3.6 THE KINETICS OF ENZYMATIC DEGRADATION AND THE STRUCTURE OF PROTEINPOLYSACCHARIDE COMPLEXES OF CARTILAGE

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The structure of proteinpolysaccharide complexes of cartilage and the problem of the arrangement of chondroitin sulphate and protein have been investigated by several workers. Results of physical investigation on the size and shape of the macromolecule support the random coil configuration (Webber and Bayley, 1956; Bernardi, 1957a,b); in the models proposed by Mathews and Lozaityte (1958) and by Partridge, Davis and Adair (1961) several chondroitin sulphate residues are linked to a protein core. The last group of workers obtained evidence of the model from the study of the products of enzymatic degradation of the complex. Webber and Bayley (1956), on the other hand, had suggested an end-to-end arrangement of the polysaccharide and protein chains.

In the present work we have repeated some physical measurements on a new preparation of proteinpolysaccharide and studied the kinetics of enzymatic degradation of the macromolecule. This method should distinguish between the possible models and should give additional information on the arrangement of the two moieties. It has been demonstrated (Mark and Tobolsky, 1950) that when a linear polymer is randomly degraded $1/M_n$ is a linear function of time. Charlesby (1954) extended the demonstration to the case of M_w. The rate of change of molecular weight versus number of split bonds depends upon the structure of the polymer. The case of proteinpolysaccharide offers the advantage that two types of bonds can be broken by mucolytic or proteolytic enzymes. The two models considered are expected to behave similarly when digested by proteolytic enzymes, every break splitting the chain into fragments of comparable mass randomly distributed. The enzymatic attack on polysaccharide units on the other hand is expected to give different rates of decay of molecular weight. Hyaluronidase would split only fragments of much smaller size than that of the parent molecule having a structure like that represented by model 1 (*Figure 1*) and M_n would drop slowly. The same enzyme would degrade the complex represented by model 2 at a faster rate, the effect of the attack being analogous to that of papain. Assuming a given number of residues and molecular weights for polysaccharide and protein, the kinetics can be calculated for both models. An example of such a calculation is shown in *Figure 2*.

Experiments were carried out measuring the rate of change of M_w by light scattering and checking the linearity of activity of the enzyme by titration of broken bonds. Proteinpolysaccharide was prepared from nasal septa of cattle according to Muir (1958), and the properties of the product are summarized in *Table 1*. The light scattering apparatus described by Wippler and Scheibling (1954) was used. The material (0·01 per cent, w/v) was dissolved in 0·015 M acetate buffer pH 4·4 containing 0·15 M NaCl. A portion of 30 ml was made free from dust by centrifuging and poured into

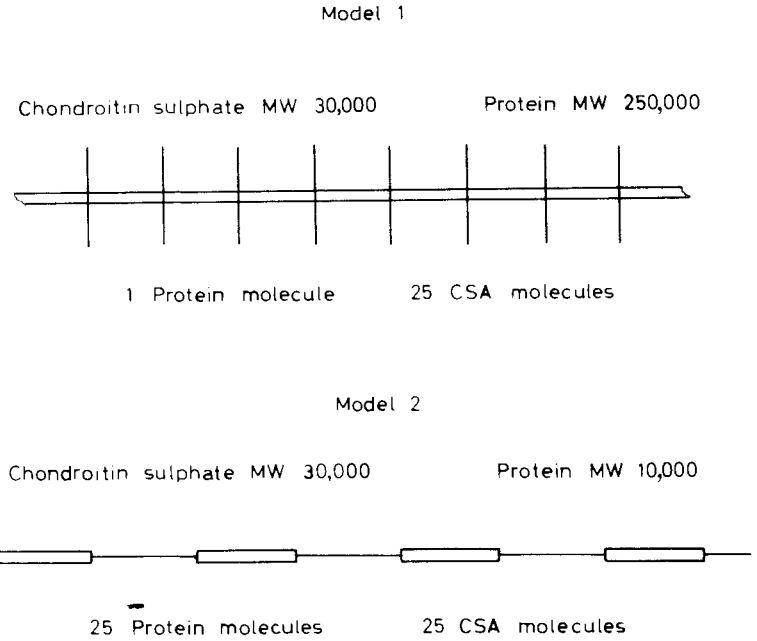

Figure 1. Comb-like and end-to-end models of proteinpolysaccharide. Values for molecular weights and number of chains are arbitrarily chosen for purpose of calculation of kinetics of degradation of *Figure 2*

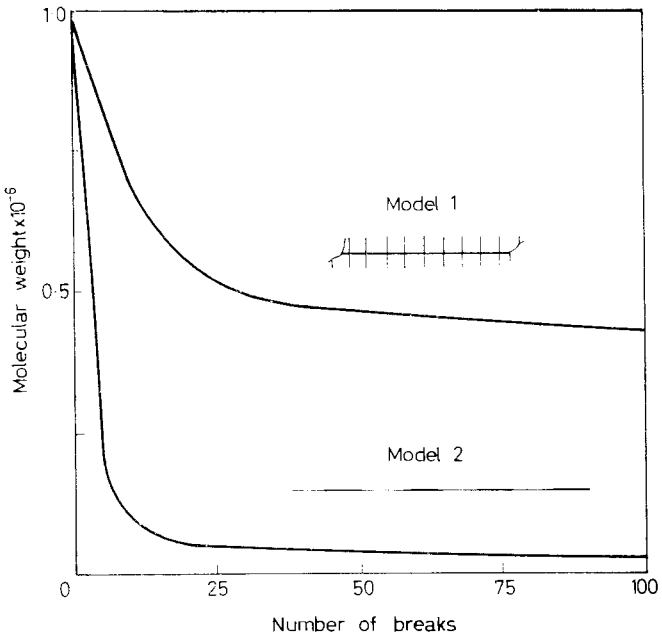

Figure 2. Kinetics of degradation of different models of proteinpolysaccharide by hyaluronidase

the cell of the light-scattering assembly. After determination of initial M_w, 0·2 ml solution was added containing an amount of testicular hyaluronidase (Worthington) or papain sufficient to obtain maximum degradation in about 5 hours; the decay of M_w was followed. From a separate volume of the same incubation mixture, measured amounts were delivered at different times for the estimation of reducing terminals in the case of hyaluronidase digestion or $-NH_2$ terminals in the case of papain. Reducing power was estimated by a modification of the alkaline ferricyanide method; in this modification the amount of ferrocyanide produced was estimated by ultraviolet photometry. It was found that $E_{240\ m\mu}-E_{281\ m\mu}$ is proportional to the concentration of

Table 1. Properties of proteinpolysaccharide

Galactosamine	23·1 per cent
Protein	14·1 per cent
Hydroxyproline	<0·2 per cent
M_w (light scattering)	2·2 × 10^6
Sedimentation coefficient	4·0*s*
$[\eta]$	1·2 dl/g[a]

[a] Viscosity was determined by the four bulbs viscometer of Eigner (1960) at velocity gradient ranging from 30 to 100 sec^{-1}. No gradient dependence was found.

glucose in the range 1 to 10 μg, 281 mμ being the isosbestic point of ferri- and ferrocyanide and 240 mμ a point where extinction of ferrocyanide is high and that of ferricyanide is the same as at 281 mμ. Although the method does not overcome the limitations due to the alkaline medium, it gives more reproducible results than methods based on development of blue colour with ferric ion and was suitable in the presence of polysaccharides and proteinpolysaccharides. Amino groups were estimated by the fluoriodinitrobenzene method according to Sanger (1945) on 10 ml portions. The amount of bound dinitrophenol was estimated either by photometry or by the use of ^{14}C–FDNB and determination of radioactivity. In some instances the proteinpolysaccharide was precipitated with ethanol and reaction with FDNB carried out on both precipitate and supernatant.

The values observed for M_w, sedimentation and viscosity support the random coil model. When the specimen was digested by hyaluronidase the 200 reducing groups approximately present per molecule before digestion rose linearly with time (see lower line of Figure 3). By the time a 100 bonds were broken, M_w had decreased to only 75 per cent of the initial weight (upper line on *Figure 3*). About 100 $-NH_2$ terminals per molecule were found in our preparation. Agreement between photometric and radiochemical determination was within 2 per cent. When the number was increased approximately 10 per cent by papain digestion, molecular weight became half the initial value (*Figure 4*). A preliminary attempt to measure the number of DNP groups in the fraction precipitated with 60 per cent ethanol (v/v) showed that this fraction did not undergo many changes in the course of proteolytic hydrolysis and the free amino group produced during breakdown were concentrated in a fraction found in the supernatant.

The high rate of decrease of molecular weight of proteinpolysaccharide on treatment by papain and the slow rate upon hyaluronidase hydrolysis support the comblike structural model with a central core of protein. The

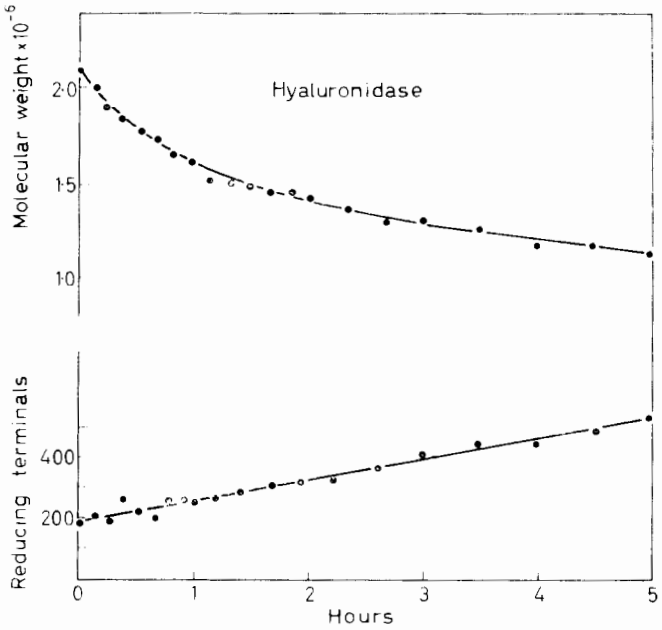

Figure 3. Kinetics of degradation of proteinpolysaccharide by hyaluronidase. Upper curve: decrease of molecular weights as a function of time. Lower curve: number of reducing terminals made available

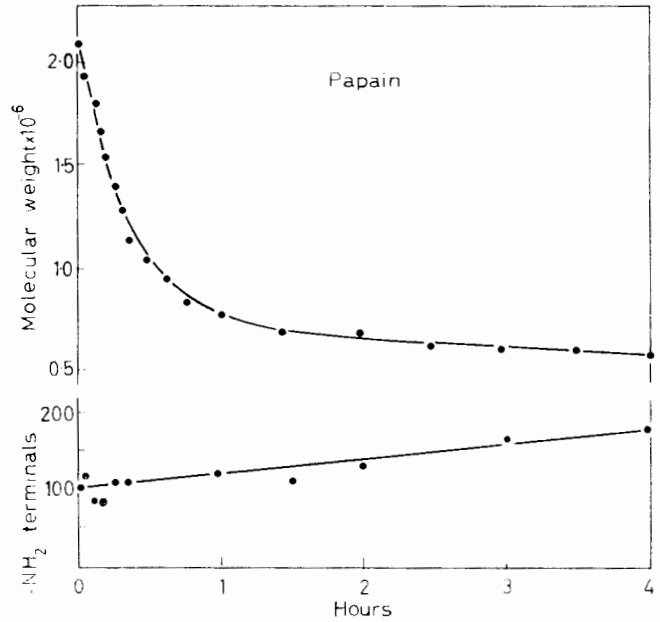

Figure 4. Kinetics of degradation of proteinpolysaccharide by papain. Upper curve: decrease of molecular weights as a function of time. Lower curve: number of $-NH_2$ terminals made available

possibility of separation of fragments with different contents of free amino groups requires further investigation since more details on the structure of the complex can be obtained by this type of analysis.

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3.7 MACROMOLECULAR ORDER IN THE GROUND SUBSTANCE

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The idea of 'multispecied macromolecular assemblies' as distinct hierarchical entities in cellular organization (Schmitt, 1963) may be extremely pertinent when considering the structural conformation of the ground substance. Many studies have emphasized the diversity of the constituents of the ground substance in different tissues, but little is known about the structural order of the molecular units. It is the purpose of this paper to discuss the evidence in support of the idea that several different macromolecules and other smaller molecules may be associated in specific three-dimensional arrays to form multispecied macromolecular assemblies as an integral part of the ground substance. The properties and configuration of such assemblies would depend in part on (*i*) the specificity of the individual components, such specificity being determined by their primary structure, (*ii*) the mode of linkage between two dissimilar molecules to form one coherent unit, (*iii*) the three-dimensional interrelationship between the various composite units one