

FREE COMMUNICATIONS XVIII

Coagulation: Fibrinogen and Fibrin.

SIZE AND SHAPE OF HUMAN FIBRINOGEN IN SOLUTION. V. Hofmann, P.W. Straub, T. Binkert, Univ. of Berne, E. Serrallach, W. Känzig, Dept. of Physics, ETH-Zurich and M. Zulauf, Biozentrum, Basle, Switzerland.

In order to obtain information on size and shape of the fibrinogen molecule in solution the translational diffusion coefficient (D_T), the rotational diffusion coefficients (D_{R1} and D_{R2}) and the sedimentation coefficients (S) have been measured on human fibrinogen with a clottability above 95%. The methods used were dynamic light scattering, nanosecond fluorescence depolarization and analytical ultracentrifugation. Dynamic light scattering yields $D_T = 2.0 \pm 3\% \times 10^{-7} \text{ cm}^2 \text{ sec}^{-1}$ at a concentration of 7 mg/ml in 0.15 M Tris-NaCl, pH 7.4. D_T is strongly dependent on concentration, being $3.4 \pm 10\% \times 10^{-7} \text{ cm}^2 \text{ sec}^{-1}$ at 0.1 mg/ml. The rotation along the minor axis as measured with the same method is $D_{R1} = 40'000 \text{ sec}^{-1}$ at 7 mg/ml. The rotation along the major axis as measured on fibrinogen labeled with dansylchloride is $D_{R2} = 1.5 \times 10^6 \text{ sec}^{-1}$. S is also strongly dependent on concentration, being 7.9 S at 0.1 mg/ml, 8.1 S at 1 mg/ml and 6.6 S at 10 mg/ml.

These results fit with an elongated molecule having an axial ratio of 7. They are compatible with a MW of 340'000 only for concentrations above 2 mg/ml, while at lower concentrations (0.1 mg/ml) they agree with a MW of approximately half the accepted value.

BINDING OF CALCIUM TO FIBRINOGEN : SOME RELATED PROPERTIES. G. Marguerie. Lab. Hématologie, IRF, Centre d'Etudes Nucléaires, Grenoble, France.

The calcium binding properties of bovin fibrinogen have been studied using equilibrium dialysis method. At pH 7.5 fibrinogen has 3 specific calcium binding sites of high affinity and several non specific binding sites of low affinity. Direct titration of the calcium induced proton release indicates that the binding center is a chelate. Thermal acid denaturation is found to be markedly influenced by the presence of Ca^{++} , suggesting that structural features are related to the binding. However the circular dichroism spectra show that no generalized conformational change is induced when Ca^{++} is bound to the protein.

The plasminic digestion of fibrinogen is also found to be specifically influenced by Ca^{++} . The velocity of the initial cleavages is slightly reduced in the presence of calcium. It is therefore suggested that the C-terminal part of the α chain is involved in the binding.

Considering the dimeric structure of the fibrinogen molecule, the presence of only 3 calcium binding sites of high affinity suggests the existence of "salt bridges" between the constitutive polypeptide chains.