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METHOD OF PRODUCING SOLUBLE COLLAGEN

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This invention relates to a process for depolymerizing the collagen fibril, and to the soluble collagen monomers that result from the depolymerization process.

Collagen is an important structural protein and occurs in many tissues of the animal body. Depending on the kind of tissue and the age of the animal, some collagen may be extracted using organic acids in the pH range 2 to 4.5. However, the majority of the collagen, particularly in the older animals, will not be extracted under these conditions and is referred to as acid-insoluble collagen.

The fundamental biochemical unit of collagen is the fibril. Fibrils have diameters ranging roughly from 50 A. to 1000 A. The lengths of the fibrils are indeterminant but are usually at least several hundred times that of the diameters. The fibril is considered to be built-up from a fundamental building block called tropocollagen. Tropocollagen is considered to be a rod-like particle of about 3000 A. in length and 15 A. in width and to have a molecular weight of about 300,000. This fundamental unit may be considered to be a monomer unit and the fibril may be considered to be a polymeric form of this unit.

The monomer units forming the fibril are linked together by several types of forces. Some of these forces are relatively weak such as hydrogen bonds and electrostatic charges. A fibril held together by such forces may be put into solution with cold dilute saline or solutions of organic acids since such solutions may rupture these relatively weak bonds. The result is a solution of the monomer units or of simple aggregates of the monomer units of the fibril and the disappearance of the fibril as an entity.

If the fibril is linked together with stronger forces such as covalent bonds (in addition to the weaker forces discussed above), it will not dissolve in solutions of dilute organic acids and is referred to as acid-insoluble collagen. While such a fibril will swell to several times its diameter by the imbibition of fluid and form viscous dispersions, it will not lose its identity. In contrast to soluble collagen these fibrils even when swollen may be readily centrifuged from a dispersion at moderate speeds (4000 r.p.m.) in the centrifuge and may be readily visualized in the phase contrast microscope.

Tissues such as hide or tendon, especially in adult animals, are composed largely of fibrils held together or cross-linked by the stronger, covalent type of bonds. When such tissues are swelled in dilute organic acids and mechanically finely subdivided, a dispersion of swollen fibrils rather than a solution of collagen is obtained.

It is a feature of the present invention to convert such dispersions to solutions of collagen.

I have discovered that the treatment of the swollen fibrils with certain enzymes results in the formation of soluble collagen.

In the process of the present invention collagenous tissues such as skin or tendons are cleaned mechanically and washed free of dirt and adhering noncollagenous tissues. The tissues are finely divided and extracted successively with 0.1 M disodium phosphate and 10% NaCl to further remove noncollagenous proteins and mucopolysaccharides. After washing out the salt the finely divided tissues are swollen in a dilute organic acid and further dispersed while in the swollen state. The resulting dispersion of fibrils (about 0.1% solids) is strained through a coarse filter such as cheesecloth to remove particles which are only partially swollen by the acid. The purified dispersion

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of fibrils may now be exposed to enzymic attack or the acid swollen collagen fibrils may be precipitated, collected and reswollen in dilute acid solution to a higher concentration, usually .2 to .3%. The above processes are done in the cold to avoid the possibility of denaturing the collagen.

It is essential that the fibril be in a swollen state prior to enzyme treatment. Acetic acid at a concentration of 0.3% by volume is a convenient dispersing medium giving a final pH of 3.5. Other organic acid swelling agents such as lactic, malonic or cyanoacetic acid may also be used. The pH of the acid swelling solution is in the range of 2 to 4.5. Above pH 4.5 the fibril is no longer in a suitably swollen state. The pH per se of the dispersion is not so important for enzymic action as is the swollen state of the fibrils achieved through the lowered pH. If the fibrils could be swollen sufficiently at neutral or alkaline pH values, it is believed that the depolymerization would still take place.

The temperature at which the enzymic depolymerization occurs is also important. This may range from 0 to 30° C. but preferably it should be at about 25° C. Mammalian collagen fibrils swollen in acid medium undergo a modification at about 33° C. thereby becoming readily attacked by proteolytic enzymes. This attack does not involve a depolymerization of the fibril but it is a proteolysis degrading the collagen to amino acids and peptides. Since the enzymes concerned with depolymerization also have proteolytic activity, the control of temperature becomes of great importance in this process.

The salt concentration of the collagen dispersion also has an effect on the enzymic depolymerization through its effect on the degree of swelling of the fibril. For example, in 2 M NaCl the swelling of the fibril is completely inhibited and no depolymerization takes place even under conditions otherwise suitable for the reaction.

Enzymes suitable for depolymerization of the swollen collagen fibril are the elastases such as pepsin and particularly the sulfhydryl containing enzymes such as papain, bromelain, pinguinain and ficin.

Enzyme concentration may range from .001 to 1%. The speed of the enzymic depolymerization depends on a number of factors including the type of fibrils. In general, fibrils from hide depolymerize more readily than those from tendon and fibrils from young animals depolymerize more readily than those from older animals. The enzyme to substrate ratio also is of some importance. If the ratio of enzyme to substrate (fibril) is sufficiently large, the fibril may be deswollen and the depolymerization actually inhibited.

While the mechanism of the depolymerization of the collagen fibril is not known, it is thought that the acid in swelling the fibril opens it to enzymic penetration and exposes certain sites involved in cross-linking the monomers. It is these cross-links that are believed to prevent the solubilization of the fibril and maintain its integrity. By permitting the enzymes to penetrate the fibril and attack the exposed sites, the collagen fibril may be broken down to its monomer components which are soluble.

It will be understood that the foregoing general description and the following detailed description as well as explanatory and exemplary but do not restrict the invention. The process for the depolymerization of swollen collagen fibrils and the nature of the product obtained will be more fully understood from the following detailed description and examples.

Example I

A single flexor tendon from the leg of a steer is mechanically trimmed of fat and fascia, frozen and sliced on the meat slicer. The slices 2.5 g. (wet weight) are extracted overnight with cold 0.1 M Na₂HPO₄. The slices are then