On phosphate release in actin filaments

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In their article recently published in Biophysical Journal (1) Burnett and Carlsson present a theoretical analysis of experimental data that we have previously published and analyzed (2). Our work provided evidence for a random mechanism of inorganic phosphate (Pi) release subsequent to chemical cleavage of ATP on individual actin filaments. Burnett and Carlsson examine the alternative possibility of cooperativity during Pi release in actin filaments. We find that the presentation of our work by Burnett and Carlsson is misleading or incorrect in several instances, and wish to clarify the following points.

In our paper, depolymerization traces of individual filaments were measured, and each individual curve was fitted in order to investigate the possibility of different Pi release mechanisms. For the sake of comparison with Burnett and Carlsson (1) we focus here on direct fits of depolymerization traces, which are detailed in the Supporting Text of our paper, but the same conclusions are drawn when fitting the inverse of the depolymerization velocity, as we have done in the main text (2). The best fits were obtained for a random Pi release model, and the release rates were found to be similar, but not identical (due to experimental noise) for each curve, with an average value of r_d = 0.0074 s^{-1} (0.0068 s^{-1} with the inverse depolymerization velocity method). Our experimental data used by Burnett and Carlsson (1) consists in the depolymerization trace of one single individual filament, which they have extracted from Fig. 2B of ref. (2). When comparing this individual depolymerization trace with traces computed for a random Pi release, Burnett and Carlsson use the average rate constant of r_d = 0.0074 s^{-1} and the resulting curves do not agree well with the data (Fig. 6D of ref. (1)). However, this is not a fit of this data. Our fit of this individual trace results in r_d = 0.0045 s^{-1} (or r_d = 0.0043 s^{-1}, as stated in the caption of Fig. 2 of ref. (2) , using the inverse depolymerization velocity method), and the quality of this fit is very good, with $\Delta^2 = 1.2 \cdot 10^{-2}$ μm^2, better in fact than the best fits with cooperativity shown in the inset of Fig. 6 (1).

Measuring the depolymerization traces of individual filaments, as we have done, allows one to look for details that would disappear when curves are averaged. As can be seen in Fig. 6D of ref. (1), for a random Pi release there is little difference between individual curves, and it therefore makes sense to compare individual experimental traces to an average theoretical curve. In contrast, for the cooperative model, as shown in Fig. 6C of ref. (1), each individual filament displays its own sharp

transitions between ADP and ADP-Pi domains, which are smoothed out when averaging several traces. Comparing an individual experimental trace to an average theoretical curve, as done by Burnett and Carlsson – "the average trajectory (…) provides a good fit to the data, as shown in Fig. 6C" (1) – makes no sense in this case. In order to argue in favor of cooperativity, Burnett and Carlsson should have demonstrated that sample filament traces (blue curves in Fig. 6C of ref. (1)), and not the mean filament time-course (red curve in Fig. 6C), provide a good fit to our single filament data (black dots in Fig. 6C).

As suggested by Burnett and Carlsson, comparing the depolymerization traces of filaments elongated at different rates (i.e. using different actin concentrations) is a good way to test whether Pi release is a purely random mechanism or involves cooperativity (Fig. 7 of ref. (1)). The authors write: "Thus, repeating the experiment of Jégou et al. with varying G should provide additional constraints on the cooperativity". Our paper however seems to have been overlooked here too, since this experiment has actually been performed and reported as follows in ref. (2): "Filaments elongated at different actin concentrations, i.e. different velocities, or for different durations all displayed the same age-dependence of depolymerization rate (Figure 2F), confirming that the ADP-Pi content depends only on the age of the F-actin, as expected for a random Pi release mechanism." Further in the article (2), we show (Fig. 4E) examples of ADP-Pi profiles obtained with a ten-fold difference in actin concentrations. Experiments over a broader range of actin concentrations might reveal some cooperativity, but our current data is in better agreement with a random Phosphate release mechanism than with a vectorial or highly cooperative mechanism.

References

1. Burnett, M. M., and A. E. Carlsson. 2012. Quantitative analysis of approaches to measure cooperative phosphate release in polymerized actin. Biophys J 103:2369-2378.
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