

PLATELET MICROTUBULE SUBUNIT PROTEINS. N. Crawford. Department of Biochemistry, University of Birmingham, Birmingham, England

Microtubule subunit proteins have been isolated and purified from pig platelet homogenates and reassembled *in vitro* to form structures similar to those seen in the intact platelet. The major subunit protein tubulin has many of the properties of neurotubulin; it is a 6S dimer protein which binds colchicine and can be split into two non-identical monomers [α and β] in discontinuous alkaline SDS polyacrylamide gel electrophoresis. Present also in the preparation are two/three high molecular weight proteins [8-12% of the total protein] which are similar to those seen in brain tubulin preparations and appear to be essential components in the reassembly process. An antibody has been raised to platelet tubulin and used for the fluorescent visualisation of cell microtubules. Their morphological distribution has been contrasted with that of structures stained with anti-actin antisera and the effect of colchicine, cytochalasin B and low temperatures on the specificity of these antibodies has been investigated. Some views will be presented on the equilibrium which exists in platelets between microtubular subunit proteins and the fully formed microtubules. Because of the possible cytoskeletal importance of the microtubules in shape change phenomena and their role in controlling membrane properties, factors which directionally influence this equilibrium may be important sites of action for antithrombotic drugs.

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MACROMOLECULES RELEASED FROM PLATELET STORAGE ORGANELLES. D.S. Pepper, S.E. Scotland Regional Transfusion Service, Edinburgh, Scotland.

Much attention has been paid to the low molecular weight substances released from platelets since the release reaction was first discovered. The release of macromolecules on the other hand has received much less attention, and of the published work on platelet macromolecules most has been devoted to the known plasma protein equivalents which are either synthesized *de-novo* or adsorbed by the platelet prior to their subsequent release. Whereas it is fairly easy to show that the physical and/or immunological properties of these molecules (notably albumin, fibrinogen and IgG) are slightly but reproducibly different from those of plasma, it is very difficult (if not impossible) to prove that they are truly synthesized within the platelet as such, rather than undergoing modulation during the release reaction or isolation procedure. Whilst these "plasma equivalent" proteins are of academic interest, they have less practical application than those which are "platelet specific" i.e. are not normally found in native, platelet free plasma. Unfortunately, there are several technical problems in the study of such proteins, and we have chosen to study them by a combination of techniques, namely biological activity, immunological activity and physico-chemical properties. Each approach has some drawbacks, but in combination, much progress is possible and we can now identify at least four antigens, four biological activities, and two physically distinct proteins, which are not necessarily related. With the recent interest in the biological activity of released macromolecules, it is now desirable that the various groups exchange samples and antisera in order to identify each antigen and biological activity.

PRIMARY STRUCTURE OF HUMAN PLATELET FACTOR 4. F.J. Morgan, G.S. Begg and C.N. Chesterman. St. Vincent's School of Medical Research and University of Melbourne Department of Medicine, St. Vincent's Hospital, Melbourne, Victoria, Australia.

The amino acid sequence of human platelet factor 4 (PF4) has been studied. PF4 is a platelet specific protein with antiheparin activity, released from platelets as a proteoglycan complex, whose measurement may provide an important index of platelet activation both *in vivo* and *in vitro*. These studies were undertaken to characterize fully the PF4 molecule. PF4 is a stable tetramer, composed of identical subunits, each with a molecular weight based on the sequence studies of approx. 7,770. Each PF4 subunit contains 69 amino acids, including 4 half-cysteine (# 10, 12, 36, 37), one tyrosine (# 59), 3 arginine and 8 lysine, but no methionine, phenylalanine or tryptophan residues. The basic residues are predominantly in the C-terminal region. The tryptic peptides were aligned after studies which included tryptic digestion of citraconylated RCM-PF4, and automated Edman degradation of RCM-PF4 and citraconylated tryptic peptides. No glycopeptides were detected. This structural information should enable clear distinction to be made between PF4 and other platelet proteins such as β thromboglobulin. The provisional amino acid sequence of each subunit is:

Glu-Ala-Glu-Glu-Asp-Gly-Asp-Leu-Gln-Cys-Leu-Cys-Val-Lys-Thr-Thr-Ser-Gln-Val-Arg-Pro-Arg-His-Ile-Thr-Ser-Leu-Glu-Val-Ile-Lys-Ala-Gly-Pro-His-Cys-Cys-Pro-Thr-Ala-Gln-Ile-Leu-Ala-Thr-Leu-Lys-Asn-Gly-Arg-Lys-Ile-Pro-Leu-Asp-Leu-Gln-Ala-Tyr-Leu-Lys-Ile-Lys (Lys, Lys, Ser, Glx, Leu, Leu)