Evidence of vanadyl-myosin coordination upon myosin photocleavage induced by decavanadate: An EPR study

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Myosin is a highly specialized protein involved in the process of muscle contraction that converts the chemical energy of ATP hydrolysis to mechanical work. This activity is greatly enhanced when actin binds to myosin forming the actomyosin complex. The inhibitory effect of decavanadate (V_{10}) towards the myosin ATPase activity has been recently reported [1,2]. Besides, the ability of decavanadate to bind to myosin and to induce protein photocleavage has been observed at our laboratory. Concentrations as low as 50µM decavanadate (5µM V_{10} species) induces 30% of protein cleavage whereas 500 µM metavanadate is needed to attain the same extent of cleavage. After irradiation, V_{10} species is rapidly decomposed, only upon protein addition, forming oxovanadium (+4) species (vanadyl) during the process. In the present work, it is confirmed by EPR spectroscopy that typical signals of vanadyl (+4) are obtained, when the enzyme reacted with V_{10}. It is suggested that the vanadyl coordination, based on the measured coupling constants of a// from studies of model complexes**,** are two equatorial oxygen atoms, from two waters molecules, one equatorial nitrogen, from a carboxyl group (aspartate or glutamate) and one equatorial nitrogen, from an amino group (lysine). It is shown that the presence of ATP increases by 2.5 the coordination of vanadyl to the protein and that actin does not affect this cluster. It is suggest that during the mechanism of V_{10} promoted photocleavage reaction on myosin, decameric vanadate is rapidly reduced into vanadyl species coordinated with the protein near the active binding site.

1) T. Tiago, M. Aureliano, R.O. Duarte, J.J.G. Moura, Inorg. Chimica Acta 2002, 339, 317-321.
2) T. Tiago, M. Aureliano, C. Gutiérrez-Merino, Biochemistry 2004, 43, 5551-5561.

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