DISSOCIATION OF ANIMAL 7 S γ-GLOBULINS BY CLEAVAGE OF DISULFIDE BONDS

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It is yet an open question, whether the antibody molecules are composed of a single peptide chain or of more chains and by what types of bonds they are hold together. To answer this question is important for the study of the mechanism of antibody synthesis, as it can help to determine whether the whole antibody molecule is synthesized on a single template specific for each antibody, or whether a part of molecule is common for all antibodies, eventually whether the whole primary structure is the same in all antibodies and the specifity is determined only by the secondary and tertiary structure. The N-terminal amino acid analyses give different results in different animal species (Porter 1960). These results cannot be considered fully authoritative, as peptide chains with masked terminal amino groups or cyclic structures may always be present. It is evident, that the enzymatic splitting of the antibody molecule into smaller subunits, even though very successful in the investigation of antibody-combining and antibody-producing sites of the γ-globulin molecule (Porter 1959, Škvařil 1960, Nisonoff 1960), cannot answer the question of the number of peptide chains in the molecule since some peptide bonds are hydrolyzed in the course of the splitting. Disulfide bonds (intrachain or interchain) are essencially important for the native structure of antibody (Karush 1958) and by their reduction the molecules of human

γ-macroglobulin (Deutsch & Morton 1958, Jirgensons et al. 1960) as well as of human 7 S γ-globulin (Edelman 1959) are dissociated. The aim of our work was to investigate whether the cleavage of disulfide bonds using an efficient and specific method (i.e. non-proteolytic) will give rise to smaller subunits at least in some animal species.

In these experiments γ-globulins of normal animals were used. γ-globulins from five mammal sera was prepared using the rivanol precipitation as the first step and sodium sulfate precipitation of the rivanol filtrate as the second step.[x] Human γ-globulin was a commercial preparation. All γ-globulins were essentially pure by paper electrophoresis and contained only the 7 S component, no macroglobulin being present. The admixture possessing the sedimentation coefficients of 9 to 12 S was less than 5%. For the cleavage of disulfide bonds the S-sulfonation procedure in 8 M urea was used (Pechère et al. 1958). S-sulfo γ-globulins dialyzed against water or salt solutions are strongly aggregated as seen by sedimentation analyses. When freeze dried they become quite insoluble in water or salt solutions. They are soluble in solvents containing detergent or in strong urea or formamide. For sedimentation and viscosity measurement of most preparations 0.1 M phosphate buffer pH 7.0 in 6 M urea was used. Rabbit S-sulfo γ-globulin was examined in 0.1 M veronal buffer pH 8.6 with 1% sodium dodecyl sulfate, as it was not completely disaggregated in 6 M urea. For better comparison the sedimentation and viscosity analyses of γ-globulins were carried out in the same solvent as those of the corresponding S-sulfo derivative. Sedimentation coeficients were corrected to the viscosity of water and temperature of 20°C by usual way and

[x] Bovine and horse γ-globulin prepared by a very similar technique were kindly supplied by F. Škvařil from the Institute for Production of Vaccines and Sera, Prague.

extrapolated to zero concentration.

The decrease of sedimentation coeficient following the S-sulfonation is in all cases so marked (Table I), that it can-

Table I

Physico-chemical constants of γ-globulins and their S-sulfo derivatives

Species	Solvent[+)]	γ-globulin			S-sulfo γ-globulin		
		$^{o}s_{20,w}$ S	$[\eta]$ (g/100 ml)$^{-1}$	M	$^{o}s_{20,w}$ S	$[\eta]$ (g/100 ml)$^{-1}$	M
Pig	A	6.0	0.10	157,000	1.9	0.22	42,000
Cow	A	5.9	0.11	161,000	2.0	0.27	43,000
Horse	A	6.2	0.12	181,000	1.9	0.23	43,000
Man	A	6.0	0.10	157,000	1.9	0.27	46,000
Rabbit	B	6.5	0.08	159,000	3.4	0.12	74,000

[+)] A - 0.1 M phosphate buffer pH 7.0 in 6 M urea
B - 0.1 M veronal buffer pH 8.6 with 1% sodium dodecyl sulfate

not be explained by unfolding of the molecule only. In order to elucidate better the changes involved, the intrinsic viscosity of each preparation was measured and molecular weights calculated on the base of the sedimentation and viscosity data (Scheraga & Mandelkern 1953). For the shape-dependent constant β the value of 2.27×10^{6} was used. This value corresponds to the actual shape of the γ-globulin molecule in aqueous solutions and was also calculated by Scheraga & Mandelkern (1953). As the constant β is very little sensitive to the changes of shape of the molecule, only negligible error is to be expected when using the same value for γ-globulin in 6 M urea or 1% detergent. In the case of S-sulfo derivatives, in which the chains are certainly closer to the state of complete unfolding, greater error may be expected. But

considering the S-sulfo γ-globulin as a flexible chain molecule (this being an extreme case) the molecular weights of S-sulfo γ-globulins are calculated according to Mandelkern & Flory (1952) to be only 14.6% lower. Critically evaluating the above results the dissociation of γ-globulins into smaller subunits after S-sulfonation is highly significantly confirmed. As it is probable that the subunits from one molecule of γ-globulin are not identical, the calculated molecular weight must be considered as average values and no direct conclusions as to the number of peptide chains in a molecule can be drawn. The lowering of the molecular weight after cleavage of disulfide bonds appeared in several animal species regardless of the quantity and quality of the terminal amino acids. Peptide chains with masked terminal amino groups are evidently present in more cases than was known till now (Putnam 1953, Press & Porter 1960).

The presented results suggest, that the multi-chained structure is a general property of γ-globulins. Fractionation of S-sulfo γ-globulins, chemical analysis of the subunits and investigation of biological activities of individual chains of purified antibodies will further elucidate the structure of antibodies and their relation to unusual types of γ-globulin.

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