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Diffusion of 2,2,6,6-tetramethylpiperidine 1-oxyl derivatives of variable hydrophobicity in tropocollagen I solution

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ABSTRACT

Electrochemical time-of-flight was used to measure the diffusion coefficients of 2,2,6,6-tetramethylpiperidine 1-oxyl derivatives, C_nTPA, (3 to 7 CH_2 groups), in tropocollagen I solution, as a function of the chain length and the cross-linking with glutaraldehyde. The values of the diffusion coefficient of C_nTPA in pure aqueous electrolyte follow the Stokes–Einstein law, i.e. the diffusion coefficient is inversely proportional to the size of the redox probe. Different behavior is observed in 0.5% (w/v) tropocollagen solution where the molecules with longer alkyl chains show larger diffusion coefficients than the smaller molecules. This behavior is explained in terms of electrostatic interactions between tropocollagen chains and the C_nTPA molecules. The measurements of the diffusion coefficients of C_nTPA in 0.5% tropocollagen cross-linked with glutaraldehyde indicate that while the C_7TPA and C_5TPA probes exhibit lower diffusion coefficients upon addition of 0.05% GA and 0.1% (v/v) GA respectively, the other C_nTPA molecules exhibit either unchanged or increased diffusion coefficients under the same conditions thus indicating the presence of hydrophobic pockets selectively interacting with C_nTPAs. These results demonstrate the utility of electrochemical time-of-flight in measurements of diffusion coefficients in complex biopolymeric media.

1. Introduction

Collagen is a main component of connective tissue [1]. It is also one of the most promising biomaterials for targeted drug delivery systems, scaffolding, and mechanical parts fabrication [2–4].

The tropocollagen consists of rod-like polypeptide chains about 300 nm in length and 1.4 nm in diameter. The conformational stabilization of the collagen triple helix is often explained by its imino-rich structure [5,6]. Since the imino residues are incapable of hydrogen bonding and are not uniformly distributed, they create local hydrophobic "pockets" within the collagen structure where the sequence shows multiple repeats of proline and hydroxyproline in the X and Y position (general sequence for helical peptides can be expressed as Gly-X-Y). In fact, the NMR study by Charlton et al. has shown that the association between the polyphenol and proline rich peptides is of a hydrophobic nature even though the collagen matrix is in general hydrophilic [7].

Collagen used in drug delivery systems is rapidly biodegradable *in vivo* without chemical modifications. In order to stabilize its structure, intramolecular and intermolecular cross-linking is employed [8]. The commonly used cross-linking agent, glutaraldehyde (GA), forms a bridge by reacting with side chain ε-amino groups [1,9,10]. The addition of GA to the solution of tropocollagen leads to the formation of both intramolecular as well as intermolecular cross-linking resulting in a polymeric gel-like network. The cross-linking with GA not only changes the structure of collagen, but it also potentially increases the hydrophobic character of the matrix. In fact, partial hydrophobicity of collagen cross-linked with glutaraldehyde or modified by methylation of the matrix was previously reported via water swelling experiments [11,12].

Hydrophobic interactions between collagen and small molecules are important in designing new drug delivery systems. The mechanism of association of small molecules with collagen is also important to understand the process of the collagen fibril formation [3,13,14]. Therefore there is a need to systematically study the problem of hydrophobic interactions between small molecules and collagen matrix both in the native and cross-linked form.

The electrochemical time-of-flight (ETOF) technique is particularly useful in measurements involving polymeric systems because it does not require prior knowledge of the redox probe concentration, thus the possible adsorption of matrix molecules on the electrodes does not complicate the interpretation of experimental data. In addition, in polymeric systems the exact concentration of the redox probe could be difficult to determine, so the value of ETOF in studying this type of systems cannot be underestimated [16].

In this report we describe the measurements of diffusion coefficients of several (2,2,6,6-tetramethylpiperidine 1-oxyl (Tempo) amide derivatives (C_nTPA, n=3–7) in a tropocollagen I solution both in the native form and cross-linked via reaction with glutaraldehyde. The C_nTPA molecules are modified with alkyl chains of various lengths (Fig. 1) and thus exhibit different hydrophobicities. Our goal is to

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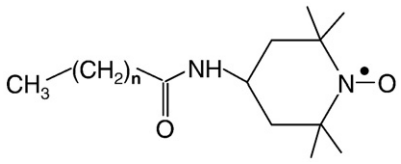

Fig. 1. Structure of C_nTPA; n denotes the number of CH_2 groups in the structure.

examine the correlation between the hydrophobicity of the Tempo probes and their diffusion rates in collagen matrix as a function of GA cross-linking.

The relative hydrophobicity of C_nTPA can be expressed as a solubility in water and in our case it varies from 3×10^{-3} M for C_3TPA to 8×10^{-5} M for C_7TPA (Table 1) [17]. These molecules, frequently used as ESR spin probes, are also kinetically facile and reversible redox probes [18].

The ETOF technique employs a photolithographically fabricated dual-band microelectrode device (Fig. 2A) with one electrode serving as a generator of the probe and the other as a collector [19,20]. As shown in Fig. 2B the oxidized form of a redox probe is produced at the generator electrode and its time of arrival at the collector electrode is monitored as a current–time transient (Fig. 2C). The transit time (τ), defined as time at which the recorded current reaches 1/3 of the plateau current, is proportional to the diffusion coefficient of a probe (D) and the distance between the electrodes (d):

$$\tau = K \times d^2/D. \quad (1)$$

The factor K depends on the geometry of the device and definition of the transit time and can be easily determined experimentally with a calibration procedure [19,20]. The value of the K coefficient (Eq. (1)) was determined previously to be equal to 0.23. The measured value of K agrees very well with the previous findings for devices of similar geometry [21].

2. Materials and methods

2.1. Materials

The C_nTPA molecules were a generous gift from Dr. Marcin Majda, UC Berkeley. The synthesis and purification of C_nTPA are described elsewhere [17]. The hydrochloric acid (trace element grade, Aldrich), disodium hydrogen phosphate (≥99%, Fluka), mercaptopropyltrimethoxysilane (MPS, 95%, Aldrich), glutaraldehyde (25% in water, Aldrich), and sodium chloride (99%, Aldrich) were used as received. House-distilled water was passed through Millipore purification system. The resistivity of the resulting water was 18.3 MΩ cm.

2.2. Preparation of collagen matrix

2.2.1. Extraction and purification of collagen

The extraction of collagen was carried out according to the procedure developed by Ho et al. using rat tail tendons (Pel-Freeze) [22,23]. Briefly, after tendon extraction, 1 g was dissolved for 4 h in 0.01 M HCl. The resulting suspension was centrifuged at 30,000 g and only supernatant was collected and filtered through 20, 5, and 0.45 μm filters. NaCl was added to the solution until it reached 0.7 M and the mixture was centrifuged again at 30,000 g. This time the pellet was collected and dissolved in 0.01 M HCl overnight. After dissolution, dialysis against a phosphate buffer (pH=7.4) was performed twice. The resulting solution was centrifuged again and the pellet was dialyzed twice: once against the 3% acetic acid and then against D.I. water. The purified collagen was lyophilized and kept at 4 °C until needed. This procedure yields 290 to 310 mg of pure collagen. The collagen extraction method described above produces soluble tropocollagen.

2.2.2. Preparation of the collagen solution

Five mg of purified collagen was dissolved overnight in 1 ml of 0.01 M HCl. When GA was used for cross-linking, it was added 24 h in advance to allow for complete reaction (it has been shown that the reaction is completed within 2 h in our experimental conditions) [10]. The reaction of GA with collagen in acidic pH is much slower and less efficient than in alkaline or neutral pH, thus the control over the resulting matrix is better [10,24]. The matrix solution was equilibrated to 21 °C before the measurement.

2.3. Characterization of collagen solutions

2.3.1. Circular dichroism measurements

The spectra were recorded with Jasco J-810 spectropolarimeter. A scan speed of 50 nm/min and 0.2 cm cell were used. A reference spectrum containing 0.01 M HCl was subtracted from the collagen containing samples. Collagen solutions treated with various concentrations of GA and incubated for 24 h were diluted to 5.8×10^{-7} M concentration prior to measurement. The dilution procedure prevents saturation of the detector and quenches any further reaction of collagen with GA.

2.3.2. Viscosity measurements

The viscosity of the solution was measured using capillary viscometers (Conning). The temperature was kept constant at 21 °C.

2.3.3. Determination of cross-linking degree

The degree of cross-linking was measured as a loss of free ε-amino groups of lysine upon cross-linking with GA. The amount of free amino groups was determine using 2,4,6-trinitrobenzenesulfonic acid (TNBS, Pierce) as described by Li et al. [25] and Sheu et al. [26] Briefly, to a 100 μl of GA treated 0.5% (w/v) collagen matrix 400 μl of 0.1 M sodium bicarbonate solution and 250 μl of 0.1% TNBS (in 0.1 M sodium bicarbonate) were added and placed in the water bath (40 °C) for 2 h. After the reaction was completed, 300 μl of 12 M HCl was added to collagen and the temperature was raised to 60 °C. The solubilization of the collagen was achieved after 60 min. The samples were transferred to a spectrophotometer (Shimadzu UV-2401 PC) and the absorbance was measured at 355 nm. The degree of cross-linking was determined as a % loss of free ε-amino groups and is equal to: $(1-(Abs_{GA}/Abs_{coll}))\times100$, where Abs_{GA} represents the intensity of the cross-linked collagen and Abs_{coll} represents the intensity of the native collagen matrix.

2.4. ETOF micro-band device

The scheme of the device is presented in Fig. 2A. In this arrangement, 10 μm parallel band electrodes are spaced by a 20 μm gap. Each electrode, about 4 mm long, is independently connected to the potentiostat via contact pad. The fabrication of the devices follows standard photolithographic procedure and is described elsewhere [15,27]. MPS (mercaptopropyltrimethoxysilane) was used as a gold adhesive layer.

2.5. ETOF measurements

The CH Instruments bipotentiostat (model 760B) was used for all voltammetric (three electrodes configuration) and ETOF (four electrodes configuration) measurements. Platinum wire was used as a counter

Table 1
Solubility of the C_nTPA

Probe	Solubility [M]
C_3TPA	3×10^{-3}
C_4TPA	2×10^{-3}
C_5TPA	7×10^{-4}
C_6TPA	3×10^{-4}
C_7TPA	8×10^{-5}

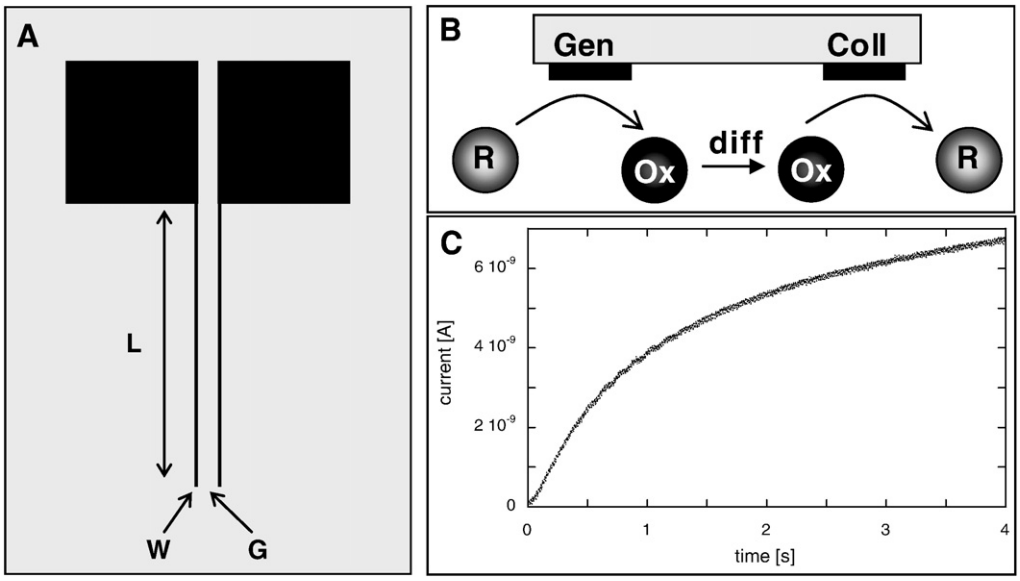

Fig. 2. Electrochemical time-of-flight technique: (A) generator–collector individual device; L=2 mm, W=10 μm, G=20 μm, (B) the principle of operation, R = reduced form and Ox = oxidized form of the redox probe, and (C) example of the ETOF transient recorded in 0.1 mM solution of C_3TPA in 0.01 M HCl.

electrode and silver wire as a pseudo-reference electrode. The band microelectrodes were tested before each ETOF experiment and the device was calibrated according to a previously described procedure [15]. The 0.01 M HCl was used as a supporting electrolyte. Dissolution of collagen in 0.01 M HCl results in formation of molecular collagen matrix. At the same time the C_nTPA probes show reversible voltammetric behavior in this electrolyte. The addition of GA does not change the voltammetry of C_nTPA within the tested concentration range. The values of diffusion coefficients of C_nTPA in collagen matrix solutions with and without GA clearly indicate the lack of chemical reaction between the C_nTPA and collagen. Therefore the quenching of the reaction of GA with collagen was not performed. All voltammetric and ETOF measurements were recorded at 21 °C.

2.6. Statistical analysis

The results are reported (where applicable) as an average ± standard deviation. Single factor ANOVA and Student's t-test were used for the hypothesis testing and parameters evaluation with $p<0.05$ were considered statistically significant.

3. Results and discussion

3.1. Characterization of collagen matrix

We have initially characterized the solutions of native and cross-linked collagen using circular dichroism (CD) and viscosity measurements. The 0.5% (w/v) collagen matrix was prepared by dissolving lyophilized collagen in 0.01 M HCl. The collagen dissolved in acidified water forms molecular collagen (tropocollagen) solutions and does not aggregate into fibrillar structure [28–30].

The circular dichroism spectra for 0.5% collagen in 0.01 M HCl upon addition of GA show characteristic ππ* and nπ* amide transitions at 197 nm and 220 nm respectively (Fig. 3). The intensities of both peaks do not depend upon the GA concentration. Previously we have shown that collagen self-assembly into a fibrillar structure (upon the change of pH from 2 to 6) results in a large change in the molar ellipticity of both peaks [15]. In our experiment shown in Fig. 3, the cross-linking with GA *does not* result in the change of molar ellipticity indicating that the native helical structure of collagen is preserved. This result also suggests the lack of aggregation of collagen molecules within the range of GA added (0–0.2% v/v) [15]. Thus it appears that the solutions containing both molecular collagen and molecular collagen cross-linked with GA up to 0.2% GA concentration are homogenous and do not contain fibrillar collagen.

We have also performed viscosity measurements of collagen dissolved in 0.01 M HCl upon addition of GA. Fig. 4 shows essentially monotonically increasing viscosity of the collagen matrix upon addition of GA indicating efficient cross-linking process. The absence of plateau in Fig. 4 suggests that, for the studied concentration range, some of the lysine residues in the collagen do not participate in the cross-linking process for the GA concentrations used [31].

To determine the degree of cross-linking we have performed colorimetric assays with 2,4,6-trinitrobenzenesulfonic acid (TNBS). The TNBS is often used in the quantification of primary amino groups in collagen [32]. We have followed modified procedure previously described by Sheu et. al. [26] and Li et. al. [25] (see Materials and methods section). Fig. 5 shows that cross-linking with GA in our experimental conditions results in the loss of 55–75% primary amine groups. This result is in agreement with viscosity data, indicating that the collagen matrix is not fully cross-linked under our experimental conditions.

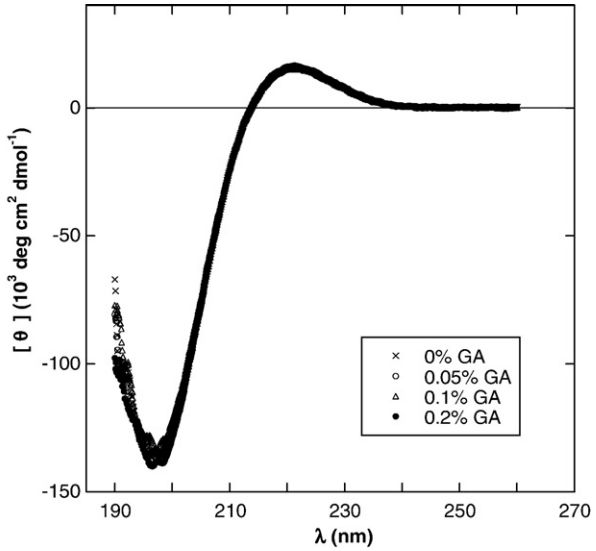

Fig. 3. CD spectra of 0.5% (w/v) collagen solution in 0.01 M HCl upon addition of GA (% v/v). All spectra were recorded for the same concentration of collagen 5.8×10^{-7} M.

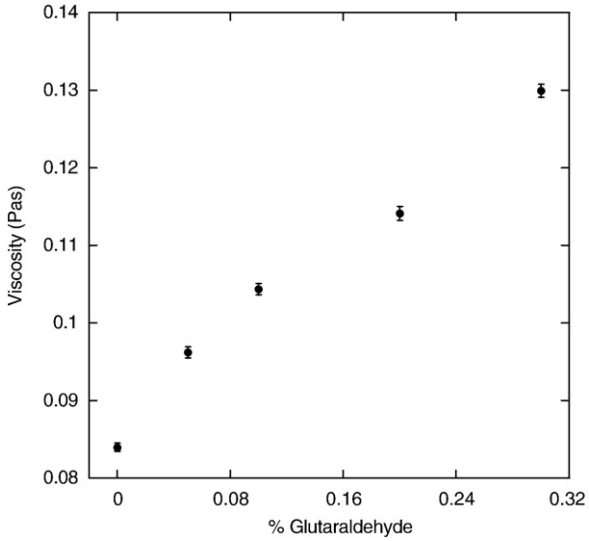

Fig. 4. Viscosity of 0.5% collagen solution measured upon addition of GA. Standard deviation on the figure is comparable with the point size and was calculated for four measurements.

3.2. Diffusion of molecular probes within native collagen matrix solution

Diffusion coefficients of the C_nTPA molecular probes were measured using electrochemical time-of-flight method as described previously [15]. Briefly, the comparison of the unknown transit time of C_nTPA (τ_1) and the known transit time of $Ru(NH_3)_6^{2+}$ (τ_2) and its known diffusion coefficient ($D_2 = 7.8\times10^{-6}$ cm^2/s) yields the absolute value of C_nTPA diffusion coefficient (D_1):

$$D_1 = (\tau_2/\tau_1)D_2. \quad (2)$$

For example, using Eq. (2) we determined the diffusion coefficient of C_3TPA^+ in 0.01 M HCl to be $5.8\pm0.1\times10^{-6}$ cm^2/s. The diffusion coefficients for the remaining C_nTPA probes were determined using the same method. The same device geometry was used through all of the ETOF experiments reported in this work.

Fig. 6 shows the diffusion coefficients of C_nTPA probes in pure electrolyte solution containing 0.01 M HCl in water (open circles), and in 0.5% collagen dissolved in 0.01 M HCl (closed circles). The nominal concentration of C_nTPA was 1×10^{-4} M, except C_7TPA, where concentration was 5×10^{-5} M.

The values of the diffusion coefficient of C_nTPA measured in pure electrolyte decrease from 5.8×10^{-6} cm^2/s for $n=3$ to 4.6×10^{-6} cm^2/s for $n=7$. This trend is consistent with the behavior predicted by the Stokes–Einstein law. In general, the molecules with longer hydrocarbon chain are expected to have a larger hydrodynamic radius and therefore diffuse slower. We need to keep in mind, however, that the conformation of the hydrocarbon chain of the probe affects the hydrodynamic radius in a complex fashion and therefore some deviations from the expected relationship between the length of the hydrocarbon chain and the diffusion coefficient can be expected.

For each C_nTPA probe, the diffusion coefficient measured in 0.5% collagen is smaller than the diffusion coefficient measured in pure electrolyte solution. The viscosity of 0.5% collagen dissolved in 0.01 M HCl electrolyte is about 80-fold larger than the viscosity of pure 0.01 M HCl [15]. Yet the diffusion coefficients of C_nTPA in 0.01 M HCl decrease less than 2-fold upon addition of collagen. Thus this change of diffusion coefficient of molecular probes does not follow the Stokes–Einstein relation. This is an expected result since the diffusion coefficient in polymer of this type is related to the local microscopic viscosity within the network of polymeric chains rather than the macroscopic intrinsic viscosity [33,34]. Indeed, several literature reports treat the value of a diffusion coefficient of a molecular probe as a measure of the average pore size of the matrix [33,34]. Thus it appears that the moderate change in the value of diffusion coefficient of C_nTPA in electrolyte upon addition of 0.5% collagen reflects the formation of a porous structure of collagen matrix as described earlier [15].

In 0.5% collagen solution, the probes with longer aliphatic chains diffuse faster than the smaller probes. This trend is opposite to the one observed in pure electrolyte solution. Indeed, the D^{coll}/D^o ratio (insert in Fig. 6) varies from 0.59±0.04 for C_3TPA probe to 0.87±0.05 for C_7TPA molecule. The C_nTPA probes exist in solution as "1+" cations. In general, the larger (with smaller charge density) and more hydrophobic probes diffuse faster in 0.5% collagen solution and smaller and more hydrophilic probes appear to be retained longer. We hypothesize that the repulsive ionic interactions between the parts of the collagen matrix and the C_nTPA molecules explain the relationship presented in the insert in Fig. 6 (n vs. D^{coll}/D^o).

The ionic interactions between the C_nTPA and collagen molecule are repulsive since the C_nTPA probes are positively charged in the solution and the experiment is performed below the isoelectric point

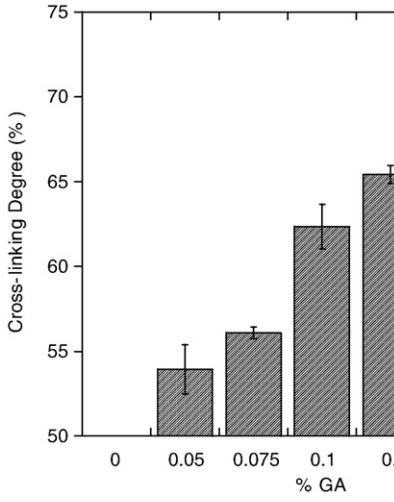

Fig. 5. Cross-linking degree measurement for 0.5% collagen matrix cross-linked with variable concentration of GA in 0.01 M HCl. The error bars reflect the standard deviation calculated for 6 measurements.

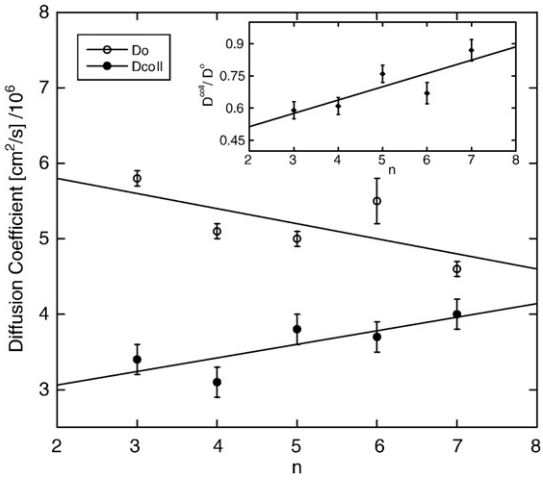

Fig. 6. The diffusion coefficients of C_nTPA measured in 0.01 M HCl (D^o, open circles) and in 0.5% collagen matrix solution (D^{coll}, closed circles). The error bars indicate standard deviations calculated for 10 measurements. The lines are to guide the eye. The insert in the figure shows the variation of the ratio (D^{coll}/D^o) of averages and standard deviation of the ratio is calculated using propagation of error analysis from standard deviation of D^o and D^{coll}.

of collagen thus the protein is also positively charged [35]. The ionic charge density is smallest for the largest C_nTPA probe, thus the openings between the collagen chains (or pores) in the matrix will show strongest electrostatic repulsion with the smallest probe leading to the smallest diffusion coefficient for this probe. In other words, the average effective size of the "pore" within the matrix structure is partly determined by the electrostatic interactions between the particular redox probe and the positively charged collagen molecules. Somewhat analogous behavior was recently observed in the diffusion studies in polyacrylic acids upon their neutralization with various alkali metal hydroxides [36]. It is worth noting that the possible hydrophobic interactions between the parts of collagen and C_nTPA should result in lower diffusion coefficients for more hydrophobic probes. The opposite effect is observed in our experiment further confirming that the observed effect should be interpreted in terms of electrostatic interactions between the positively charged collagen and the positively charged redox probe.

3.3. Diffusion of molecular probes within GA cross-linked collagen matrix

The collagen used in drug delivery systems is usually stabilized by cross-linking. This procedure improves the stability of the matrix against biodegradation. The most common cross-linking procedure, addition of GA to collagen, results in reaction between the lysine residues and GA and leads to the formation of imine bonds. It was postulated that this reaction might increase the hydrophobic character of the matrix because of the loss of possible protonation sites and incorporation of an alkyl chain from GA molecule [37].

To study the effect of cross-linking on the diffusion of C_nTPA in collagen we have used ETOF to measure the diffusion coefficients of the C_nTPA molecules in the tropocolagen solution treated with different amounts of GA (D^{GA}). Fig. 7 shows the diffusion coefficients for C_nTPA molecules normalized with respect to diffusion coefficients measured in non-cross-linked 0.5% collagen solutions (D^{coll}) for each probe. In this approach, $D^{coll}/D^{GA}>1$ indicates that a probe is retained longer in a solution upon addition of a particular amount of GA. Inspection of the data shown in Fig. 7 indicates that for C_nTPA ($n=3$, 4 and 6) the diffusion coefficient increases or remains unchanged upon cross-linking ($D^{coll}/D^{GA}<1$). Different behavior is seen for C_nTPA ($n=5$ and 7) where the diffusion coefficient initially decreases (statistically significant change, $p<0.05$) and reaches a minimum for GA concentrations equal to 0.05% and 0.1% respectively. Thus our data indicate

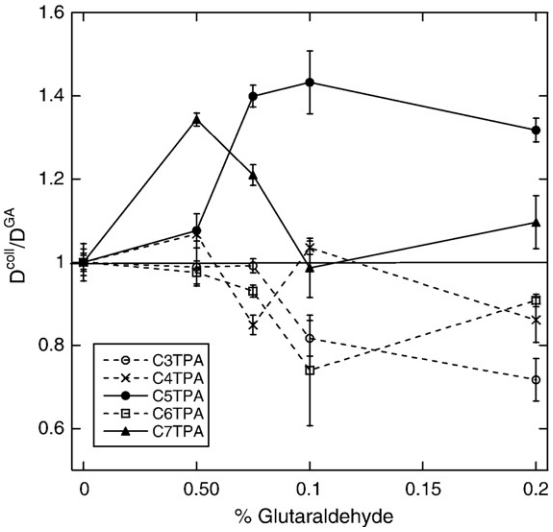

Fig. 7. Normalized diffusion coefficients (D^{coll}/D^{GA}) of C_nTPA in 0.5% collagen matrix solution plotted vs. GA concentration. The error bars reflect the standard deviation calculated for 10 measurements.

that C_nTPA molecules containing 5 or 7 carbons are retained within the cross-linked matrix while other probes show either constant or increasing rates of diffusion.

The addition of GA leads to geometrically smaller pores with a pore size depending upon the concentration of GA. Furthermore as cross-linking progresses the number of protonated lysine residues decreases thus reducing the overall charge of the collagen molecule. Therefore the interplay between the sterical hindrance due to the physically smaller pore openings and the electrostatic effect associated with the probe/matrix repulsion is expected to influence the observed diffusion coefficient of C_nTPA.

In principle, the average pore size in the GA cross-linked collagen could be estimated by modeling this system using the effective medium (Brinkman) model together with the Carman–Kozeny model [33,34]. In these models, the changes in diffusion coefficient of a tracer molecule can be linked to the change of a pore size within the matrix. However, major assumption in this model is that there are no interactions between the probe and the medium. Clearly, each of C_nTPA shows different interactions with collagen and the strength of these interactions varies with the chain length. Moreover the Carman–Kozeny–Brinkman model assumes random distribution of pores modeled as cylinders within a 3-D space. Thus the higher level organization of the pores cannot be described with the existing theory.

Since the cross-linking lowers the average charge density on the collagen molecules, the diffusion coefficient of the smaller C_nTPA molecules (thus with a large charge density on the probe) should increase with the increasing extent of cross-linking. This effect is somewhat analogous to the D vs. n dependence shown in Fig. 6 for non-cross-linked collagen and C_nTPAs with variable size. Again, the lower electrostatic repulsion between the C_nTPA and the charged collagen matrix results in larger diffusion coefficients. Indeed for $n=3$ and 4 the diffusion coefficient, in general, decreases with the increase of GA concentration (and thus increase of cross-linking). Yet the opposite behavior is seen for C_nTPA $n=5$ and 7. Those probes are retained longer within the cross-linked matrix. Furthermore the maximum retention is observed for both probes at different concentrations of GA (0.05% and 0.1% for $n=5$ and 7 respectively).

Therefore it appears that the cross-linking of collagen indeed introduces some degree of hydrophobicity into the system. It is worth noting that these dependencies are complex since five different parameters should be considered: the radius of the probe, the radius of the matrix pore, hydrophobic character of the probe, hydrophobic character of the matrix and the geometric factors related to the ability of a particular probe to interact with the hydrophobic spots in the matrix. The combination of these parameters leads to the maximum retention of C_5TPA for 0.1% of GA and to the maximum retention of C_7TPA for 0.05% of GA.

The observed change in the diffusion coefficient of molecular probes is not large, but statistically significant. It appears that the retention of C_nTPA ($n=5$ and 7) molecules is very sensitive to the length of the aliphatic chain, thus it must be related to the size and geometry of the hydrophobic regions (pockets) formed in the cross-linked collagen. It is worth noting that C_6TPA is not retained in any of the above cross-linking conditions, thus the hydrophobic pockets interact rather selectively with the alkyl chain of the probe. This selectivity implies that the hydrophobic pockets formed under particular cross-linking conditions have sizes comparable to the size of the particular C_n chain in the C_nTPA.

We note that the D^{coll}/D^{GA} value for C_6TPA (Fig. 7) is abnormally low. However the diffusion coefficient of C_6TPA in water appears to be higher than that of C_5TPA thus indicating smaller than expected hydrodynamic radius of the probe. We believe that this abnormal value could be a result of alkyl chain conformation as discussed earlier. Thus it appears that the effects related to the size and shape of the molecule dominates over hydrophobic interactions between the C_6TPA and the cross-linked collagen.

The data reported above suggest that the size of hydrophobic pockets can be controlled by the degree of cross-linking. It is also reasonable to assume that the GA used for the cross-linking participates in the formation of the pocket. The ability to control the formation of the hydrophobic pockets of variable size and geometry would open new possibilities of using collagen matrices for the delivery of hydrophobic drug molecules. This could be achieved, for example, by varying the structure of the cross-linking agent. Therefore, by changing the concentration, length, and the hydrophobicity of the agent, the shape and the character of the cavities incorporating the drug can be tuned.

4. Conclusions

The diffusion coefficients of C_nTPA in pure electrolyte show behavior predicted by Stokes law, i.e. the diffusion coefficient is inversely proportional to the diameter of a molecule. The opposite relationship is observed in 0.5% (w/v) collagen I matrix solution where the molecules with longer alkyl chains show larger diffusion coefficients than the smaller molecules. This effect is explained in terms of electrostatic interactions between the positively charged gel and the positively charged redox probe. The measurements of the diffusion coefficients of C_nTPA in 0.5% collagen upon cross-linking indicate that C_7TPA and C_5TPA are retained within the matrix upon addition of 0.05% GA and 0.1% (v/v) GA respectively, while C_3TPA, C_4TPA and C_6TPA molecules show unchanged or larger diffusion coefficients upon additions of GA. Thus, the diffusion coefficients of C_nTPA measured within the GA cross-linked matrix show the presence of hydrophobic interactions. The interactions can be attributed to the formation of small hydrophobic pockets. The hydrophobic pockets seem to interact selectively with the hydrocarbon chain of C_nTPA probes. The size of the pocket and thus the specificity towards particular length of C_nTPA depends upon the extent of cross-linking. The reported findings might provide new insights in the design of an effective matrix for controlled delivery of small hydrophobic drugs.

Acknowledgments

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