Supplementary Abstracts

Fibronectin, Fibrinogen and Fibrin

1103

ISOLATION, PASTEURIZATION, AND LYOPHILIZATION OF HUMAN PLASMA FIBRONECTIN. Shirley I. Miekka, Thomas F. Busby, Kenneth C. Ingham and Doris Menache. American Red Cross Blood Services Laboratories, Bethesda, MD 20014 U.S.A.

The goal of this research is to develop a suitable method for purification of plasma fibronectin for potential replacement therapy in patients with acquired deficiencies. Using a modification of published affinity procedures, a simplified one-step method has been achieved. Plasma or cryoprecipitate is chromatographed on gelatin-agarose and the unadsorbed and loosely adsorbed proteins are removed by washing with pH 7.5 buffer (0.05 M Tris HCl, 0.05 M ε-amino caproic acid, 0.02 M sodium citrate) followed by 1 M NaCl. The bound fibronectin is eluted simply by decreasing the buffer pH to 5.5 (0.02 M sodium citrate). The eluate can then be neutralized and further processed as desired. Sedimentation velocity measurements suggest that the elution may be related to a conformational change in the fibronectin molecule below pH 7.

Studies of thermal stability have been initiated with the objective of defining conditions under which the protein can withstand pasteurization (10 h, 60°C) to minimize the risk of hepatitis. Using an increase in the fluorescence of added 1,8-anilinonaphthalene sulfonate to monitor thermal denaturation, a variety of potential stabilizers was tested. No protection was afforded by gelatin, heparin, citrate, phosphate, EDTA, $CaCl_2$, glycine, glycerol, or PEG; however, neutral sugars increased the midpoint of the denaturation curve to well above 60°C. Lyophilization was found to have no detectable deleterious effect on fibronectin. Freeze-dried samples were readily soluble in water, showed no change in sedimentation behavior, and retained the ability to augment the uptake of gelatin-coated particles by rat liver slices.

1104

COMPETITIVE ENZYME IMMUNOASSAY FOR FIBRINOGEN AND FIBRINOGEN/FIBRIN DEGRADATION PRODUCTS. T. Ho and A. Gray. Diagnostic Div., Abbott Lab., N. Chicago, Illinois U.S.A.

A competitive enzyme immunoassay for fibrinogen and fibrinogen/fibrin degradation products (FDP) is described, in which a peroxidase - fibrinogen conjugate competes with fibrinogen or FDP for binding to a fibrinogen antibody attached to polystyrene solid phase. After this competition reaction is completed, unbound fibrinogen or FDP is removed by washing the polystyrene-antibody complex with water. The extent of the conjugate binding to antibody is observed by adding a chromogenic substrate for the peroxidase reaction. Peroxidase activity measured spectrophotometrically at 492 nm is inversely proportional to the fibrinogen or FDP concentration in the serum sample. This enzyme immunoassay requires only 50 ul of human serum per assay and can be performed in as little as 45 minutes. Using this method, serum collected from normal healthy volunteers give a mean FDP level of 1.8 ± 0.7 ug/ml (n=24). With a properly anticoagulated plasma, the assay can be used to determine immunological fibrinogen level in plasma sample. This enzyme immunoassay is unaffected by the presence of rheumatoid factors in some sera, which has been reported as a source of error in latex agglutination technique. The assay has the added advantage of measuring directly the concentration of FDP in serum, without making serial dilution of the sample.

1105

A QUANTITATIVE NORMAL FIBRIN PLATE ASSAY. E. M. Devine, Abbott Laboratories, North Chicago, Illinois 60064 USA

Astrup and co-workers introduced the normal fibrin plate assay method for assaying plasminogen activators in 1952. Over the years, this technique has been used as a semi-plasminogen activators such as urokinase. Our laboratory designed experiments to investigate and establish parameters for the fibrin plate assay which would give a maximum and reproducible response to quantities of urokinase. The parameters investigated included molarity (ionic strength) and pH of the buffers used for both the fibrin clot and the unknown sample. To establish these conditions, a series of experiments were performed involving parallel line assays. With this procedure, the response to each parameter studied was compared at several different levels of activity. Statistical comparisons were made to determine how changes in the measured parameters would influence the sensitivity and precision of the assay. The outcome of these studies led to the development of a routine normal fibrin plate assay for urokinase using a sample pad and a standard curve with an activity range of 0.1 to 0.8 IU. These conditions yielded a linear response on a semilogarithmic scale. When assaying urokinase, the results of the conventional fibrin plate assay (one level unknown vs. four levels standard) and the dose response-parallel line-fibrin plate assay (four levels unknown vs. four levels standard) are equivalent. The coefficient of variation for both assays was 2 to 5 percent.