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Diffusion of ions in a calcium alginate hydrogel-structure is the primary factor controlling diffusion

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ABSTRACT

The diffusion of solutes has been evaluated in an alginate hydrogel as a function of its structure. The role of solute and gel charge on the diffusion measurements were of particular interest. Diffusion coefficients were measured using fluorescence correlation spectroscopy as a function of solute charge and size, bulk solution ionic strength and pH, and gel density. Diffusion coefficients of fluorescent dextrans with hydrodynamic radii up to 6 nm were reduced by 30% in a 1.8% (w/w) hydrogel whereas they were reduced by only 2% in a 0.2% (w/w) hydrogel. The role of ionic strength was examined for various concentrations (0.1–100 mM) and compositions of ions (Na^+, Ca^{2+} or mixtures thereof). The diffusion coefficient of a small charged probe (rhodamine 6G, $R6G^+$) did not change significantly with increasing ionic strength when sodium was used as the counter ion. The diffusion coefficient was only moderately influenced by the charge of solutes (from +1 to −2). Similarly, pH variations from 3 to 9 had little impact on the diffusion coefficients of $R6G^+$ in the gel. On the other hand, the addition of Ca^{2+} had a significant impact on gel compactness, which led to a significant reduction in solute diffusion. For the calcium alginate hydrogels, structural modifications resulting from Ca binding were much more important than electrostatic effects due to modifications of the gel Donnan potential.

1. Introduction

The diffusive properties of solutes in gels are key to a large number of environmental (Chang & Huang, 1998; Chen, Hong, Wu, & Wang, 2002; Davis, Llanes, Volesky, & Mucci, 2003), pharmaceutical (Pillay, Dngor, Govender, Moopanar, & Hurbans, 1998) and biotechnological (Lim & Sun, 1980) applications. The diffusion of a solute through a hydrogel will depend upon both physical (e.g. obstruction (Amsden, 1998a; Giddings, Kucera, Russel, & Myers, 1968; Johansson, Skantze, & Lofroth, 1991)) and chemical (e.g. hydrogen bonding, electrostatic effects (Fatin-Rouge, Milon, Buffle, Goulet, & Tessier, 2003; Nilsson, Nordenskiold, Stilbs, & Braunlin, 1985)) interactions between the two phases, which are dependent upon the physicochemical properties of both the solute and the gel. For example, solute size (Petit, Zhu, & Macdonald, 1996) and charge (Johansson, Skantze, & Lofroth, 1993) and the extent of gel crosslinking (Amsden, 1998b) have been well documented to influence diffusion in hydrogels. For calcium alginate hydrogels, electrostatics has also been thought to play an important role in the diffusion of sodium (Lundberg & Kuchel, 1997) and bovine serum albumin (BSA) (Amsden, 2001) at various ionic strength and pH (Kalis, Davis, Town, & Van Leeuwen, 2009a). Indeed, a decreased mobility of charged solutes was attributed to an increased electrostatic interaction between the solute and the gel (Holte, Tonnesen, & Karlsen, 2007; Lundberg & Kuchel, 1997). Several recent papers (Davis, Kalis, Pinheiro, Town, & Van Leeuwen, 2008; Kalis et al., 2009a; Kalis, Davis, Town, & Van Leeuwen, 2009b) have confirmed a large Donnan potential in alginate gels, resulting in significant cation partitioning. Nonetheless, the precise role of gel (and solute) charge on diffusion is not clear. For example, ionic strength increases (or pH decreases) can lead to contrasting effects on solute mobility in gels by (i) decreasing the gel Donnan potential, leading to decreased partitioning of solutes with the gel (Sangi, Halstead, & Hunter, 2002); (ii) decreasing the double layer thickness on the pores, leading to an increase of the effective pore size in the hydrogel (Golmohamadi, Davis, & Wilkinson, 2012); (iii) reducing electrostatic repulsion among the gel fibers, leading to a compression of the gel structure and decrease in the physical pore size; or (iv) increasing the homocoagulation of the diffusing solutes, effectively decreasing diffusion (Wilkinson, Gendron, & Avaltroni, 2008). In order to quantitatively determine the effects of charge on solute diffusion, especially in highly charged gels such as alginate, further investigation is thus required.

In this paper, the diffusion coefficients of a number of charged probes with relatively small sizes were evaluated in order to assess the relative importance of charge interactions on diffusion through a negatively charged calcium alginate hydrogel. Ionic strength (I) and pH of the bulk medium and the nature of the diffusing solutes

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were systematically varied in order to determine the role of charge effects on diffusion in an alginate hydrogel.

2. Experimental

2.1. Materials

Medium viscosity alginate, D-glucono-δ-lactone (GDL), rhodamine 6G (R6G), calcium nitrate and calcium carbonate were purchased from Sigma–Aldrich. Rhodamine 110 (R110) and ultrapure nitric acid were acquired from Fluka. Fluorescently labeled dextrans (molar masses = 3k, 10k, 40k and 70k) and other fluorescent probes – tetramethylrhodamine, methyl ester (TMRM); Oregon green 488 carboxylic acid, succinimidyl ester (Oregon 1C); Oregon green 488 carboxylic acid (Oregon 2C) – were purchased from Invitrogen. For all fluorophores except Oregon 1C and 2C, small quantities of fluorophore were added to Milli-Q water ($R > 18\,\mathrm{M}\Omega\,\mathrm{cm}$) to obtain stock solutions in the micromolar concentration range. Oregon 1C and 2C were dissolved in 1 mM morpholineethanesulfonic acid (MES) at pH 7.2. Samples were prepared by dilution of the stock solutions into an electrolyte solution with a controlled pH and ionic strength in order to obtain a final probe concentration of 20 nM (pH: 3–9, I: 0.1–100 mM). Dilute HNO_3 (Fluka), sodium hydroxide (Sigma) and sodium nitrate (Fluka, analytical grade) were used to adjust the pH and ionic strengths of the solutions. pH was measured using a Metrohm 744 pH meter, calibrated with standard NBS buffers. All products were used without further purification.

2.2. Hydrogel preparation

The hydrogel was prepared according to the method of Draget, Ostgaard, and Smidsrod (1989). Simply, sodium alginate solutions were stirred overnight. The next day, calcium carbonate ($CaCO_3$) particles were dispersed into the viscous alginate solutions and degassed under vacuum. Finally, a freshly prepared solution of D-glucono-δ-lactone (GDL, 30 mM) was added to the mixture and stirred for 2 min, after which the gel was poured into cylindrical wells (for swelling measurements) or FCS coverslips (for FCS experiments). The cylindrical gel pieces (diameter = 1 cm; height = 0.5 cm) were left 24 h to solidify and then equilibrated in the desired experimental solutions for another 24 h. In a few selected experiments, hydrogels were first immersed in a 50 mM $Ca(NO_3)$–20 mM $Na(NO_3)$ mixture for 48 h prior to transfer into 20 mL of a pH and ionic strength controlled experimental solution for an additional 48 h (solutions were refreshed 3×).

2.3. Diffusion measurements

Diffusion coefficients of fluorescent solutes were measured by fluorescence correlation spectroscopy (FCS) using a Leica TCS SP5 laser scanning microscope equipped with an argon ion (Ar^+) laser (excitation at 488 or 514 nm) and a DPSS Nd:YVO_4 laser (excitation at 561 nm). An avalanche photodiode detector was used to quantify fluorescence intensity fluctuations in the small volume (ca. 1 μm^3) defined by the confocal optics of the instrument. Fluorescence was measured in the emission ranges of 500–530 nm or 607–683 nm. For any given set of calibrations/experimental measurements, the position of the laser from the bottom of the coverslip was kept constant.

Diffusion coefficients measured by FCS are attributed to the Brownian motion of a fluorescent solute (*self*-diffusion) after its equilibration with the hydrogel (i.e. chemical potential gradient equal to zero). Such measurements can be contrasted with measurements of *mutual* diffusion, which are directional and driven by concentration gradients. The characteristic time that a fluorescent probe spends in the confocal volume, τ, is determined from an autocorrelation function, $G(t)$:

$$G(t) = a + \left(\frac{1}{N}\right)\left(1 + \left(\frac{t}{\tau}\right)^{\delta}\right)^{-1}\left(1 + \frac{1}{p^2}\left(\frac{t}{\tau}\right)^{\delta}\right)^{-0.5} \quad (1)$$

where a is the limiting value of $G(t)$ for $t \to \infty$; N is the average number of fluorescent particles diffusing through the confocal volume, t is the measurement time, δ accounts for anomalous diffusion in the gel (Banks & Fradin, 2005) and p is the structural parameter (ratio of the transversal, ω_{xy}, to the longitudinal, ω_z, dimension of the confocal volume: $p = \omega_z/\omega_{xy}$). Diffusion coefficients are determined from the measured values of τ:

$$D = \frac{\omega_{xy}^2}{4\tau} \quad (2)$$

Prior to measurements, ω_{xy} is obtained from a calibration of the confocal volume using R110, which has a known diffusion coefficient of $4.42 \times 10^{-10}\,\mathrm{m^2\,s^{-1}}$ (Wilkinson et al., 2008).

Fluorescent probes (typically 2×10^{-8} M) were added to the top of a small quantity (0.25 mL) of gel in each of the 8 wells of an FCS cell; solutions were refreshed at least three times over the 24 h period leading up to the FCS measurements. For each experimental condition, diffusion coefficients were measured in the bulk solutions and in the hydrogels under identical conditions. Diffusion was examined as a function of the pH and ionic strength of the bulk solution, the charge and size of the fluorescent probe and the weight fraction of the gel. For each experimental condition, results were obtained at a minimum of 8 different locations in the gel. In addition, experiments were repeated with freshly prepared gels on different days. Means and standard deviations were obtained from all of the repeated measurements (combination of different days and different gel locations). Acquisition times of 100 s were used to optimize the signal-to-noise ratio.

2.4. Swelling measurements

It was assumed that the swelling of the gel due to changes in the physicochemistry of bulk solution would have an influence on solute diffusion. Swelling was quantified using a swelling factor (S), which was determined from the ratio of the mass of the hydrogel before (m_1) and after (m_2) its equilibration in the experimental solutions:

$$S = \frac{m_2}{m_1} \quad (3)$$

As above, gels were allowed to equilibrate with external solutions for at least 48 h, with at least 3 renewals of the experimental solutions over this period.

3. Results and discussion

3.1. Effect of probe size and gel concentration on the diffusion coefficient

The diffusion coefficients of solutes in hydrogels are generally smaller than those in the bulk solution due to the presence of the polymer network that limits the free volume available for diffusion (Amsden, 1998a, 2001; Aslani & Kennedy, 1996; Martinsen, Storro, & Skjark-Braek, 1992; Muhr & Blanshard, 1982; Wang & Spencer, 1998). This effect was indeed seen for the diffusion of the dextran size standards for several different concentrations of alginate (Fig. 1). The diffusion coefficient of the largest dextran (hydrodynamic radius, r_h, of 6.2 nm) decreased by ca. 20% in the 0.2% (w/w) alginate and by ca. 50% in the 1.8% (w/w) hydrogel when compared to measurements in water (i.e. D_g/D_w of 0.8 and