Hysteresis Phenomena in Swelling of Collagen Biomatrix in Animal Hides

INTRODUCTION

Collagen is a naturally occurring and abundantly available amphoteric biopolymer. It is present as a fibrous network in animal hides (or skins) and is chiefly used for leather making. For this purpose, hides are first cleaned in pretanning processes comprising liming (at pH $\simeq$ 12), deliming, and pickling (treatment with acid, pH $\simeq$ 2) to obtain a purified collagen matrix free of noncollagenous matter. The amphoteric protein attains a net negative charge in the liming step and swells; it subsequently becomes neutral in the deliming process and deswells, and the matrix attains a net positive charge in the pickling operation and swells again. The matrix thus undergoes a pH (or swelling) cycle. It is important to study the nature of the volume changes involved during these processing steps, since this strongly influences the transport rates of different species.

Several studies have been reported separately for the swelling of the native, limed, and pickled collagen.[1-5] These studies are concerned mainly with finding the effect of salt, temperature, types of alkali or acid, etc., on the swelling processes. The practical aspect concerning the volume changes involved in the native matrix as it traverses the pH cycle has not been studied. The objective of the present work was to study volume changes in a native collagen matrix when subjected to cyclic pH variations.

EXPERIMENTAL

Pieces of collagen sheet (of dimensions $\sim 4 \times 3 \times 0.3$ cm and of dry weight of $\sim$ 2 g) in its native state were equilibrated at 40°C with a large excess of aqueous solutions at the desired pH level. The solution was changed frequently and a suitable preservative was added to prevent any decomposition of the protein. The equilibration was assumed to be complete when no change in either pH or volume was observed for about 24 h. Equilibration typically takes about 3–7 days depending upon the pH. On reaching the equilibrium, the matrix volume was determined using the method described below.

A method[6] based on the Archimedes' principle was used for volume determination from the apparent weight of the matrix in hydrocarbon solvents (kerosene and *n*-heptane). It was observed[6] that measurement of the apparent weight in a hydrocarbon solvent is not subject to appreciable errors from the possible effects such as absorption of solvent and shrinkage of the matrix. Thus, if w_1 and w_2 are the apparent weights of the swollen piece in liquids 1 and 2, respectively, then volume of the matrix, V, can be determined from eq. (1):

$$V = \frac{w_2 - w_1}{\rho_2 - \rho_1} \tag{1}$$

where ρ_1 and ρ_2 are liquid densities. The accuracy of volume measurement by this method was found to be $\sim \pm 3\%$.

MATERIALS

Collagen was obtained from a buffalo hide with minimum chemical treatment so that it may be considered to be its native state. The nonaqueous treatment suggested by Bowes and Kenten[1] was used for this purpose. The method consists of dehydration of the pieces with repeated acetone treatments, mechanical removal of epidermis and flesh layers, and removal of all fat content by treatment with petroleum ether. The final pieces have an isoelectric pH of 8.0. Double-distilled and deionized water was used in all experiments. pH of the pieces were adjusted with a dilute solution of HCl in the acidic region while a dilute solution of sodium hydroxide was used to adjust the pH in the alkaline region.

RESULTS AND DISCUSSION

Swelling data were collected in the pH range 2–12, which is generally encountered in pretanning operations. The pieces were swollen according to two separate pH sequences—(A) 6-2-12-2-12 and (B) 6-12-2-6—and the data are shown in Figures 1 and 2, respectively. The points are numbered according to the order in which the pH was varied. The same collagen piece was used for all experiments covered in a given pH sequence.

The data for sequence A in Figure 1 show that the matrix volume undergoes an irreversible change after the acid treatment at pH 2 (i.e., at point b), but the subsequent volume changes for the pH cycles 2-12-2-12 are reversible and fall on the same average curve b-c-d. The swelling behavior of the matrix for sequence B is slightly different, as shown in Figure 2. The matrix undergoes two irreversible changes: one after the alkali treatment at pH 12 (point d) and the other after the acid treatment at pH 2 (point f). The subsequent volume changes after the acid treatment are once again reversible. Further, the data show

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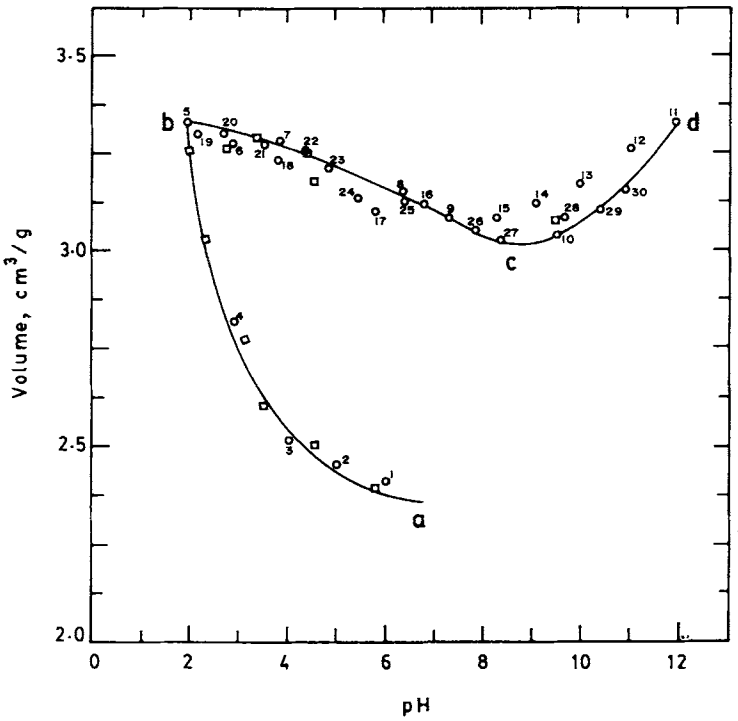

Figure 1 Volume changes of the native collagen matrix as the pH varies according to sequence A: (○) piece 1; (□) piece 2.

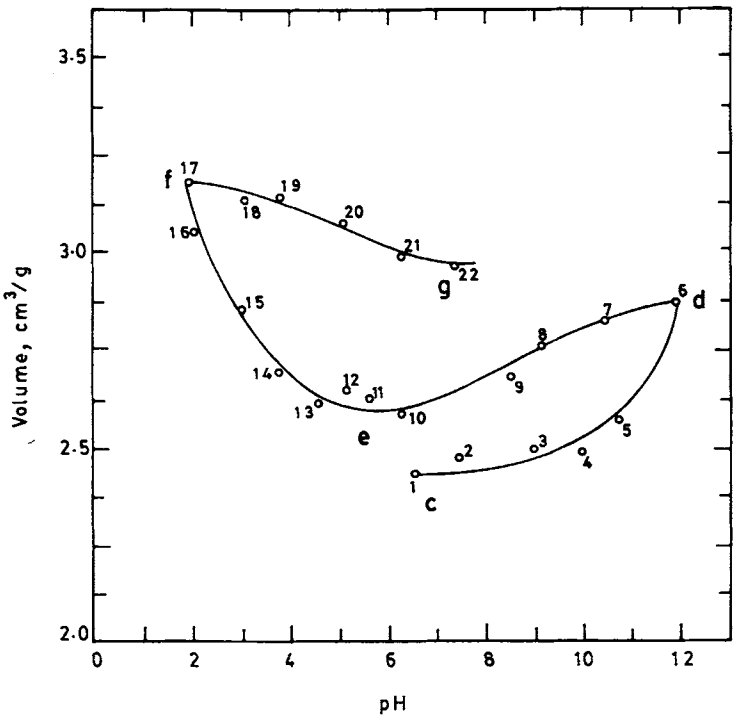

Figure 2 Volume changes in the native collagen matrix as the pH varies according to sequence B.

that changes after the acid treatment are more drastic than those after the alkaline treatment. These observations may be rationalized on the basis of severing of certain weak cross-links as described below.

Collagen matrix has a fibrous structure with different levels of organization.[7,8] The primary building block of the matrix is a collagen protofibril or molecule that comprises three (α-)polypeptide chains wound together by primarily different types of hydrogen bonds and also by specific covalent linkages.[9] These hydrogen bonds are responsible for the crystalline portions of the molecule.[7] The protofibrils are stacked end-to-end and side-to-side by Schiff-base intermolecular linkages[10,11] to form a fibril. A few parallelly arranged fibril clusters make fibers. Finally, several fiber bundles are woven to form a three-dimensional structure.

The covalent linkages are stable and are not broken even when subject to hydrothermal treatment.[12] Schiff-base linkages are labile. Consequently, as the pH becomes more acidic or basic, the matrix weakens due to the rupture of these links that reform as the pH becomes neutral, restoring the matrix strength.[13] The hydrogen bonds, however, can irreversibly break. It was suggested[7] that breakage of some peptide linkages (i.e., hydrogen bonds between imino and carbonyl groups of the adjacent keto-imide groups belonging to different polypeptide chains in the helix) takes place when the matrix is subject to prolonged treatment with strong acids or bases. Once the bonds are broken and the groups separated, these linkages may not reform since they are effective over only short distances (< 5 Å). This suggests modifications in the crystalline order of the structure, which was confirmed by indirect measurements such as stress–strain isotherms,[14] shrinkage temperature,[15] and membrane properties.[16,17] The irreversible volume changes that occurred at pH values 2 and 12 may therefore be attributed to this structural modification of the matrix. A similar permanent swelling was also observed[7] when pickled collagen (at pH $\sim$ 3.3) was subject to aqueous HCl solution at pH 2. The greater degree of the observed irreversibility at pH 2 for sequence B (shown in Fig. 2) may be due to the stronger effect of acidic solutions as compared to the effect of alkaline solutions. Further, the absence of irreversibility at pH 12 for sequence A (shown in Fig. 1) may be due to the fact that the weak peptide linkages were already ruptured in the acid treatment at pH 2.

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