SOME PHYSICO-CHEMICAL PROPERTIES OF CONNECTIVE TISSUE PROTEINS

All kinds of connective tissue, as well as organs with latter as a component, contain collagen. In relation to the function and location of organs collagen content in them varies greatly - from 20-30% in tendons and skins to 0,5-2% in hearts, livers, muscles (% of the organ weight) /1/.

Connective tissue is important for the viability of organisms; in addition, being collagen accumulator, it is of interest for the technical utilization of this protein in the production of leather, gelatine, glue, catgut, sausage casings and a number of artificial collagenous materials.

In the recent years, due to extensive studies of collagen, various sciences, e.g., biochemistry, technology, medicine, biology, biophysics, veterinary science, etc., paid great attention to the aspect concerning the causes of so-called "collagenous deseases", or "collagenesis". In the normal connective tissue there is a certain relationship among fibrillar structures, cells and the main substance. Changes in this relationship cause deseases of the connective tissue /2, 3/, which are based upon developing processes of collagen structures disorganization. These changes are usually conversions of the connective tissue, rich with fibrous structures, into the one in which cellular elements prevail.

In cases of other pathological changes of the connective tissue, a reverse phenomenon can be observed, viz., decreases in the main substance content, the dehydration and infiltration of the fibrillar structures, a partial loss of tissue elasticity and extensibility /3/. Similar consequences result from organism ageing accompanied with changes in the connective tissue. The processes occuring in cases of the above abnormalities in the connective tissue are, obviously, determined with the stability of collagen highly-ordered structure at the molecular level and depend upon the formation and the availability of intra- and intermolecular bonds and upon their changes under various factors.

Earlier, data were presented on the changes of the structure of certain connective tissue in the preparation of collagen solubilization products and on the reconstitution of artificial materials from them /4, 5, 6/.

Investigations of some aspects of the physico-chemical properties of collagen, the main protein of the connective tissue, in vitro facilitate greatly studies into the processes which take place in collagen in vivo.

Many studies in the recent years were concerned with connective tissue physico-chemical properties. E.g., procollagen and hydroxyproline contents in animal hides under different physiological and pathological conditions were studied, as well as the possibility of including radioactive glycine into procollagen and collagen of healthy and scurvied animals /3, 7/; procollagen molecular weight and other characteristics were determined /7, 8/. Molecular weights of collagen-like proteins, determined with different methods, vary rather greatly. Procollagen molecular weight as found by Bresler et al. /8/ in 1950 was 70,000. Orekhovitch and Shpikiter /7/, studying procollagen sedimentation and viscosity, calculated its molecular weight as being about 700,000; Peng and Tsao /9/ obtained the value 400,000, and Boedtker and Doty /10/ - 350,000.

Along with the determination of the molecular weight, of great interest are studies into certain physico-chemical characteristics of collagen solubilization products: the degree of their homogeneity, the possibility of their fractionation, the molecular weight of individual fractions and their electrophoretic mobility. The objects of the studies were purified products of collagen solubilization (C.S.P.) of cattle tendons. C.S.P. were obtained as a result of consecutive alkali-salt and acid treatments of tendons until a homogeneous, highly viscous solution with the concentration of 0.8-1% (by protein) was prepared /4/.

Tendons C.S.P. molecular weight and homogeneity were studied on chromatographic columns packed with Gel-Cephadex G-200 and Cepharose 2B. Protein solutions for the tests were prepared as follows: 2 g of dry collagen (freeze-dried from C.S.P.) were added to 200 ml of 8 M urea solution prepared with tris-buffer (pH 12.5). After centrifugation the protein was precipitated with

acetone or a saturated solution of sodium sulfate, and the precipitate was dialyzed against distilled water. The dialyzed precipitate was solved in 100 ml of 0.05 N KOH (pH 12.5). For elution, 5 M area solution with pH 12.5 in the 2.4x5.6 column with Cephadex G-200 (superfine) was used.

The protein solution was applied in the quantity of 3 ml. Flow rate was 3 ml/hr. C.S.P. chromatographic data indicate that cattle tendon collagen is not homogeneous by its structure and contains about 6 fractions having molecular weights of 3.0×10^5 (the 1st fraction) to $0,8 \times 10^5$ (the 6th fraction). These fractions have the following molecular weights: the 1st fraction - 3.0×10^5; the 2nd fraction - 1.9×10^5; the 3rd fraction - 1.7×10^5; the 4th fraction - 1.3×10^5; the 5th fraction - 1.1×10^5; the 6th fraction - $0,8 \times 10^5$.

By the chromatographic data it can be calculated that the product tested contains, mainly, three big fractions with the average molecular weight $(3.0 \pm 0,5) \times 10^5$; $(1.8 \pm 0.3) \times 10^5$ and $(1.0 \pm 0.2) \times 10^5$.

The availability of three fractions in C.S.P. can be explained by rather severe treatment of collagen-containing raw materials, which results in the mixture of collagen loosening products. Such a state is possible in the processes providing selective splitting of cross- and, in some cases, of longitudinal links in collagen structure, that is observed in the preparation of C.S.P. These data agree with the three-helix structure of collagen macromolecule and with solubilization of α, β and γ-components under certain conditions /11/.

Then, we studied chromatographic separation of this protein on the 2.4x5.6 column with Cepharose 2B. Elution was carried oit under milder conditions (carbonate buffer with pH 8,6 and flow rate of 15 ml/hr). On Cepharose 2B (it can separate proteins with the molecular weight up to 20,000,000) tendon collagen yields four main fractions with the following molecular weights: the 1st one - 12.0×10^5; the 2nd one - 1.5×10^5; the 3rd one - 1.3×10^5; the 4th one - 0.2×10^5. The difference in the number of fractions and in the molecular weights between columns filled with Cephadex G-200 and Cepharose 2B can be explained with the use of different eluating solutions having different pH-values.

An increased number of fractions of much lower molecular weights on Cephadex G-200 can be approximately explained with urea presence in the eluate, which is known to be capable of breaking intermolecular hydrogen bonds in multicomponent proteins.

To study collagen protein electrophoretic mobility, the method of disc electrophoresis in 4% polyacrylamide gel prepared with 8 M urea (the pH-values of the upper and the lower buffers with 8 M urea being adjusted to 9) was used. Electrophoresis lasted for 2 hours, with the resistance of 5 ma/2 ml gel and with the voltage of 200 v.

Collagen protein was found to be heterogeneous in electrophoretic mobility and to yield 4 fractions having different mobility. We performed electrophoresis of collagen without urea as well, and established that the number of phoretic fractions did not change and that each fraction had a similar electrophoretic mobility.

The data obtained confirm once again our suggestions that C.S.P. are polymolecular solutions which, obviously, contain macromolecules and aggregates thereof, as well as splinters of fine structure of collagen with a broad range of molecular weights. C.S.P. heterogeneity depends on the methods and materials used, or on selective breakage of intermolecular bonds in collagen structure.

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