TOPOLOGICAL STUDY OF HUMAN COMPLEMENT TERMINAL COMPLEX

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ABSTRACT

Complement terminal complex was prepared through the classical pathway of the complement fixation on sheep erythrocytes sensitized with sheep hemolysin and human serum. The complex was purified both by Sepharose-6B CL column chromatography and by density gradient centrifugation through 10-50% sucrose. The purified complex comprised of C5b, C6, C7, C8, C9 and C9 dimer in the molar ratio of 1:1:1:1:2-3:1 as calculated from densitometry of Coomassie blue-stained protein patterns.

Two-dimensional SDS-gel electrophoresis of the complex with 40 mM 2-mercaptoethanol in the second dimension produced subunit cleavages of C5b into C5b α-and β-chain, of C8α γ into C8 α-and γ-subunits and of C9 dimer into C9 monomer. In addition C5b. β-chain was found to be linked to C8α and/or C9 through disulfide bonds.

Treatment with copper-o-phenanthroline reagent, in the concentration range of 10-100 μM copper and 50-500 μM o-phenanthroline, at both 4^{o} and room temperature, could not induce additional disulfide bond formation in the complex.

Crosslinking of complement terminal complex with dithiobis succinimidyl proprionate (DSP), methyl-4-mercaptobutyrimidate (MMB) and dimethyl suberimidate (DMS) (0.2 mM at room temperature for 1 min, 5 min and 3 min, respectively) followed by analysis on 7.5% SDS-polyacrylamide gel electrophoresis indicated a decrease in the protein bands corresponding to C7, C8 and C9 dimer. In addition, decrease in

C9 band was specifically caused by DSP, in C5b by MMB and in C6 by DMS. Two-dimensional SDS-gel electrophoretic analysis of the crosslinked complex indicated that the five components, C5b, C6, C7, C8 and C9, had been partially crosslinked and had moved to the high molecular weight region of the gel.

Identification of the components of the complement terminal complex separated by SDS-polyacrylamide gel electrophoresis by means of electroblotting onto nitrocellulose paper followed by reaction with ^{125}I-labelled antibodies or FITC-conjugated antibodies revealed the presence of dimers of C6-C7, C6-C8 and C7-C8.

From this biochemical investigation of the topology of human complement terminal complex, it is suggested that the components C6, C7 and C8 are located close together, that C5b is close to C8 and/or C9 and that the C9 components are grouped together.