Supporting Information

To assess the purity of the protein preparations, they were analyzed by SDS-PAGE as shown in Fig. S1 for typical preparations. Samples of kinesin constructs were loaded at 1 μg/lane based on the protein concentration calculated from the molar concentration determined from A_{280} and the molecular mass. Gels were 8-16% acrylamide and stained with CoomassieR. The only significant contaminants of DKH412 are two smaller bands that may be proteolysis fragments. Serial dilution indicates that they represent less than 4% of the main band. Samples of TT910-952 and NF910-952 at 1 μg/lane are also included for comparison. TT910-952 as two minor bands that are likely proteolysis fragments. NF910-952 does not stain well because of its small size (5.35 kDa). Also included is the bovine serum albumin (BSA) supplied as a reference standard by Pierce Chemical Company at 1 and 0.5 μg/lane. Although different proteins can stain to different extents, the bands for DKH412, TT910-952, NF910-952 and BSA are of very similar intensity at the same nominal 1 μg/lane protein concentration even with allowance for the presence of aggregates in the BSA. If the true stoichiometry of tails:heads was 1:1 at the equivalence point and the apparent half site stoichiometry was due to an incorrect determination of the DKH412 concentration, then the intensity of the DKH412 band at 1 μg/lane would be equal to the BSA band at 0.5 μg/lane and not 1 μg/lane. The observed intensity of the DKH412 band at 1 μg/lane is, however, similar to that for BSA at 1 μg/lane and clearly much greater than that for BSA at 0.5 μg/lane.

The half site titration with tails is also not due to half the DKH412 being inactivated or denatured because the basal ATPase activity is equal to that expected based on the concentration of K412 and the k_{cat} value. The expected k_{cat} value was determined from the rate of ADP dissociation that is the rate limiting step in steady state turnover {rrr}. To determine the rate of dissociation of unmodified ADP, the DKH412•ADP complex was diluted into a 20-fold excess of 2'-deoxy-mATP in A25 buffer with 25 mM KCl and 2 mM PEP to duplicate the conditions for determination of the steady state ATPase rate. Under these conditions, an increase in fluorescence occurs with a rate of 0.00849 ±0.00034 s^{-1} as the ADP is released and replaced with the chase of 2'-deoxy-mATP. This rate is similar to the 0.0098 s^{-1} value reported for DKH405 using ^{32}P-labeled ATP (1). The steady state turnover rate of DKH412 at 1 μM ATP was measured in parallel experiments as 0.00828 ±0.00026 s^{-1} calculated using the DKH412 concentration determined by A_{280}. Thus DKH412 was fully active and the concentration of actively turning over sites equals the concentration of sites calculated from the A_{280}.

References

1. Jiang, W., Stock, M., Li, X., Hackney, D. D. (1997) Influence of the kinesin neck domain on dimerization and ATPase kinetics, *J.Biol.Chem.* 272, 7626-7632.