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Collagen: Medical and Surgical Applications

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Collagen: Medical and Surgical Applications*,†

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INTRODUCTION

Collagen is found in biologic systems as a supporting material for a wide variety of cells and membranes. Its fibrous nature is well suited to biologic plasticity in both strength and form. Its natural occurrence as the primary structural protein of animals suggests that it might be used as a structural element in "synthetic" biomaterials. The molecular nature of collagen and its biologic reactivity must be understood, however, before collagen biomaterials can be designed and fabricated. Over the past 20 years, a broad and detailed account of the molecular biology of collagen has become available. The present report will review some of this material, with special reference to those aspects important to the use of collagen as a biomaterial, and illustrate some of the uses that these materials can serve.

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STRUCTURE OF COLLAGEN

The basic molecular unit of collagen is a triple helix [1] composed of three similar, but not identical, polypeptide chains. This fundamental molecular unit, termed tropocollagen (TC), is a rigid rod about 2800 Å long, with a diameter of about 14 Å, and has a molecular weight of about 300, 000 [2-4]. The three subunit polypeptide chains of TC are held in their helical configuration by hydrogen bonds and packed into polyproline-type helixes where every third residue is glycine [5]. Covalent bonds also exist, however, between individual polypeptide chains, accounting for a variability in collagen structure after denaturation. TC molecules occur in nature bonded together in bundles, or fibrils, with a characteristic quarter-stagger array. This quarter-stagger configuration of the molecules gives rise to a 700 Å axial periodicity of native collagen fibrils when visualized in the electron microscope after staining with phosphotungstic acid.

The intramolecular covalent bonds that exist between individual polypeptide strands of TC have been the subject of intense investigation. When collagen is denatured and separated on sephadex columns, or in the ultracentrifuge, a variety of subunits may be obtained, rather than the three that would be predicted on the basis of a triple-stranded molecule. The subunits can be either three strands of similar molecular weight (α-chains), two polypeptide strands linked together (β-chains), or all three covalently bonded (γ-chains). There are at least two different α-chains, accounting for a further heterogenicity in β-chains. Vertebrate collagen lacks cysteine, so the intramolecular bonds cannot be accounted for by disulfide linkages.

Proteases, other than collagenase, effect the intramolecular (as well as the intermolecular) cross-links of collagen, without destroying the body of the collagen triple helix. End regions of collagen, which are different from the major portion of the helix in that they contain relatively little hydroxyproline and are relatively rich in tyrosine, appear to be the areas attacked by proteases and appear to be important areas for the cross-linking properties of collagen [6]. Studies by Rubin et al. [7] indicated that proteolytic enzymes (a) release peptides from collagen, (b) do not alter the triple helical configuration of the molecule, (c) interfere with the intramolecular bonding in that β-chains tend to become α-like, and (d) interfere with the intermolecular bonding in that the viscosity of enzyme-treated collagen solutions does not increase like that of native collagen on changes of ionic strength or pH. These end regions, or telopeptides, are probably near the N terminal portion of the molecule and, since they do not contain glycine as every third residue, cannot be packed in a helix but are probably randomly coiled. The resultant exposure of polypeptide linkages probably accounts for the increased sensitivity to proteolytic enzymes in this region.

The chemical nature of the intramolecular cross-links is still being investigated, but evidence points toward aldol-type condensations of two lysine-derived aldehydes on adjacent chains [8, 9].

Denaturation of collagen occurs by heating to between 37° and 42°C depending on pH and ionic environment, and results in the formation of gelatin. The loss of helical conformation is reflected in a loss of the highly negative optical rotation of collagen and concomitant decrease in viscosity.

IMMUNOCHEMISTRY OF COLLAGEN

Collagen is a poor antigen, but antibodies that are produced against TC have many combining sites specific for the telopeptide region [10]. Thus, treatment of TC with proteases results in a loss of complement fixing activity with anti-TC serum. Nevertheless, antibodies can be made in rabbits to enzyme-treated collagen. These antibodies appear to be directed against the helical portion of the molecule. Antibodies specific for native TC are species specific [11], but the cross-reactivity of enzyme-treated TC has not yet been determined.

APPLICATION TO MEDICINE AND SURGERY

The picture of collagen that emerges from these studies is that of a fibrous protein with a biologically important end region. The end region can be removed by proteolytic attack and accounts for important reactive and antigenic sites on the molecule. By treating animal hides and skins with proteolytic enzymes, large amounts of collagen can be solubilized, purified, and antigenically altered at the same time. This enzyme-solubilized collagen is the raw material for a variety of medically useful materials. The physical states in which collagen may be useful medically are as follows: (a) as a gel or viscous fluid for vitreous body replacement and coating of dacron-woven vessel prostheses, (b) as clear films for corneal replacements, (c) as a membrane for artificial kidneys, membrane oxygenators, blood vessel prostheses, or coverings for burns, (d) as filaments for weaving blood vessel and heart valve prostheses, and (e) as a coascervate for microscapsules which could be used in a variety of ways.

Collagen Gels and Solutions

Enzyme-solubilized collagen is soluble in acidic solutions of low ionic strength, but collagen fiber tends to precipitate out with increasing pH, increasing ionic strength, and increasing temperature.

One of the important medical uses of collagen is as a vitreous replacement in the eye for both diseased vitreouses and also to increase intraocular pressure to treat detached retinas. To be of use, the collagen must therefore remain clear and viscous in a physiologic environment with a relatively high salt content, a pH of 7.4, and a temperature perhaps as high as 40°C. Two approaches have been used to solve this problem: first, reacting collagen with ascorbic acid and second, irradiating collagen with UV light. The latter approach appears to be the most promising.

Collagen and ascorbic acid interact and result in a decrease in fiber formation in salt solutions, as temperature increases. However, partial denaturation of collagen may occur at ascorbic acid concentrations above 30 mg %, and the melting temperature is decreased at even lower concentrations. The problem of clarity was solved by this maneuver, but not the problem of stability at elevated temperatures. Collagen-ascorbic acid complexes have been placed in animal eyes and they remain clear and are nonreactive.

Ultraviolet irradiation of collagen results in the appearance of free radicals. If oxygen is excluded from the system, collagen molecules interact and form a random molecular mesh which is clear and becomes a gel. The characteristics of the gel can be altered by the duration of UV irradiation and also by the solvent system. By appropriate manipulation of these factors [12] a stable, clear gel is obtained which is completely nonreactive when placed in animal eyes and hopefully will be useful in treating a variety of ocular diseases.

Collagen Films

One of the major causes of blindness is corneal disease, and we have naturally wondered if collagen could be used as a corneal replacement. Clear collagen films may be cast on polyethylene plates and cross-linked with UV irradiation. Again, if oxygen is excluded, the cross-links are very firm and allow the material to be used surgically. Dr. Michael Dunn of the Department of Surgery (Ophthalmology) at Cornell Medical Center has placed such clear films intralamellarly in rabbit corneas. [13]. These have remained clear and without inflammatory changes for over a year and a half. Examinations of stained sections of such grafts by Dr. A. Whitley Branwood at Cornell have shown that the grafts do not become replaced by scar tissue but rather by what appears to be normal corneal stroma. The clarity of the material, its lack of tissue reaction, and the possibility that it becomes replaced by normal corneal tissue all indicate that it may be useful in corneal grafting.

Collagen Membranes

Mixtures of collagen solution and collagen fiber can be cast into membrane form by extruding through a double rotating annular noz-

zle into a coagulating bath. The membranes that are formed can be cross-linked with UV irradiation. The strength and permeability of the membranes depend upon their thickness and degree of cross-linking. Thickness is controlled by the per cent of collagen in the extruding mixture and the ratio of soluble to fibrous collagen, and cross-linking is controlled by the duration of UV irradiation [14]. By altering these parameters, membranes of a wide variety of strengths and permeabilities can be obtained. Their versatility is enhanced further by the presence of many free acidic and basic groups on the membranes available either for blocking, to result in a membrane with only a positive or negative charge, or for reaction with other compounds (lipids, heparin, antibiotics, etc.). Such membranes have been used to dialyze dogs and rabbits. The animals have not experienced adverse reactions and membranes have been prepared which have better creatinine and urea permeabilities than do cellophane membranes. Preparations for using such membranes in clinical hemodialysis units are now under way.

Membranes may also be structured into tubular form by wrapping the membranes around Teflon rods, gluing together with collagen, and cross-linking with UV irradiation. These tubes are very difficult to work with surgically, but Dr. Lande and Dr. Subramanian at Cornell have been able to suture such tubes into femoral arteries and have simply hung the tubes in the vena cava or atrium of dogs. The arterial grafts have not lasted more than 4 or 5 days because of clotting at the anastomosis site. The collagen surfaces, however, were free of clots. In fact, studies done by Dr. Nachman at Cornell revealed only minimal platelet clumping on collagen membranes and no platelet damage.

Collagen Filaments

Woven grafts will probably be more useful for vessel replacements than will collagen membrane wrapped in tubular form. Collagen can be spun into filaments and cross-linked by chrome tanning or by UV irradiation. The filaments can then be knitted into the desired design. The advantages of this material as a vascular prosthesis include the freedom of collagen surfaces from clot formation and the ease with which cells grow on these surfaces.

Collagen Coacervates

Gelatin, or heat-denatured collagen, can become a coacervate under suitable conditions. The resultant microcapsules can be cross-linked, again by either UV irradiation or by chrome tanning, and in the process biologically important materials can be incorporated into the coacervate and subsequently into the microcapsules. The same techniques may be applicable to collagen and may permit prolonged circulation of such capsules containing enzymes or hemoglobin.

DISCUSSION

Understanding of the molecular biology of collagen has suggested many applications of this protein to problems of biomaterials in medicine. Collagen has a very low order of antigenicity and thus can be implanted without significant danger of later rejection. It can be cross-linked in many ways, but most significantly by physical means such as UV irradiation. This technique is effective and precludes the introduction of toxic substituents. The amino acid structure of collagen provides many reactive amino acid and carboxyl groups which are available for coupling with other agents. This property, in fact, suggests a new area for biomaterial development—the coupling of synthetic polymers to natural materials such as collagen. Such processes should strengthen collagen and, if the protein retains its desirable attributes, should open the way for many more uses in biology and medicine.

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