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Transport Properties of Charged Membranes Undergoing Conformational Transitions

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Summary. The permeability and reflection behavior of cross-linked collagen films in dilute salt solutions have been investigated by measurements of net volume flow, isotopic exchange of THO and of Ca^{45}, and osmotic pressure. Complementary measurements of swelling, membrane resistance, membrane potential, and streaming potential are presented. Measurements were performed in the pH range of 5 to 1.5, at temperatures between 25 and 52 °C, and in the presence of KCl, 10^{-2} M or $CaCl_2$, 10^{-3} M. Under the conditions adopted, the membrane carries a net positive charge and undergoes large changes in degree of swelling (Donnan effect) and structure (crystal → amorphous transition). The results indicate that when pH is lowered the filtration coefficient L_p decreases in the crystalline state (pH 5 to 3), increases during the conformational transition (pH 3 to 2), and decreases in the amorphous state (pH <2). It appears that L_p is affected more by such properties as structure and porosity (i.e., mechanical resistance to flow) than properties related to the charged character of the membrane. The reflection coefficient σ increases when pH is lowered until pH ~ 3, and decreases upon further lowering of pH. Such behavior is described in terms of the competition between swelling (due to both the Donnan effect and the melting transition) and fixed-charge density. Values of fixed-charge density derived on the basis of a theoretical expression for σ were found to be in good agreement with independent titration data.

In a previous study [7, 8] we investigated the transport properties of cross-linked collagen membranes near the isoelectric point. Transport properties were investigated in a range of salt concentration broad enough (~ 0.1 to ~ 5 M) to include characterization of the isothermal permeability behavior when the membrane was in the crystalline state, in the amorphous state, and while undergoing the melting transition.

The characterization of membrane properties for relatively simple systems undergoing a phase transition is of particular interest for the understanding of the behavior of some biological membrane phenomena, where recent evidence [2, 17] indicates that a conformational transition might be involved.

In the present study we extend the characterization of the transport properties of cross-linked collagen membranes to the case in which the phase transition is induced by alteration of the degree of ionization of the polymer at low ionic strength.

Materials and Methods

Doubly-oriented collagen films, 25 μ thick, obtained by casting a dispersion of fibrils from steer tendons, were kindly supplied to us by the Ethicon Company. The characteristics of these membranes have been previously described [7, 8, 9]. To assure insolubility of the membrane in the amorphous state, a cross-linking reaction was performed by equilibrating the films for 5 min in a 0.1 % solution of *p*-benzoquinone.

Dimensional changes of the membrane and the occurrence of the melting transition were determined by following the isothermal variation of length with pH, the change of the elastic modulus, and the X-ray diffraction pattern, as previously described [7, 15]. The degree of swelling V_s/V_0 (the volume of swollen membrane relative to the dry volume) was determined from the weights and densities of the swollen and unswollen membrane. The thickness of the swollen membrane (clamped at constant area in the flow cell) was calculated from the dry thickness and from the value of the degree of swelling of the unrestricted membrane.

Resistance Measurements

For the measurements of the membrane resistance a 250 μ thick membrane was used in connection with a small cylindrical cell (about 3 cm long and 1 cm in diameter) having two movable black platinum electrodes at each end. An impedance bridge was employed. The system was immersed in an insulator (oil) and thermostated. A thermocouple was fitted to a small hole to control the temperature of the cell solution. Since the cell volume is rather small as compared to the volume of the membrane, the pH of the external solution varied during the equilibrium time. For that reason, periodic changes of the external solution were performed, until a constant value of the system conductance was attained. (For all the other measurements, performed with a thinner membrane in a much greater cell (~ 120 cm^3 of volume) the maximum change of the pH of the external solution during the equilibrium time was calculated to be of the order of 2% and therefore such procedure appeared unnecessary.) The measurement was performed by determining the resistance as a function of the distance between the two electrodes. When this procedure was followed with and without membrane, the resistance *vs.* electrode distance plot yielded two parallel lines. The distance between these lines was taken as the difference ΔR between the resistance of the membrane and that of a geometrically equal volume of solution. (The value of the resistance of the membrane itself can be obtained once the values of the thickness of the membrane and of the specific resistance of the solution are known.)

Membrane Potential Measurements

Electric potential measurements [11] were performed at 52 °C while the membrane was clamped between solutions of different salt concentration. The solution in the two half-cells was maintained under forced circulation. Calomel electrodes with agar-

agar bridges and Ag–AgCl electrodes were employed. Potential differences were recorded by means of a Keitley 610A electrometer with a sensitivity of 0.2 mV.

Streaming Potential Measurements

Streaming potential measurements were performed, as previously described [7], sealing Ag–AgCl electrodes to the cell employed for the volume flux measurements. Temperature was maintained at 52±0.1 °C. Potential differences $\Delta\psi$ were recorded by means of a Solatron L.M. 1420 digital voltmeter with a sensitivity of 2.5 μV. The cell was operated under different nitrogen pressures ΔP up to 35 cm Hg. The streaming potential-pressure ratio was deduced by the slope of the $\Delta\psi$ *vs.* ΔP plot.

Volume Flow Determination

The volume flow per unit time and unit area J_v was determined by following the rate of flow through the membrane (clamped between two half-cells containing aqueous solutions of identical composition and pH) under an applied pressure gradient ΔP of about 20 cm H_2O. The apparatus and the details of the method have already been described [7]. The temperature was kept constant, usually at 52±0.1 °C. The rate of flow was found to be constant after about 0.5 hr. The same membrane was used for determining the volume flow at different (decreasing) pH values and fixed salt concentration. The estimated precision on J_v was of the order of ±3%.

Tracer Measurements

The tritiated water flow under free diffusion at zero hydrostatic pressure gradient was measured using the same experimental conditions adopted for the volume flow determinations. Following the procedure previously described [7], 0.5 ml of THO containing 100 μC activity were added to one side of the membrane and the rate of flow was followed using a liquid scintillation spectrometer. A similar procedure was used (adding a sample with 10 μC activity) to determine the Ca^{45} permeability coefficient.

Determination of the Reflection Coefficient

The reflection coefficient σ was determined using the same cell employed for the determination of the volume flow. Two different salt solutions were used in the two half-cells and the hydrostatic pressure (applied to the most concentrated solution side) necessary to reduce the volume flow to zero was determined by extrapolating to zero the volume flow in the J_v *vs.* ΔP plot. For KCl the two solutions were 5×10^{-3} and 10^{-2} M, while for $CaCl_2$ they were 10^{-3} and 5×10^{-3} M. The volume flow was measured by applying hydrostatic pressures which were small enough that operation in the linear region of the pressure-volume flow dependence was assured and viscoelastic deformation of the membrane was avoided.

It should be noticed that, because of the concentration gradient, the membrane can no longer be regarded as a homogenous phase during the measurements of σ. However, we believe that the difference in concentration is small enough to make the results comparable with those obtained when the concentration is equal on both sides of the membrane (measurements of J_v at two different salt concentrations, 10^{-2} and 5×10^{-3} M, did not in fact differ by more than 10%).

The theoretical value of the osmotic pressure difference $\Delta\pi$ was calculated assuming ideal behavior.

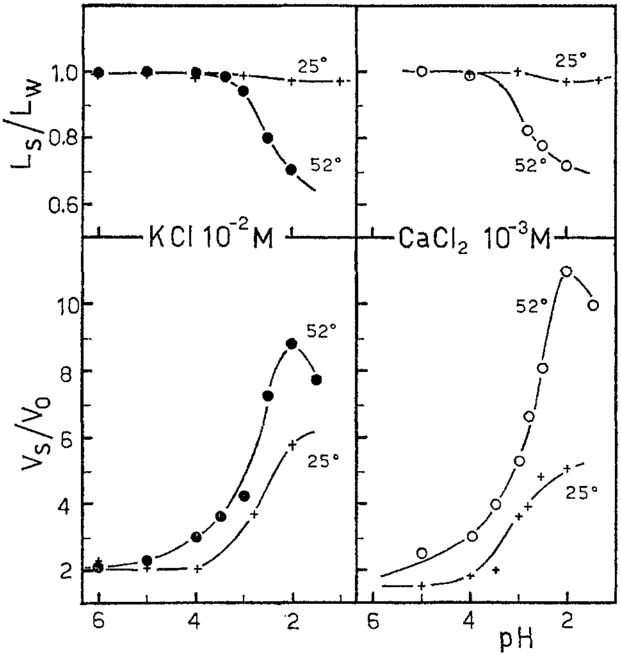

Fig. 1. Variation of length and volume of swollen cross-linked collagen films with pH at the indicated salt concentrations and temperatures. V_0 and L_w refer to the volume of the unswollen sample, and to the length of the sample swollen in water, respectively

Results

Dimensional Changes and Swelling

Typical data for the pH variation of length and degree of swelling for unrestricted collagen membranes are collected in Fig. 1. The large increase of swelling which occurs when the pH is lowered between 4 and 2 is primarily associated with the large osmotic forces (Donnan effect) due to the increase of the net cationic charge on the protein. The deswelling observed when pH$<$2 is attributed to the increasing concentration of the acid once the number of fixed charges no longer increases [4] with decreasing pH. The fact that the degree of swelling is larger at 52 than at 25 °C, particularly between pH 3 and 2, is attributed to the occurrence of a complete melting transition at the higher temperature. In fact, while at 25 °C no shrinkage is observed (*see* Fig. 1) and the X-ray diagram indicates the occurrence [15] of a crystalline component even at pH 2, the shrinkage observed at 52 °C (*cf.* the L_s/L_0 *vs.* pH curve) indicates the occurrence of a complete melting transition at such higher temperature.

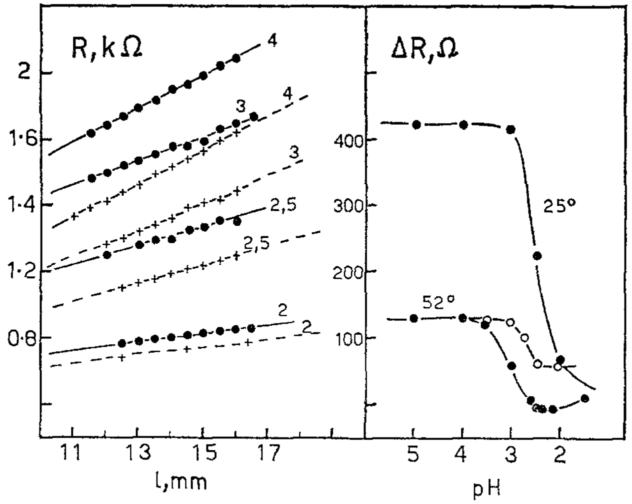

Fig. 2. *Left:* Typical variation of the resistance between two electrodes as a function of their distance (solid line: with membrane; dotted line: without membrane). The pH value is indicated. KCl, 10^{-2} M, T = 25 °C. *Right:* Corresponding pH variation of ΔR (obtained from the distance between solid and dotted lines reported on the left side) at different temperatures for KCl, 10^{-2} M (•), and $CaCl_2$, 10^{-3} M (○)

This body of results is in line with results previously obtained [3, 4, 15] for cross-linked collagen tendons, indicating that at low temperatures the increase in degree of swelling due to Donnan effects may be accompanied by a large decrease of the degree of crystallinity without complete melting or shrinkage. Donnan effects and complete melting (i.e., shrinkage) occur, instead, simultaneously at relatively high temperatures, the exact value of the melting temperature depending upon the chemical composition of the collagen, the degree of cross-linking and the solvent type [15].

The swelling data reported in Fig. 1 reveal some differences in the effect of the different salts. In particular, a larger swelling is noticed with $CaCl_2$ than with KCl (note that the ionic strength of the latter is greater than that of the former). As discussed elsewhere [1], these effects may be related to the fact that the binding constant to collagen of Ca^{++} is greater than that of K^+.

Resistance and Membrane Potential

The results of typical experiments of resistance are reported in Fig. 2. The decrease of ΔR (*see* Materials and Methods) observed when the pH is lowered below ~3.5 reflects the large increase of swelling of the membrane with a consequent enrichment of mobile ions. The negative value of ΔR

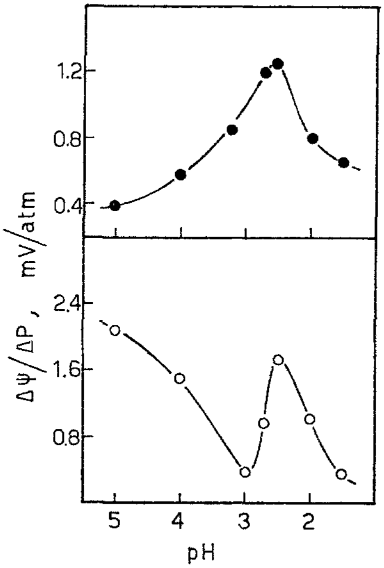

Fig. 3. pH variation of the streaming potential-pressure ratio $\Delta\psi/\Delta P$ for cross-linked collagen membranes. T = 52 °C. • = KCl, 10^{-2} M; ○ = $CaCl_2$, 10^{-3} M

at pH ~2.5 (KCl 10^{-2} M, T = 52 °C) indicates that the specific conductivity of the membrane is even higher than that of the corresponding external solution.

The potential difference between two KCl solutions of different salt concentrations on the sides of the membrane revealed, even at pH = 5, the occurrence of a positive charge on the membrane. The potential difference observed at pH = 5 approached the theoretical value for a perfect anion selective membrane given by the Nernst equation [11] $\Delta\psi = (RT/zF) \ln c_s'/c_s''$ when the c_s'/c_s'' ratio of the concentrations on the two sides of the membrane was small (~2).

Streaming Potential

Excess pressure applied to one side of the membrane forces the electrolyte through the membrane pores. The counterions-fixed charge strong interaction will cause a space separation of charges and therefore an electric field. The potential difference $\Delta\psi$ between the two external solutions is given, by irreversible thermodynamics treatment, by the expression [12]:

$$\Delta\psi = \frac{FXL_p\Delta P}{L_E\varphi_w} \tag{1}$$

where L_E is the electric conductance of the membrane, L_p is the filtration coefficient defined as $(J_v/\Delta P)_{\Delta\pi=\Delta\psi=0}$, X is the fixed-charge density expressed in moles of the monomeric charged unit per unit swollen volume, F is Faraday's constant and φ_w is the volume fraction of water inside the membrane.

The pH variation of the streaming potential per unit pressure is reported in Fig. 3 for KCl and $CaCl_2$. The increase of $\Delta\psi/\Delta P$ on lowering pH (down to ~2.5) observed in the presence of KCl may be attributed mainly to the increase of the fixed charge, while the decrease at lower pH is attributed to the prevailing effect of the ionic strength and consequent increase of conductance, as theoretically expected from Eq. (1).

Somewhat more puzzling is the behavior in the presence of $CaCl_2$ where $\Delta\psi/\Delta P$, which attains a large value at pH ~5, exhibits a decrease on lowering pH between 5 and 3; its trend seems to be mainly related to the variation of L_p (*cf.* Fig. 4).

Filtration and Permeability Data

The linear relationships between flow and forces, derived by irreversible thermodynamics treatment, when $\Delta\pi=0$, are [12]:

$$J_v = L_p \Delta P + L_{pE} \Delta\psi \tag{2}$$

$$I = L_{Ep} \Delta P + L_E \Delta\psi \tag{3}$$

where I is the electric current, and L_{Ep} is the phenomenological cross-coefficient which connects the volume flow to the electromotive force (due to streaming potential). Substituting the value of L_{Ep} given by Eq. (3), recalling that under the experimental conditions adopted $I=0$, and that $L_{Ep}=L_{pE}$ [12], one gets

$$\frac{J_v}{\Delta P} = L_p - L_E \left(\frac{\Delta\psi}{\Delta P}\right)^2. \tag{4}$$

The term $L_E(\Delta\psi/\Delta P)^2$ may be evaluated from measurements of conductance and of streaming potential. With the data reported in Figs. 2 and 3 it appears that the latter term accounts to only 0.1% at pH ~5, and 6% at pH ~3, of the $J_v/\Delta P$ value experimentally measured in KCl 10^{-2} M. Thus, in our case, the effect of the streaming potential may be neglected and the filtration coefficient may be simply equated to the $J_v/\Delta P$ ratio.

L_p values thus obtained for different salts and at different temperatures are reported in Fig. 4 as a function of pH. A strong dependence of the filtration coefficient upon pH and temperature is observed. There is a tendency

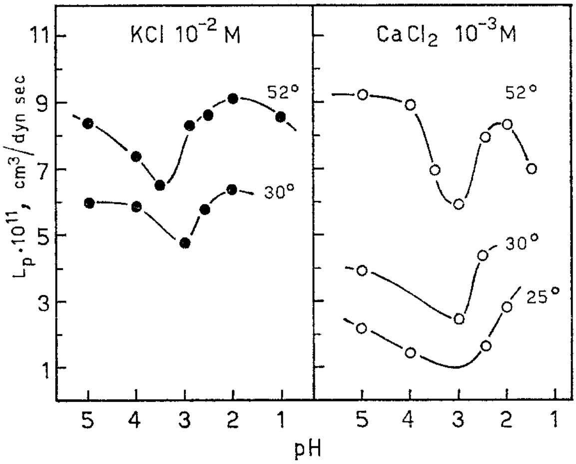

Fig. 4. pH variation of the filtration coefficient L_p for cross-linked collagen films in different salt solutions at several temperatures

for L_p to decrease with decreasing pH in the crystalline state until pH ~ 3. Between pH 3 and 2, when maximum swelling and a strong reduction of the degree of crystallinity occur, L_p increases with decreasing pH. There is a further inversion of the trend of L_p at lower pH, corresponding to the deswelling of the amorphous network (*cf.* Fig. 1). The pH variations of the filtration coefficient appear to be larger at 52 °C (when the melting transition is complete) than at 25 or 30 °C. The decrease of L_p with decreasing temperature may be attributed to the corresponding decrease of water mobility.

The permeability of tritiated water, ω_T, at zero volume flow was calculated [7] using Fick's law, conveniently written as

$$\omega_T \Delta t = \frac{V_1 \Delta \alpha}{\mathrm{RTA}(\alpha_2 - \alpha_1)} \tag{5}$$

where V_1 is the volume of the compartment at lower isotopic activity; $\Delta \alpha$ is the variation of THO activity during the time Δt; A is the area of the membrane; and $\alpha_2 - \alpha_1 \cong \alpha_2$ is the difference in THO activity between the two compartments. Values of ω_T were used to calculate the mobility of tritiated water, u_T, defined as

$$u_T = \frac{\omega_T \Delta x}{\varphi_w} \tag{6}$$

where Δx is the thickness of the membrane. u_T values are reported in Fig. 5 as a function of pH. Also included in Fig. 5 are the values of the water mobility, u_w, obtained using the relationship [13]

$$u_w = \frac{L_p \Delta x}{\varphi_w \bar{v}_w} \tag{7}$$

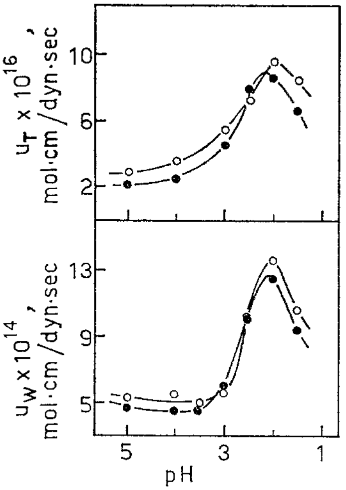

Fig. 5

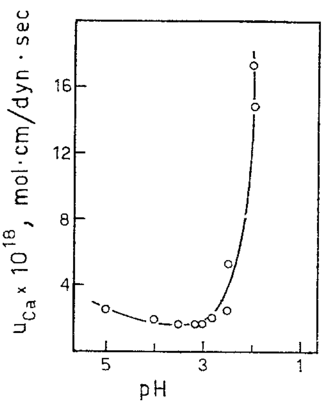

Fig. 6

Fig. 5. Mobilities of THO (u_T) and of water (u_w) as a function of pH for cross-linked collagen membranes in KCl, 10^{-2} M (•) and $CaCl_2$, 10^{-3} M (○). T = 52 °C

Fig. 6. Mobility of Ca^{45} as a function of pH for cross-linked collagen films. T = 52 °C. $CaCl_2$, 10^{-3} M

(where $\bar{v}_w$ is the partial molar volume of water), in conjunction with the L_p values reported in Fig. 4. This relationship is valid as far as the drag of water by the solute can be neglected.

A large increase of mobility is observed when pH is lowered below 3, while between pH 5 and 3 the mobilities are not greatly affected by pH.

The numerical difference between the two mobilities which are reported in Fig. 5 may be discussed in terms of the porosity of the membrane [7, 13, 14, 18]. In fact, the term $g = L_p/\bar{v}_w\ \omega_T$ is generally used as an indication of the porosity of the membrane. In the present case, $g \gg 1$ in line with previous results [7] and in favor of the model of permeation through a kind of porous pathway.

The mobility of Ca^{45}, u_{Ca}, as a function of pH is reported in Fig. 6. u_{Ca} is nearly constant in the crystalline state of the membrane, as in the case of water mobility, while there is a large increase of mobility at pH < 3. [Notice that the value of u_{Ca} is about 100 times smaller than that of u_T (pH 5–3).]

Reflection Coefficient

The reflection coefficient σ introduced by Staverman [16] and defined as

$$\sigma = \left(\frac{\Delta P}{\Delta \pi}\right)_{J_v = 0}, \tag{8}$$

where $\Delta\pi$ is the osmotic pressure difference between the two external solutions, gives, as is well known, an indication of the rejection properties of the membrane. In general, $\sigma=1$ only for an ideally semipermeable membrane (i.e., permeable only to one species, but not to others), while $\sigma=0$ for a porous membrane completely permeable to all species present in the system. For the discussion of our results, we shall make use of the expression of σ derived by Kedem and Katchalsky [12] for the permeation of a mono-monovalent electrolyte through a charged membrane

$$\sigma=1-\frac{\omega_s\bar{v}_s}{L_p}-\frac{\bar{c}_s\varphi_w}{X t_1^0} \tag{9}$$

where ω_s is the permeability coefficient of the salt when $J_v=0$; $\bar{v}_s$ is the partial molar volume of the salt; $\bar{c}_s$ is the arithmetic mean of the external salt concentration on both compartments; t_1^0 is the transport number of the counterion in free solution; and the other symbols are as previously defined. A similar expression has also been derived [12] for a mono-divalent electrolyte (the only difference being related to the requirement of Donnan equilibrium of the charged species).

The hypothesis under which such relationships have been derived (highly swollen membrane with moderate charge density in very dilute salt solution) apply rather well to the presently studied system (only about 10% of the amino-acid residues of collagen may be positively charged), and Eq. (2) was found to be in good quantitative agreement with experimental results [6, 13].

Reflection coefficient data for KCl and $CaCl_2$ solutions are reported in Fig. 7. An inversion point in the trend of σ at pH ~ 3 may be observed. The greater value of σ for $CaCl_2$ than for KCl is attributed to the lower ionic strength of $CaCl_2$, as well as to the higher Donnan exclusion for the divalent cation [13].

Discussion

The behavior of the filtration coefficient as a function of pH exhibits features similar to those previously observed [8] when the salt concentration was varied at constant pH. In fact, when the membrane is still essentially crystalline (pH $> \sim 3$), the increase in degree of swelling observed on lowering pH is accompanied by a corresponding decrease of L_p. As previously discussed [7], this decrease of L_p suggests that swelling in the crystalline state improves the homogeneity of the membrane, perhaps by closing structural voids. Again, analogouslv with the behavior previously observed

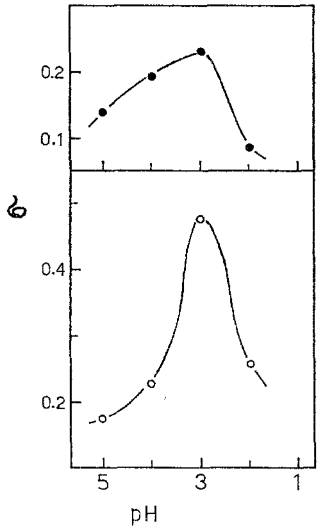

Fig. 7

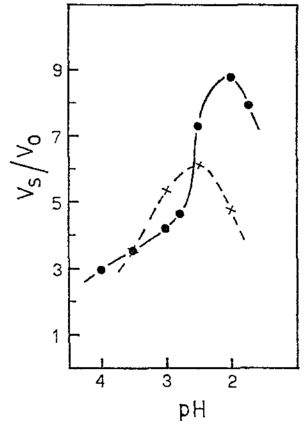

Fig. 8

Fig. 7. Reflection coefficient as a function of pH for cross-linked collagen membranes in KCl, $5\times10^{-3}-10^{-2}$ M (•) and $CaCl_2$, $10^{-3}-5\times10^{-3}$ M (○). T = 52 °C

Fig. 8. Experimentally observed pH variation of the degree of swelling (solid line) for unrestricted collagen networks at 52 °C compared with the corresponding variations (dotted line) calculated using Flory's theory [5] for the swelling of ionic network

[7], during the transition, and even at 25 °C when the degree of crystallinity is strongly reduced by swelling (pH 3 to 2), the increase in swelling is accompanied by a corresponding increase of L_p. Thus, the conversion of the rigid crystalline structure into a structure where the amorphous component prevails corresponds to an increase of permeability, irrespective of the mechanism by which the transition is initiated. Moreover, the amorphous membrane, whether charged or not [8], behaves as a homogeneous system in which a reduction or an increase of swelling is accompanied, respectively, by a reduction or an increase of the filtration coefficient.

The behavior of the mobilities of THO and water is in line with the above findings. The fact that the mobility is nearly constant between pH 5 and 3 indicates that the frictional interactions do not vary appreciably, as long as the crystalline structure prevails. On the other hand, the frictional interactions decrease strongly at pH < 3, indicating that as the degree of crystallinity tends to zero, the solution swelling the membrane is in a relatively looser state.

At variance with the near-isoelectric system previously investigated [7, 8], the present system is of interest because of the combined effect of swelling, fixed charges and phase transition on the permeation of the membrane to ionic species. The pH variation of the reflection coefficient may be interpreted in terms of Eq. (9). Substituting plausible values of the filtration coefficient and of the salt permeability, the second term in Eq. (9) is found to be negligible with respect to the others. Therefore, the expression of σ reduces simply to

$$\sigma = 1 - \frac{\bar{c}_s \varphi_w}{t_1^0 X}. \tag{9a}$$

Since $\bar{c}_s$ and t_1^0 may be regarded as constant on lowering pH (at least down to pH$\sim$2.5, *see below*), the trend of the reflection coefficient should be determined mainly by the competition between swelling and fixed-charge density.

Table 1. Comparison between the density of fixed charges X_σ as derived from Eq. (9a) using reflection data (KCl 10^{-2} M) and that (X_t) obtained using titration data for native collagen [10]

pH	φ_w	n_0 (mEquiv/g)	$X_t \cdot 10^2$ (mole/liter)	$\varphi_w/X_t \cdot 10^{-2}$ (liter/mole)	$X_\sigma \cdot 10^2$ (mole/liter)
5	0.56	0.09	0.5	1.1	0.38
4	0.67	0.3	1.3	0.5	1.24
3.5	0.72	0.5	1.57	0.46	1.38
3	0.76	0.7	2.1	0.36	1.67
2.5	0.86	0.83	1.48	0.58	2.2–1.5
2	0.89	0.9	1.3	0.68	3.3–1.3
1.5	0.87	0.9		0.67	

This expectation is fully supported by the data collected in Table 1. The value of φ_w reported in the second column was derived from the experimentally observed variation in the degree of swelling with pH (*cf.* Fig. 1). The number n_0 of milliequivalent of HCl fixed by one gram of collagen, and the corresponding charge density, reported in the third and fourth columns, were determined using typical titration data for native collagen, as reported by Gustavson [10] (titration data for collagens of different origin did not significantly differ in the pH region under consideration). When the ratio (reported in the fifth column of Table 1) φ_w/X is used in conjunction with Eq. (9a), it appears that in the crystalline state (pH$>$3) X increases more than does φ_w, thus leading to the experimentally observed increase of σ, while at pH$<$3 the decrease of σ with pH is accounted for by the prevailing increase of φ_w. In the last column of Table 1 we report values of X calculated using Eq. (9a), and the experimental σ and φ_w values. The agreement with the data obtained from independent titration measurements up to pH 3 is satisfactory.

It should be observed that when pH <2.5 the mean concentration of the two external solutions can no longer be regarded as a constant (the amount of HCl added to lower the pH cannot be neglected). In this case, even the approximation under which Eq. (9) has been derived, i.e., $c_s' < X$, where c_s' is the salt concentration inside the membrane, is incorrect and the term c_s' must be added to X. However, an evaluation of these corrective terms reveals that, even at low pH, the above interpretation is qualitatively correct.

The foregoing discussion has been based on the concept that, upon lowering pH, an increase of the fixed net charge on the membrane causes both an increase in degree of swelling, due to Donnan effects, and the crystal → amorphous transition. The latter, however, causes an increase in the degree of swelling, which, in principle, could be distinguished from that due to Donnan effects. To this end we present the data in Fig. 8, where the solid line is the experimentally observed variation in degree of swelling with pH (equal to that in Fig. 1), and the dotted line was calculated using Flory's theory for the swelling of polyelectrolyte networks not undergoing a conformational transition. When the external electrolyte concentration is comparable to that of fixed charges, the latter theory yields [5]

$$\left(\frac{V_s}{V_0}\right)^{\frac{5}{3}} = [(X^0/2S^{\frac{1}{2}})^2 + (\tfrac{1}{2} - \chi_1)/v_1]\, v_e/V_0 \qquad (10)$$

where X^0 is the concentration of fixed charges referred to the unswollen network; S is the ionic strength; χ_1 is an interaction parameter; v_1 is the molar volume of the solvent; and v_e/V_0 a parameter characterizing the degree of cross-linking of the network. Using the density of charge data of Table 1, taking [4] $\chi_1 = 0.5$, and fitting the network structure parameter v_e/V_0 to the experimentally observed value of V_s/V_0 at pH $= 3.5$, the dotted line in Fig. 8 was calculated. We regard this curve as an approximation for the variation of degree of swelling with pH for an ionic network not undergoing conformational transitions in the pH range under consideration, and having an elastic modulus equal, at pH $= 3.5$, to that of our collagen membrane. When values of the degree of swelling, obtained from the dotted line of Fig. 8, are used in conjunction with Eq. (9a), it is apparent that the decrease of σ with decreasing pH, when pH < 3, is explained only by the contribution to swelling given by the phase transition.

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