and the size of the force step?

COPPIN: Not yet.

STEFAN HIGHSMITH: Can the dwell time, the time between steps, be accommodated to the biochemical cycle rates?

COPPIN: We haven't actually measured the ATPase activity under these identical conditions, but that's something we certainly plan to do, and will try to correlate the two.

DAVID WHITE: But the answer is yes in principle it can be, because you could do those transients, and Justin (Molloy) will show you those on myosin this afternoon.

STEVE BLOCK: Chris, this harks back to a conversation we had earlier, but I thought it might be useful to share it with other people. The forces that you see when you go into the isometric clamp situation are somewhat higher—7, 8, 9 pN—than the stall forces that have been seen in steady state measurements by Joe Howard's group and my own group, of about, say, 6 pN. I was wondering if you wanted to comment on why you see this discrepancy and what you think might be going on.

COPPIN: As I see it, there are at least two possibilities. One is simply that for some unknown reason, the head can produce more force when there's an active resistance. I don't have any model to explain that. One easier possibility is that if there is such a thing as a powerstroke, that powerstroke could take place faster than the feedback. So that here's my motor head and here's the trap trying to counteract the force (he demonstrates). It's moving step, step, step, and then it reaches the normal stall force. If it can make one step that's really fast, faster than the feedback, it's going to make one more step, and raise the force maybe from 5 to 5.2 or 5.3 pN or so. And then the feedback pulls the bead back to where it was, and you can get an extra 2.5 pN or so of force out of that. That's one possibility, and we'll have to control for that.

JOE HOWARD: Chris, what do you measure in the orthogonal direction? Do you see steps in the orthogonal direction? Or is it possible that the different forces are because it's moved over to a different protofilament? And so there's a different spacing between the motor and its binding site on the filament.

COPPIN: If we manage to pick an axoneme that's lined up with one of the axis of the quadrant photodiode detector, then there are no steps at all in the orthogonal direction. Sometimes, there a very low-frequency drift, but there are no steps that correlate with what we see in the longitudinal direction.

TOSHIO YANAGIDA: Recently, we have measured the force from a single kinesin molecule with a microneedle, a different method, and we found that the peak force is about 9 pN or so, so that our results are consistent with his results.