AMINO ACID SEQUENCE STUDIES ON THE β-CHAIN OF HUMAN FIBRINOGEN. K. Watt, D. Goldbaum, B.A. Cottrell, T. Takagi and R.F. Doolittle. Department of Chemistry, University of California, San Diego, La Jolla, California U.S.A.

The β-chain of human fibrinogen contains 480 ± 15 residues, sixteen of which are methionines. In this vein, we have isolated and characterized all seventeen cyanogen bromide peptides. The arrangement of most of these fragments has been achieved by the identification of key overlap peptides derived from enzymatic digestion of β-chains, on the one hand, and a characterization of β-chain fragments isolated from fragments D and E, on the other. In some cases the alignment is based only on homologies with the α- and/or γ-chains, and in a few instances some definite ambiguities still exist. For the most part, however, the general arrangement is in hand. Of particular interest is the plasmin-sensitive segment which is a part of the interdomainal connection, which in turn we believe is a three-stranded coiled-coil punctuated by an unusual arrangement of disulfide bonds. The positioning of the other cysteine residues in the β-chain is also of considerable interest, since it sheds light on the extent of connections between portions of the three non-identical chains in the parent molecule.

AMINO ACID SEQUENCE STUDIES ON HUMAN FIBRINOGEN: ARRANGEMENT OF INTERCHAIN DISULFIDE BONDS BOUNDING THE REGIONS OF THREE-STRANDED ROPES. R.F. Doolittle, D. Goldbaum and L.R. Doolittle. Department of Chemistry, University of California, San Diego, La Jolla, California U.S.A.

Human fibrinogen contains 29 disulfide bonds, only three of which are involved in holding the two dimeric halves of the molecule together. Of the remainder, twelve others are arranged in four sets of three bonds each. Thus, each half of the molecule has two of these unusual arrangements separated by a three-stranded rope consisting of approximately 110 residues in each chain. Alignment of the three non-identical chains at the appropriate cysteine residues participating in these assemblies has revealed not only significant homology but also a rhythmic occurrence of polar and nonpolar amino acids consistent with the existence of coiled α-helices. Indeed, other workers had predicated the existence of such coiled-coils on the basis of fiber diffraction studies a generation ago, hypothesizing that they were likely interdomainal connections holding the distal portions of a Hall and Slayter-type molecule to the central region. Our data indicate that these predictions were essentially correct. To reinforce the point we have constructed a detailed molecular model of the connecting regions, even to the point where the hypothetical atomic coordinates have been recorded. The model is consistent with virtually all physical data and illuminates details of how molecular packing may occur during formation. It also delineates the geography of bond splitting during fibrinolysis, especially with regard to the boundaries of fragments D and E.

PARTIAL CHEMICAL CHARACTERIZATION OF S-CARBOXYMETHYLATED CHAINS OF RABBIT FIBRIN(OGEN): EVIDENCE FOR SEPARATE CHAIN BIOSYNTHESIS. B. Alving, G. Murano, and D. Walz. Bureau of Biologics, Bethesda, Maryland, U.S.A., and Wayne State University, Detroit, Michigan, U.S.A.

The purpose of this study was twofold: 1) chemically characterize the isolated polypeptide chains of rabbit fibrin(ogen), and 2) explore their mode of biosynthesis. The three S-carboxymethyl polypeptide chain derivatives of rabbit fibrin (α, β and γ) were isolated by cation exchange chromatography. Their amino acid composition was similar to the human with a methionine distribution (mole/mole) as follows: γ = 9; β = 14, α = 14. Their molecular size, (SDS electrophoresis) was estimated as follows: γ = 46,000; β = 54,000; α = 63,500. The N-terminal amino acid sequence (12 steps) of the β derivative was: Gly-His-Arg-Pro-Ile-Asp-Arg-Arg-Arg-Glu-Glu-Leu-. To determine whether the three chains are synthesized sequentially (one continuous chain, later split into three) or in parallel, turpentine-stimulated male New Zealand rabbits were given ∿ 40 μCi of [^{75}Se] selenomethionine (SeM) and its incorporation into fibrinogen (F) was followed. F was clotted from plasma samples, washed, reduced, and constituent chains separated by gel electrophoresis in the presence of SDS-urea. The radioactivity of each chain (expressed as percent of total F radioactivity) was determined, and the specific methionine radioactivity calculated for each chain isolated at 20, 25, and 30 min after SeM injection. During this interval the specific activity of the α and the γ chains was essentially the same (within 3%) while that of the β chain was 42 to 97% greater than that of the α chain. The similar activity of the α and γ chains during the early phase of SeM incorporation suggests that these two chains are not synthesized sequentially, rather they are synthesized in parallel.