Pore Size Distribution in Collagen Fiber Using Water Vapor Adsorption Studies

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The pore size distribution in collagen fibers was determined using the sorption characteristics of water vapor. Collagen fibers were found to have pores of radius ~55 Å and micropore analysis showed that collagen has micropores of radius ~4.5 Å. When the noncollagenous components were removed, the ~55 Å radius pore was absent and there was a new pore of radius ~75 Å. Neither the removal of noncollagenous components nor tanning caused any change in the micropore structure.

INTRODUCTION AND THEORY

Moisture sorption characteristics of collagen fiber were studied by plotting a graph of the percentage of moisture content along the Y-axis and relative humidity along the X-axis (1, 2). The results obtained were analyzed (3–6) using the BET equation. The role played by the polar groups in the sorption behavior of proteins in general was dealt with by Pauling (7) and of polypeptides by Mellon (8) and Rao (5). In light of the recent suggestion made by Brunauer *et al.* (9), it was thought that it would be interesting to study the pore size distribution in collagen fibers using its moisture sorption characteristics.

In the evaluation of the pore size distribution, it is assumed that the pores are cylindrical in shape and capillary condensation occurs in the pores according to Kelvin's equation

$$r_k = -\frac{2.\sigma \cdot V \cdot \cos\theta}{R.T. \ln p/p_0}$$

where σ = surface tension; V = molar volume of the condensing vapor; θ = angle of contact of the liquid with the wall; r_k = radius of the pore; p/p_0 = relative humidity expressed in decimal.

Kelvins' radius of the pore denotes the radius of the pore minus the thickness of the adsorbed film of the water vapor. The thickness of the film at different relative humidities can be calculated from the values of the number of adsorbed layers given by Brunauer *et al.* (9) and the thickness of each adsorbed layer given by McClellen and Harnsberger (10). The pore size distributions were evaluated using the BJH method (11), and the desorption branch of the sorption isotherm curve was used for the calculation. For computation of the micropores the "MP-method" suggested by Brunauer *et al.* (12) was employed, using the adsorption branch of the moisture sorption isotherm. The total surface areas obtained by the BJH-method (up to radius ~20 Å) and the MP-method were added up and compared with the surface area obtained from the BET equation.

EXPERIMENTAL

Collagen fibers were removed from a freshly slaughtered cow hide belly portion. They were

TABLE I

Computation of Mesopores Using the BJH Equation for Control and Enzyme Treated Collagen

p/p0	% of water adsorbed		t[a] (Å)	r_p[b]	V_p (ml)[c]		A_p[d] (m²/100 g)		BET surface area
	Control	Enzyme treated			Control	Enzyme treated	Control	Enzyme treated	
0.95	57.5	51.1	12.225	185.8	8.4				
0.93	50.0	45.0	11.100			6.8	904	731	Control = 262 m²/gm
0.90	44.5	37.5	9.900	132.4	6.2	8.5	1841	2060	
0.85	37.0	30.5	8.550	91.0	8.7	8.0	3753	3818	
0.80	31.0	27.5	7.650	63.7	7.2	3.4	6023	4891	Enzyme treated = 245 m²/g
0.75	27.0	25.3	6.750	48.8	4.6	2.4	7908	5875	
0.70	24.5	23.0	6.375	39.5	3.1	3.0	9502	7393	
0.65	22.0	21.5	5.925	33.0	3.1	1.7	11380	8423	
0.60	20.5	20.0	5.475	28.3	1.6	1.7	12475	9624	Tanned (Myrab and chrome)
0.55	19.0	18.5	5.025	24.5	1.5	1.7	13699	11011	
0.50	17.0	17.0	4.650	21.2	2.5	1.8	14868	12709	375 m²/g

[a] t = thickness of the layers on the pore walls computed from (9, 10).

[b] r_p = Radius of the pore, = Kelvins radius of the pore + t.

$$r_p = \frac{(r_p)_1 + (r_p)_2}{2}.$$

[c] V_p = volume of the pore.

[d] A_p = area of the pore walls.

thoroughly washed in distilled water. The fibers were preserved in analar acetone at 4°C. One group of these fibers was taken for the enzyme treatment to remove the noncollagenous components. Nishihara enzyme at 4°C, pH 5.3, and at an enzyme substrate ratio of 1:300 was used for this purpose for a period of 96 hr (13). Both groups of fibers were tanned with myrab, wattle, chrome, syntan, formaldehyde, and aluminum sulfate using the procedure of Mohanaradhakrishnan and Ramanathan (14).

The atmosphere inside several desiccators was conditioned at different relative humidities from 0 to 95% at 25°C ±2 temperature, using P_2O_5 and various salt solutions. Small collagen fiber bundles were placed in small weighing bottles and conditioned at 0% relative humidity. After 48 hr, the desiccator was opened and the weighing bottle was immediately closed. The weight of the bottle with the fiber bundle was quickly determined. After weighing, the fiber bundles were conditioned at the same relative humidity and after 24 hr the weight was once again determined. The experiment was repeated until two consecutive readings did not vary by more than 1%. Following the same procedure, the weight of the fiber bundle was found at different relative humidity values up to 95%. The weights were also determined at various relative humidity values decreasing from 95% to 0%. Since the approximate weight was already known, the weighing could be done in a very short time and thereby small errors that could occur due to the opening of the desiccator could be minimized. The weight at 0% relative humidity was taken as the dry weight of the sample.

TABLE II

Computation of Micropores Using the MP Method for Control and Enzyme Treated Collagen

t (Å)	S (m^2/g)	Si–Si + 1 (m^2/g)	Mean r_h (Å)	Vi (ml/g)
3.5	320	—	—	—
4.0	250	70	3.75	0.0263
4.5	200	50	4.25	0.0213
5.0	160	40	4.75	0.0190
5.5	150	10	5.25	0.0050
5.75	150	—	5.6	—
		170 m^2/gm		

TABLE III

Computation of Micropores Using the MP Method for Chrome and Myrab Tanned Collagen

t (Å)	S (m^2/g)	Si–Si + 1 (m^2/g)	Mean r_h	Vi (ml/g)
3.0	425			
3.5	300	125	3.25	0.0406
4.0	250	50	3.75	0.0188
4.5	200	50	4.25	0.0213
4.75	150	50	4.625	0.0231
5.0	100	50	4.875	0.0244
5.25	75	25	5.125	0.0128
5.5	75	—	—	—
		350		

RESULTS AND DISCUSSION

Tables I–III give details of the calculations for pore size distribution by the BJH and MP methods. Results are given only for collagen fibers tanned with myrab and chrome, although results were obtained for fibers tanned using the other materials stated above, as the results were almost the same for the others.

The pore size distribution plots, viz, $\Delta V_p/\Delta r_p$ against $\bar{r}_p$ are shown in Figs. 1 and 2. The results obtained indicate that raw collagen fibers have meso pores of radius ~55 Å and micro pores of radius ~4.5 Å. After the enzyme treatment, the ~55 Å radius pores are absent, instead there is a new set of pores of radius equal to ~75 Å. The micropores of radius ~4.5 Å were not found to be altered by the enzyme treatment. A detailed work on the low angle X-ray diffraction pattern showing the intermolecular spacing of the collagen fiber in relation to the relative humidity of the environment was done by Rougvie and Bear (15). Results obtained by Rougvie and Bear show that the intermolecular spacing increases from 10.6 to 13.7 Å when the relative humidity increases from 0 to 95%. The diameter of the micropore, viz, 9 Å is near the value of the Rougvie and Bear spacing at 0% relative humidity. Smith (16) suggested a five molecular model for collagen incorporating the Rougvie and Bear spacing inside the five molecular packings. It appears that the micropore may be situated inside the five molecular packings.

Mathews (18) and Jackson and Bentley (17) have suggested a model for collagen fibril in which the noncollagenous components are situated in the interfibrillar spacing. From our observations, it seems logical to think that after the removal of the noncollagenous components the ~55 Å radius pore is widened to ~75 Å.

After tanning, the ~55 and ~75 Å radii pores were absent and only micropores of radius ~4.5 Å were found to be present. This is in agreement with the suggestions made by Heidemann and Keller (19). Using X-ray diffraction, they have shown that the tannins are deposited inside the fibrils between the proto fibrils. The results obtained show that the micropores are not affected due to tanning. This result seems to suggest that the tannins are deposited between the units comprising five molecules and not in the space enclosed by the five molecules in a unit. This is also in agreement with the findings of Ramanathan (20). It is also interesting to see that the tannins enter the same place in which the noncollagenous components are situated, for the tannins and the enzyme affect the pores of the same radius, viz, ~55 Å.

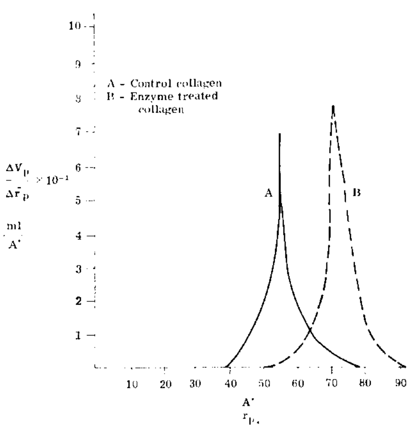

FIG. 1. Pore size distribution for raw and enzyme treated collagen fibers.

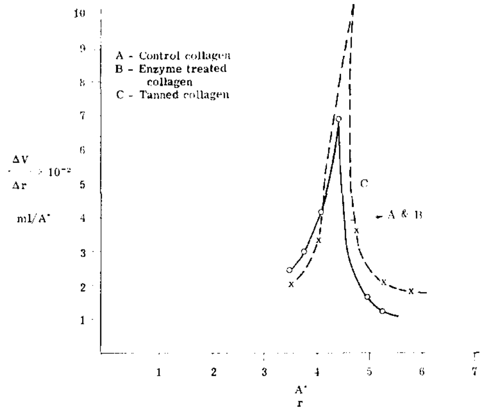

FIG. 2. Micropore size distribution for raw, enzyme treated and tanned collagen fibers.

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