

GEL STRUCTURE AND CLOTTING TIME. B.Blombäck, M. Okada
The New York Blood Center, New York, N.Y.

Ferry and Morrisson found on the basis of the mechanical and optical properties of fibrin gels that they ranged between two extreme types: fine or transparent and coarse or opaque. We have now developed a device permitting studies of flow properties of gels. The gels are formed in standardized cups (1.47x2.5 cm) which are fitted into a special holder attached to a reservoir for application of buffer solution for the flow measurement. Gels were formed by mixing thrombin or Batroxobin with fibrinogen solutions of different protein concentrations (1-4 g/l), pH (6.5-8.2) and ionic strengths (0.1-0.23). All these studies were in addition performed at different enzyme concentrations. The gels were left in the cups for complete gelation to occur (1-2 hours) and subsequently the same buffer as used for gel formation was percolated through the gels at room temperature and a constant pressure gradient. In parallel experiments the formation and optical properties of the gels formed was studied by turbidity measurement at 450 nm. After a lag-time rapid increase in turbidity occurred. The lag-time was defined as the "clotting-time".

We found that for both enzymes the flow is related to the optical properties of the gels, being fast for the opaque and slow for transparent gels. Fast flow is thus favoured by low pH and low ionic strength. The flow rate is furthermore inversely related to the gel concentration. Of particular importance was the finding that under all conditions so far studied there was, within a wide range, a direct relationship between clotting time and flow rate. This suggests that the final structure of the gels is determined by events preceeding the gel formation. These events are enzymatic activation and alignment of activated molecules. Gel formation is therefore very likely an ordered process resembling that of crystallization. Batroxobin and thrombin gels having the same clotting times displayed different flow properties suggesting that different types of polymers are formed on release of one (FPA) and two (FPA + FPB) activation peptides, respectively.

CHARACTERIZATION OF A CROSSLINK-CONTAINING FRAGMENT DERIVED FROM THE α POLYMER OF HUMAN FIBRIN AND ITS APPLICATION IN IMMUNOLOGIC STUDIES USING MONOCLONAL ANTIBODIES. J.H. Sobel, S. Birken, P. Ehrlich, R. Friedman, Z. Moustafa and R.E. Canfield. Department of Medicine, College of Physicians and Surgeons of Columbia University, New York, N.Y. 10032.

A high molecular weight crosslink-containing fragment derived from cyanogen bromide (CNBr) digests of the α polymer component of human fibrin has been isolated and characterized. The material has been used to generate monoclonal antibodies toward the goals of (1) producing fibrin-specific probes for use in the early detection of thrombosis and (2) generating monoclonal lines to single determinants in the COOH-terminal region of the A α chain for use in structural studies of fibrinogen and fibrin.

Biochemical and immunologic characterization data indicate the fragment is comprised, predominantly, of equimolar quantities of the CNBr peptides spanning residues #241-476 (CNBr 8) and #518-584 (CNBr 10) in the original A α chain. The acceptor and donor units are crosslinked via an average of 2.8-3.2 ϵ -(γ -glutamyl) lysine bonds per mole of CNBr 8 + CNBr 10 producing heterogeneously sized fragments in the range of 80,000-200,000 daltons.

Two types of monoclonal lines have been obtained. The first react with regions of primary structure and in one instance immunoreactivity could be localized to the A α tryptic peptide #253-268. The second type appear to recognize conformational determinants as shown by one antibody that reacts well with the CNBr crosslinked fragment but poorly with its constituent peptides.