DETERMINATION OF HAGEMAN FACTOR (HG, FACTOR XII) AND PLASMA PREKALLIKREIN (FLETCHER FACTOR) BY RADIOIMMUNOASSAYS

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ABSTRACT

The titers of components of the plasma kallikrein-kinin system have been measured conventionally by their biological functions. The functional assays are, however, antagonized by the presence of inhibitors and/or the absence of potentiators in test samples. Immunologic assays obviate these difficulties. We have developed specific, sensitive and reproducible radioimmunoassays (RIA) for HF and prekallikrein, and have applied these assays to some clinical conditions. Normal pooled human plasma contained approximately 40 μg of HF and 50 μg of prekallikrein per ml. RIA were able to measure concentrations of HF and prekallikrein as low as 0.1% and 0.3% that of normal pooled plasma respectively. A good correlation existed between titers measured by clotting and radioimmunoassays among 40 normal subjects (correlation co-efficient = 0.82 for HF and 0.71 for prekallikrein). There was no significant difference between the levels of HF and prekallikrein in plasma and those in serum. Both HF and prekallikrein were significantly reduced in plasmas of patients with advanced liver cirrhosis or disseminated intravascular coagulation (DIC) and in cord serums, but they were normal in plasmas obtained after strenuous physical exercise and in plasmas of patients under treatment with warfarin.

INTRODUCTION

Both Hageman factor (HF, factor XII) and plasma prekallikrein (Fletcher factor) are plasma proteins that participate both in plasma kinin-generating and blood clotting systems (Ratnoff and
Colopy, 1955; Margolis, 1958; Hathaway et al., 1965; Wuepper, 1973). The titers of these proteins have been measured conventionally by their biological functions. The titer of HF is usually assayed by measuring the effect of a test sample on the prolonged partial thromboplastin time (PTT) of Hageman trait (factor XII deficiency) plasma. The titer of prekallikrein is measured by the esterolytic or amidolytic activity of contact-activated plasma (Colman, et al., 1969; Amundsen, et al., 1977; Kluft, 1977) or by procoagulant activity for Fletcher trait (prekallikrein-deficient) plasma (Hathaway et al., 1965).

These functional assays are however, interfered with by the presence of inhibitors and/or the absence of potentiators in test samples. The clotting assay may give an erroneous titer if the sample contains an anticoagulant such as heparin that interferes with coagulant assays. The esterolytic or amidolytic assay requires the presence of other proteins (HF and high molecular weight-kininogen) for optimal activation of prekallikrein in the test sample. Immunologic assays obviate these difficulties. We have developed specific, sensitive and reproducible radioimmunoassays for HF and prekallikrein and have applied these assays to some clinical conditions.

MATERIALS AND METHODS

Citrated plasmas from normal individuals and patients were prepared as described earlier (Saito et al., 1978a). High molecular weight kininogen-deficient (Fitzgerald trait) plasma was obtained through the courtesy of Dr. R. Waldmann, Henry Ford Hospital, Detroit, Michigan.

Radioimmunoassays of HF and prekallikrein were performed by a double-antibody technique (Fig. 1 and 2) (Saito et al., 1976a; Saito et al., 1978a). A standard curve was prepared by plotting the percentage of bound (precipitated) radioactivity against the logarithm of the concentration of purified proteins or normal pooled plasma. Titers were expressed as units per ml plasma, one unit of HF or prekallikrein being arbitrarily defined as that amount present in one ml of a standard pool of 24 normal plasmas of male adults.

The amounts of HF and prekallikrein in normal pooled plasma were estimated to be approximately 40μg and 50 μg protein per ml, respectively as judged by comparison to a standard curve using purified HF and kallikrein. The minimum concentration of HF and prekallikrein detectable by RIA were approximately 0.1% and 0.3% that of normal pooled plasma (Saito et al., 1976a; Saito et al., 1978a).