

THE ROLE OF MYOSIN PHOSPHORYLATION IN REGULATING ACTIN-MYOSIN INTERACTION IN HUMAN BLOOD PLATELETS. R.S. Adelstein, B. Barylko, and M.A. Conti. Section on Molecular Cardiology, NHLBI, Bethesda, Md. USA.

Human blood platelets contain actin and myosin which are similar in many of their structural and biochemical properties to the contractile proteins found in muscle. Platelet myosin is composed of six polypeptide chains, two heavy chains of 200,000 daltons and two pair of light chains of 20,000 and 15,000 daltons. Platelets also contain a specific enzyme capable of phosphorylating the 20,000 dalton light chain of platelet myosin. The kinase has been purified 600-fold and has been shown not to require  $\text{Ca}^{2+}$  or cyclic AMP for its activity (Daniel, J.L. & Adelstein, R.S., Biochemistry 15, 2370, 1976). Phosphorylation of platelet myosin results in a 7-10 fold increase in the actin-activated myosin ATPase activity but has no effect on the myosin  $\text{K}^+$ -EDTA ATPase activity measured in 0.5 M KCl. The phosphatase responsible for dephosphorylation of platelet myosin has been partially purified. Dephosphorylation of platelet myosin results in a decrease in the actin-activated myosin ATPase activity, without affecting the myosin ATPase activity measured in the presence of  $\text{K}^+$ -EDTA in 0.5 M KCl.

Thus, using the actin-activated ATPase activity as an indication of actin-myosin interaction, myosin phosphorylation-dephosphorylation appears to function as a reversible regulatory mechanism. That this type of regulation is not confined to platelets is indicated by finding a similar mechanism in proliferative rat myoblasts grown in culture (Scordilis, S.P., and Adelstein, R.S., Biophysical J., 17, 268a, 1977) and guinea pig vas deferens smooth muscle (Chacko, S., et al., PNAS. 74, 129, 1977). B.B. is on leave from the Nencki Institute, Warsaw, Poland.

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## INVITED SYMPOSIUM VI

### Factor IX: Biochemistry and Clinical Applications.

THE ROLE OF FACTOR IX (CHRISTMAS FACTOR) IN BLOOD COAGULATION. Earl W. Davie, Kazuo Fujikawa, Patricia Lindquist, Richard Di Scipio, Kotoku Kurachi, and Ronald Heimark. Department of Biochemistry, University of Washington School of Medicine, Seattle, Washington 98195, U.S.A.

Factor IX participates in the middle phase of the intrinsic pathway of blood coagulation. The reactions leading to the activation of factor IX involve prekallikrein, high molecular weight kininogen, and factor XII. These proteins interact in the presence of a surface such as kaolin and give rise to the activation of factor XI. Factor  $\text{XI}_a$  then converts factor IX to factor  $\text{IX}_a$  in the presence of calcium ions. In this reaction, factor IX (a single-chain glycoprotein of mol. wt. ~55,000) is converted to factor  $\text{IX}_a$  in a two-step reaction. In the first step, an internal peptide bond is cleaved leading to the formation of an intermediate lacking enzymatic activity. This intermediate contains two polypeptide chains held together by a disulfide bond(s). In the second step, an activation peptide is split from the heavy chain of the intermediate giving rise to factor  $\text{IX}_a$  (mol. wt. ~45,000). Factor  $\text{IX}_a$  is composed of a heavy chain (mol. wt. ~27,000) and a light chain (mol. wt. ~16,000) held together by a disulfide bond(s). The activation mechanism is essentially identical for human and bovine factor IX. Factor  $\text{IX}_a$  is a serine protease with esterase activity and is sensitive to protease inhibitors such as antithrombin III. Factor IX is also activated by the protease from Russell's viper venom, but this reaction involves only a single cleavage in the precursor molecule. The critical step in the activation of factor IX by factor  $\text{XI}_a$  or the protease from Russell's viper venom is the cleavage of the same internal Arg-Val peptide bond and the formation of a new amino-terminal sequence of Val-Val-Gly-Gly- in the heavy chain of the enzyme.