EFFECTS OF CYSTEINE MODIFICATION ON MICROTUBULE-MOTOR FUNCTION AND TUBULIN ASSEMBLY

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE
in
Biology

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December, 1998
Blacksburg, Virginia

Keywords: cysteine, kinesin, microtubule, Ncd, N-ethylmaleimide, tubulin

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(ABSTRACT)

Chemical modification is a powerful technique for probing functionally important amino acids. N-ethylmaleimide (NEM) reacts readily with exposed sulfhydryl groups, and has previously been shown to inhibit the activity of MT-motor proteins and tubulin assembly. This project seeks to investigate the mechanisms by which NEM affects motor function and inhibits MT minus end assembly. Recombinant motor domains of *Drosophila* kinesin (DK350 and DK375), Ncd (MC1), and squid kinesin (p181) were modified by NEM. NEM treatment was shown to affect the binding of MC1, but not recombinant kinesin proteins to MTs in the co-sedimentation assay. NEM treatment decreased the MT-stimulated ATPase rates of MC1 and DK350 in an NEM-concentration dependent manner, but did not affect the rate of DK375. Observed effects with DK375, p181, and MC1 were correlated with the number of labeled cysteines determined with [^{3}H]NEM. As previously known, when NEM-treated tubulin was combined with untreated tubulin at certain ratios, assembly occurred only at the MT plus end. To investigate the mechanism by which NEM affects the polarity of tubulin assembly, tubulin was treated with NEM and assembly was analyzed using video-enhanced differential interference contrast microscopy. [^{3}H]NEM was used to follow the time course of modification and to determine the number of modified sites per tubulin subunit. After 10 minutes, one cysteine was labeled on both α and β tubulin and this was sufficient to inhibit minus end assembly. Additionally, having one subunit labeled out of five tubulin subunits was sufficient to observe this effect. Protein digestion methods were used to aid in elimination of cysteines, to characterize potential critical cysteines in MC1, α, and β tubulin.

Acknowledgments

I have always had a great appreciation and respect for my committee members, Dr. Eric A. Wong, Dr. Brenda Winkel-Shirley, and Dr. Richard A. Walker, and over the past two years I developed an even greater sense of appreciation for their knowledge and accomplishments. Research is fulfilling, yet challenging work and I thank them all for their insight and words of encouragement.

I extend a special word of thanks to my advisor, Richard Walker, who welcomed me into the lab as an undergraduate and made the laboratory an unforgettable experience and a huge part of my life. Also, I extend this thanks to my fellow graduate students in the lab, Arzu and Bettina, for their words of wisdom and helpful advice.

Finally, I am grateful to my husband, Keith, and to my mother for their support during this time. I am also thankful to my father who first instilled in me a love for science.

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