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DISCUSSION

Session Chairperson: Ivan Rayment
Scribe: Willy Wriggers

BERNHARD BRENNER: This was unregulated actin, so wouldn't you expect from Ken Holmes' contribution this morning, that the weak interaction should be nonspecific?

JOHN TRINICK: What makes us believe that our results are representative in some way for high salt concentrations are the binding and ATPase rates as a function of salt: if you plot the ionic strength of binding of S1 or S1.ADP we don't find anything new at low salt compared to high salt.

MASATAKA KAWAI: Do you have an estimate of the rate constants of the kinetic state transitions you showed in the last slide, especially the fast two: A.M. to A.M.ATP and the detaching transition?

TRINICK: My collaborator Howard White can comment on that.

HOWARD WHITE: The maximum rate of dissociation is 150 per second for mant-ATP, which could either be assigned to the rate of the M.ATP coming off or, probably more likely, to a transition before it comes off.

DAVE THOMAS: We have apparently an example here of a single biochemical state associated with a wide range of angles. Spectroscopic experiments have been suggesting this for a long time: Pjotr Fajer will present some work later, where you have a multi-angular state that should be a single biochemical state. It has been suggested that the disorder is due to many different states. At least in biochemical terms that is not true. Of course there could be many different structural states within one biochemical state.

If you now identify the weak binding, early states with the A.M.ATP state you show on the transparency, and on the right you draw a state like the one you drew for A.M., the transition going from an disordered to an ordered state will produce an average tilt of about 45 degrees of the myosin S1 and should be able to produce force without any other stereospecific state other than the final.

GERALD POLLACK: Did you perform a control experiment to make sure it was not the spray that induced the disorder?

TRINICK: Yes. For example if one just sprays buffer, one only gets the normal arrowhead configuration.

ROGER COOKE: Some years ago Tom Pollard did a somewhat similar experiment, in which he decided that it looked like rigor in steady state hydrolysis. What is the difference between his result and yours? Why is his wrong?

TRINICK: One difference between our experiment and Tom's is, he froze his specimen, and then he cleaved it into two and etched it in vacuum. And in order to etched it you have to raise the temperature to about −100 degrees. Then he shadowed it with metal. Our results are different from his, possibly because something happened during the warming, drying, and shadowing procedure, which was not necessary in our case: our specimens were never warmed up above −170 degrees.

COOKE: You could test this hypothesis by warming your probe up, cooling it off, and see if it would change the angle.

TRINICK: Yes, we could warm it and then shadow it. Technically, it probably wouldn't be an easy experiment.

IVAN RAYMENT: These are very challenging results. Are you really confident that you get uniform mixing on that timescale?

TRINICK: I don't think the mixing is uniform. We have a grid with many droplets on it. With a small molecule like mant-ATP, the question is how to determine whether the spray has arrived at the observed region. We put 10-nm colloidal gold in the spray. This particle can be then detected in the image; in fact, it gives a very dense spot on the negative. So we are absolutely certain that the spray has arrived at the observed region. But it will not be uniformly mixed; we rather get a mant-ATP gradient. Locally, however, the ATP concentration will be very large.

HOWARD WHITE: I also want to answer this. The dimension of the picture is roughly 1 micron. The rate of diffusion is a few microns per millisecond. So the nucleotide concentration should be homogeneous within the field we are looking at. However, we can also find different fields on the same grid with rigor conformations, where the specimen has not been exposed to mant-ATP.