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Determination of diffusion coefficients of peptides and prediction of permeability through a porous membrane

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Abstract

The diffusion coefficient (D) of peptide and protein drugs needs to be determined to examine the permeability through biological barriers and to optimize delivery systems. In this study, the D values of fluorescein isothiocyanate (FITC)-labelled dextrans (FDs) and peptides were determined and the permeability through a porous membrane was discussed. The observed D values of FDs and peptides, except in the case of insulin, were similar to those calculated based on a relationship previously reported between the molecular weight and D of lower-molecular-weight compounds, although the molecular weight range was completely different. The observed D value of insulin was between the calculated values for the insulin monomer and hexamer. The permeability of poly-lysine and insulin through the membrane was determined and the observed values were compared with predicted values by using the relationship between molecular weight and D and an equation based on the Renkin function. The observed permeability of insulin through the membrane was between that of the predicted permeability for the insulin monomer and hexamer. For the permeation of insulin, the determination of D was useful for estimating the permeability because of the irregular relationship between molecular weight and D. The methodology used in this study will be useful for a more quantitative evaluation of the absorption of peptide and protein drugs applied to mucous membranes.

Introduction

The delivery of therapeutic peptides and proteins needs to be optimized to obtain the most potent therapeutic effects. The dosage forms should be designed by considering many factors, including the release rate of the peptides or proteins and the degradation rate itself (Watnasirichaikul et al 2002; Kim & Peppas 2003). Especially for oral, nasal or pulmonary administration, both the release and absorption rate should be well controlled to obtain high enough bioavailability. In those processes, diffusion is the major mechanism of transport (Flynn et al 1974; Stay et al 2003). Therefore, the diffusion of such drugs immediately after administration of the delivery systems should be examined in detail (Weiss 1999; Salvetti et al 2002). The diffusion coefficient (D) of peptide and protein drugs having a relatively high molecular weight is lower than that of common therapeutic agents, and this value can vary with changes in conformation (Chittchang et al 2002; Salamat-Miller et al 2002) and interaction with other molecules (Bhat et al 1996; Nardviriyakul et al 1997; Larhed et al 1998). In some reports, the diffusivity of peptide and protein drugs during the processes of release and membrane permeation has been examined to optimize the delivery systems (Aso et al 1998; Agarwal et al 2001; Choi & Kim 2003).

In our preliminary reports, the D values of many kinds of drugs were determined by a chromatographic broadening method (CBM) and the effects of physicochemical properties and molecular interactions on the D values were discussed, although the molecular weight of the drugs was relatively low (Seki et al 2000, 2003a, b). Now, we wish to apply the same methodology to higher-molecular-weight compounds, such as peptide and protein drugs. The D values in several media were determined by CBM without molecular labelling and expensive equipment.

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The most important process related to the diffusion of drugs during their administration to mucous membranes is absorption (Nardviriyakul et al 1997; Larhed et al 1998). Since the absorption rate of higher-molecular-weight hydrophilic drugs is low, absorption enhancement is needed for the delivery of sufficiently high amounts. Such drugs may be absorbed via an intercellular pathway, which can be regarded as an aqueous channel, and the diffusivity in the channel determines the absorption rate (Pade & Stavchansky 1997; Pauletti et al 1997; Dorkoosh et al 2004). Therefore, improving the diffusivity in the channel is one way to enhance the absorption of such drugs. The size of the aqueous channel might be several nanometres and this could be similar to the hydrodynamic molecular size of therapeutic peptides and proteins (Tomita et al 1992; Hämäläinen et al 1997; Horibe et al 1997). In this case, friction affects the penetration rate through the channel, and the hydrodynamic molecular size is the most important parameter for determining the penetration rate. Since the hydrodynamic molecular size can be evaluated from the diffusivity in the medium, the absorption rate of drugs through such membranes can be predicted by determination of the D value (Adson et al 1994; Roberts et al 1998; Edwards & Prausnitz 2001).

In this study, the D values of fluorescein isothiocyanate (FITC)-labelled dextrans (FDs) and peptides were determined by CBM and the molecular-weight dependence was compared with that of parabens and steroids previously reported (Seki et al 2003a) to evaluate the suitability of CBM for higher-molecular-weight compounds. In addition, the D values of poly-lysine (MW = 16 000) and insulin (MW = 5800) were used to predict the permeation rates through a porous cellulose membrane (MW cut-off = 15 000). Insulin and poly-lysine were chosen as model penetrants because insulin is commonly used as a model drug and poly-lysine was used for studies of the relationship between the diffusivity and membrane permeation by Salamat-Miller et al (2002) and Chittchang et al (2002). The equivalent radius of the membrane pore (r_p) was determined by an equation based on the Renkin function (Renkin 1954) and the relationship between r_p and the hydrodynamic radii (r_i) of poly-lysine and insulin calculated from the D values is discussed.

Materials and Methods

Materials

FD4 (MW = 4400), FD10 (MW = 9500), FD40 (MW = 38 260), FD70 (MW = 50 700) and poly-lysine (MW = 16 000) were purchased from Sigma Chemical Co (St Louis, MO). Oxytocin, angiotensin II, [Lys^8]-vasopressin, [Arg^8]-vasopressin, α-conotoxin ImI, somatostatin, cortistatin (rat), gastrin I, endothelin-3, calcitonin, β-endorphin and gastric inhibitory polypeptide (GIP) were obtained from the Peptide Institute (Osaka, Japan). Insulin (human recombinant) and 5(6)-carboxyfluorescein (CF) were purchased from Wako Pure Chemical (Osaka) and Acros Organics (NJ), respectively. Spectra/Por Biotech Membranes (MW cut-off = 15 000; Spectrum Laboratories, CA) were used as the porous cellulose membrane. Other chemicals were of reagent grade and were used without further purification.

Determination of D by chromatographic broadening method (CBM)

The D values of FDs, CF, poly-lysine and peptides were determined by CBM as described previously (Seki et al 2000). An HPLC system (LC-10A; Shimadzu, Kyoto, Japan) equipped with a 10 m, 0.75 mm i.d., length of PEEK tubing (GL Sciences, Tokyo, Japan) was used for the studies. The HPLC system consisted of a pump (LC-10AD), oven (CTO-10AC), detector (RF-10A or SPD-6A), and integrator (CR-5A). A Rheodyne 7125 injector with a 10-μL sample loop was used for the sample injection. The PEEK tubing was placed in the oven and connected directly to the injector and detectors, and kept at 37°C. For the determination of the D of FDs and CF, RF-10A was used as the detector and operated at an excitation wavelength of 495 nm and an emission wavelength of 515 nm. For peptides and poly-lysine, SPD-6A was operated at 278 nm and 220 nm, respectively. A phosphate buffer solution (10 mM, pH 7.4) was used as the mobile phase and the solvent for the compounds. The flow rate of the mobile phase was 0.1 mL min^{-1}. In some cases, asymmetrical peaks were observed for peptides. This could be due to an interaction between the peptide and the tubing. In such cases, 10 mM phosphoric acid solution (pH 2.2) was used. Only for insulin was a symmetrical peak not observed in both solutions. Each solution containing CF, FDs, poly-lysine or peptides was injected into the system. The values of the retention time (t_R) and the eluted peak width at half height ($W_{1/2}$) were obtained by integration.

The D values were determined from equation 1, where r is the inner radius of the tubing (0.420 mm) determined from calibration runs (Seki et al 2000).

$$D = (0.231 \times r^2 \times t_R)/W_{1/2}{}^2 \quad (1)$$

The determination of D under each set of conditions was repeated 11 times and the mean value was calculated.

Determination of D of insulin by the porous membrane permeation method

The D of insulin was determined by the porous membrane method. A membrane filter having straight pores (Anodisc 25, pore size = 0.2 μm; Whatman, NJ) was used. The membrane was mounted on a 2-chamber diffusion cell with an available diffusion area of 0.95 cm^2. The whole cell set was kept at 37°C. Isotonic phosphate buffer (PBS, pH 7.4, 3.0 mL) was added to the receptor chamber and FD10 (50 μg mL^{-1}) or insulin (12.5 IU mL^{-1}) solution (3.0 mL) in PBS was added to the donor chamber. At predetermined times, the receptor medium (1.0 mL) was withdrawn and fresh medium was added to keep the volume constant. There were no marked volume changes in the donor and receptor phase at the end of the experiments. For the determination of FD10 and insulin, a fluorescence spectrophotometer (excitation wavelength

495 nm and emission wavelength 515 nm, F-2000; Hitachi, Tokyo) and a Micro BCA protein assay kit (Pierce, IL) were used, respectively. The D of insulin (D_{Ins}) was calculated from equation 2.

$$D_{Ins} = D_{FD10} \times (P_{Ins,AD}/P_{FD10,AD}) \quad (2)$$

where D_{FD10} is the D of FD10 determined by CBM, and $P_{Ins,AD}$ and $P_{FD10,AD}$ are the permeability coefficients of insulin and FD10 through the membrane, respectively.

Permeation studies through the porous cellulose membrane

To evaluate the pore size of the porous cellulose membrane, the permeability coefficients of CF and FD10 (P_{CF} and P_{FD10}) were determined. The membrane was mounted on the 2-chamber diffusion cell and then the whole cell set was kept at 37°C. PBS was added to the receptor chamber and a solution containing FD10 ($200\,\mu g\,mL^{-1}$) and CF ($500\,ng\,mL^{-1}$) in PBS was added to the donor chamber. At predetermined times, the receptor medium (1.0 mL) was withdrawn and fresh medium was added to keep the volume constant. FD10 and CF in the medium were isolated and determined by a gradient HPLC system consisting of a pump (LC-10AT), oven (CTO-6A), detector (RF-10A) and integrator (CR-5A). Separation was carried out using an analytical column (Shodex Asahipak NH2P-50 4E, 250 × 4.6 mm i.d.; Showa Denko, Kawasaki, Japan) and a gradient of 0.1 M triethanolamine + 10% acetonitrile–0.1 M triethanolamine + 10% acetonitrile + 60 mM NaCl from 30:70 to 80:20 over 30 min. The flow-rate of the eluent was $1\,mL\,min^{-1}$, the oven was operated at 45°C and the detector was operated at an excitation wavelength of 495 nm and an emission wavelength of 515 nm. The experiments were carried out four times to obtain mean values.

The r_p value of the membrane was calculated from an equation based on the Renkin function (Renkin 1954):

$$P_i = D_i\,\varepsilon/L\,[1-(r_i/r_p)]^2\,[1-2.109\,(r_i/r_p) + 2.09\,(r_i/r_p)^3 - 0.05\,(r_i/r_p)^5] \quad (3)$$

where P_i, D_i and ε/L are the permeability coefficient of the penetrant i through the membrane, the diffusion coefficient of the penetrant i in the medium used as the solvent and the ratio of the area fraction of the pore pathway to the barrier length of the membrane, respectively. Equations such as equation 3 will be useful for evaluating the permeation of drugs through porous membranes (Adson et al 1994; Horibe et al 1997; Pade & Stavchansky 1997; Tavelin et al 2003). Hydrodynamic radii (r_i) were calculated from the following Stokes-Einstein equation:

$$r_i = kT/6\pi\eta D_i \quad (4)$$

where k, T and η are the Boltzmann constant, absolute temperature and viscosity of the solvent, respectively. The values of r_p and ε/L as the membrane parameters were calculated from the values of P_i, D_i and r_i of FD10 and CF.

Permeation of poly-lysine and insulin through the porous cellulose membrane was also examined in the same way as for FD10 and CF. Insulin ($12.5\,IU\,mL^{-1}$) or poly-lysine ($1.92\,mg\,mL^{-1}$) solution in PBS was applied to the cell instead of the solution containing FD10 and CF. The concentrations of insulin and poly-lysine were determined using a Micro BCA protein assay kit. The experiments for poly-lysine and insulin were carried out 4 times to obtain mean values. The observed permeability coefficients of insulin (P_{Ins}) and poly-lysine ($P_{poly\text{-}Lys}$) were compared with the calculated values. To obtain the calculated permeability coefficients, the D values were obtained first from the molecular weight by a previously reported equation (equation 5) (Seki et al 2003a) then the r_i values were calculated from D_i by equation 4, and, finally, the calculated permeability coefficients were obtained using equation 3.

Statistical analysis

The mean values and their standard deviations (s.d.) were calculated in each experiment. The effect of disulfide linkages on the D values of peptides and the difference between the calculated and observed permeability coefficients of poly-lysine through the porous membrane were evaluated by Student's *t*-test. Statistical significance was taken as $P < 0.05$.

Results and Discussion

Determination of D of FDs and peptides

The molecular weight is the most convenient parameter for predicting the D values of molecules. In our preliminary reports, a relationship between the molecular weight and D of drugs having a molecular weight range of 152–477 was expressed by the following equation (Seki et al 2003a):

$$\log D = -0.434 \log MW - 4.059 \quad (5)$$

The molecular weight of the FDs and peptides examined in this study was over 1000, and was greater than that expressed in equation 5 (Tables 1 and 2). The relationship between molecular weight and D determined in this study (except for insulin) and a plot of equation 5 are shown in Figure 1, and the observed and calculated D values are

Table 1 Calculated and observed D of CF and FDs

	MW	Dcal[a] × 10^6 ($cm^2\,s^{-1}$)	Dobs[b] (s.d.) × 10^6 ($cm^2\,s^{-1}$)
CF	376.32	6.656	5.87 (0.09)
FD4	4400	2.290	2.39 (0.05)
FD10	9500	1.639	1.61 (0.01)
FD40	38 260	0.896	1.02 (0.01)
FD70	50 700	0.793	0.891 (0.19)

Each data point is the mean of 11 determinations. [a]Calculated D in water using $\log D = -0.434 \log MW - 4.059$ (Seki et al 2003a). [b]Observed D in buffer (pH 7.4) by CBM.

Table 2 Calculated and observed D of peptides, poly-lysine and insulin

	S-S[a]	MW	Dcal[b] $\times 10^6$ ($cm^2 s^{-1}$)	Dobs (s.d.) $\times 10^6$ ($cm^2 s^{-1}$)
Oxytocin	+	1007.2	4.34	4.30 (0.05)[c]
Angiotensin II	−	1046.2	4.27	4.20 (0.06)[d]
[Lys^8]-vasopressin	+	1056.2	4.25	4.18 (0.05)[c]
[Arg^8]-vasopressin	+	1084.2	4.20	4.27 (0.03)[c]
α-Conotoxin ImI	+	1351.6	3.82	4.22 (0.06)[d]
Somatostatin	+	1637.9	3.52	3.74 (0.05)[d]
Cortistatin (rat)	+	1721.0	3.44	3.70 (0.05)[d]
Gastrin I	−	2098.2	3.16	2.94 (0.01)[c]
Endothelin-3	+	2643.1	2.86	3.01 (0.02)[d]
Calcitonin	+	3417.8	2.55	2.76 (0.03)[d]
β-Endorphin	−	3465.0	2.54	2.39 (0.03)[d]
GIP	−	4983.6	2.17	2.22 (0.03)[d]
Poly-lysine	−	16 000	1.31	1.28 (0.03)[c]
Insulin	+			1.14 (0.06)[c,e]
monomer		5807.6	2.03	
hexamer		34 845.6	0.933	

Each data point for Dobs is the mean of 11 determinations, except for insulin, where it is the mean of 4 determinations. [a]S-S bond in the molecule. [b]Calculated D in water using $\log D = -0.434 \log MW - 4.059$ (Seki et al 2003a). [c]Determined at pH 7.4. [d]Determined at pH 2.2. [e]Determined by a porous membrane permeation method. Others (peptides and poly-lysine) were examined by CBM.

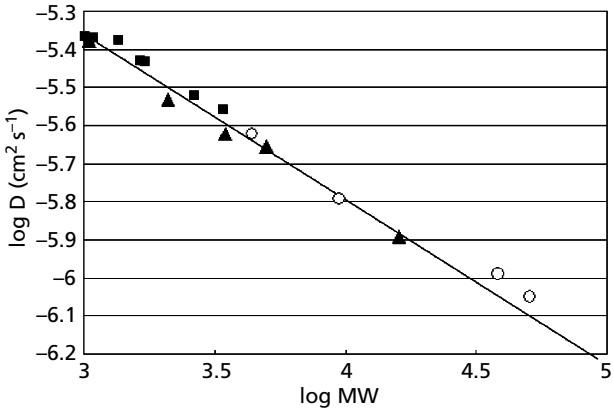

Figure 1 Relationship between the molecular weight and D determined by the CBM of FDs, poly-lysine and peptides. ○, FDs; ▲, peptides without an S-S bond and poly-lysine; ■, peptides with an S-S bond. Line, $\log D = -0.434 \log MW - 4.059$ (Seki et al 2003a). The line was obtained from the relationship between the molecular weight and D of parabens and steroids (MW 152–477).

shown in Tables 1 and 2. For most peptides and FDs, the observed D values were similar to the values calculated from equation 5, although the molecular range was completely different from that expressed in equation 5. This result suggests that molecular weight is a good parameter for predicting D values and, in addition, the CBM is useful as a method for the determination of the D values of higher-molecular-weight drugs. In the case of insulin, the observed D was lower than that calculated for the insulin monomer. Insulin is known to be a dimer or hexamer in neutral pH solution (Dathe et al 1990). Since the observed D was higher than the calculated value of the insulin hexamer, insulin could be in an equilibrium state as monomer, dimer and hexamer.

Angiotensin II, gastrin I, β-endorphin, GIP and poly-lysine have simple linear structures, whereas the other peptides have intramolecular disulfide linkages and this might affect the hydrodynamic molecular size and diffusivity of the peptides. The effect of disulfide linkages on the D was examined by studying the differences between the calculated and observed D values (ΔD). The ΔD values (calculated D − observed D) were $-0.150 \pm 0.158\,cm^2 s^{-1}$ ($\pm$ s.d.) for peptides having disulfide linkages (Figure 1, squares) and $0.084 \pm 0.105\,cm^2 s^{-1}$ for compounds without disulfide linkages (Figure 1, triangles). The mean value of peptides having disulfide linkages was significantly lower than that of compounds without disulfide linkages ($P < 0.05$). The large negative values of ΔD means that the prediction is an underestimation. The hydrodynamic size could be reduced by the linkage and the larger D values could be observed for peptides having disulfide linkages.

Characterization of the porous membrane

The porous membrane (MW cut-off = 15 000) was characterized by equation 3 based on the Renkin function. The characterization was carried out using two parameters, r_p and ε/L. The r_p is the radius of the permeation route. Since this parameter is strict only for cylindrical permeation routes having a uniform pore size, the values are the apparent pore radii for membranes having other geometry like the membrane used. The ε/L is the ratio of the occupied area fraction of the pore to the length of the route. When two penetrants of different size were applied to the membrane, if the pore was big enough compared with both penetrants, the effects of friction are low and the ratio of the permeability of the penetrants is equal to that of D in the medium. For a membrane with a smaller pore, the penetration of larger molecules is limited by the friction of the pore wall. Since the molecular weight cut-off level (15 000) of the membrane was a little higher than the molecular weight of FD10, the permeability of FD10 would be affected by the friction of the pore wall. This was the reason why the membrane was chosen for this experiment although the geometry and the physicochemical properties (e.g. charge of the pore wall) were different from those of the paracellular pathway in biological membranes. In this study, FD10 and CF were simultaneously used to determine the membrane parameters. The P_i values of FD10 (P_{FD10}) and CF (P_{CF}) through the porous membrane were $1.31 \times 10^{-6} \pm 0.31 \times 10^{-6}\,cm\,s^{-1}$ and $4.36 \times 10^{-5} \pm 0.28 \times 10^{-5}\,cm\,s^{-1}$, respectively. The ratio of P_{FD10}/P_{CF} was 0.030, and this was lower than the ratio of D_{FD10}/D_{CF} (observed D, 0.27; calculated D, 0.25). The observed P_i, observed D_i (Table 1) and hydrodynamic radii r_i (Table 3) of FD10 and CF were substituted in equation 3 to obtain the membrane parameters, although

Table 3 Hydrodynamic radius (r_i) of molecules calculated by the Stokes-Einstein equation

		r_i (s.d.) (nm)
FD10	Observed	2.026 (0.010)
CF	Observed	0.5558 (0.0085)
Insulin	Observed	2.862 (0.161)
	Calculated (monomer)[a]	1.607
	Calculated (hexamer)[b]	3.498
Poly-lysine	Observed	2.549 (0.060)
	Calculated	2.495

The values of r_i were calculated from the observed or calculated D. [a]Molecular weight used for the calculation was 5807.6. [b]Molecular weight used for the calculation was 34 845.6.

FD10 was a flexible polymer and different equations have been proposed for the permeation of such flexible polymers (Davidson & Deen 1988). The obtained values of r_p and ε/L were 4.39 ± 0.32 nm and $13.3 \pm 1.2\,cm^{-1}$, respectively. The order of the pore size appears to be similar to that of the penetration route for water-soluble higher-molecular-weight compounds in epithelia (Tomita et al 1992; Horibe et al 1997).

Permeation of poly-lysine and insulin through the porous membrane

Since the D values can be calculated from the molecular weight, the P_i values of the drugs through the porous membrane can be obtained from equations 3 and 5. The hydrodynamic radii r_i for equation 3 can be calculated from the molecular weight using equation 5 and the Stokes-Einstein equation. The values of insulin monomer (1.607 nm) and hexamer (3.498 nm) appeared appropriate considering their sizes shown by the space-filling models (Langkjaer et al 1998). Figure 2 shows the simulation curve for molecular weight and P_i through the porous membrane. The mean values of the membrane parameters ($r_p = 4.39$ nm and $\varepsilon/L = 13.3\,cm^{-1}$) were used to construct the curve. The values of P_{Ins} and $P_{poly\text{-}Lys}$ were determined ($P_{Ins} = 1.87 \times 10^{-7} \pm 0.51 \times 10^{-7}\,cm\,s^{-1}$ and $P_{poly\text{-}Lys} = 2.24 \times 10^{-7} \pm 0.17 \times 10^{-7}\,cm\,s^{-1}$) and then also plotted in Figure 2. The observed and predicted $P_{poly\text{-}Lys}$ (MW = 16 000) were $2.26 \times 10^{-7} \pm 0.17 \times 10^{-7}\,cm\,s^{-1}$ and $4.22 \times 10^{-7} \pm 1.36 \times 10^{-7}\,cm\,s^{-1}$, respectively. Although the predicted value was significantly higher than that observed ($P < 0.05$), the simulation curve could be used for rough estimations of the permeability of higher-molecular-weight drugs through the membrane. Since the Renkin function is strictly for rigid spherical molecules, application of other functions for linear flexible polymers (Davidson & Deen 1988) or a change in the standard penetrants to rigid spherical molecules will be effective in improving the prediction. The methodology described in this study will be useful for the prediction of the permeability of higher-molecular-weight drugs through porous membranes since only molecular weight is needed as a parameter for the calculation.

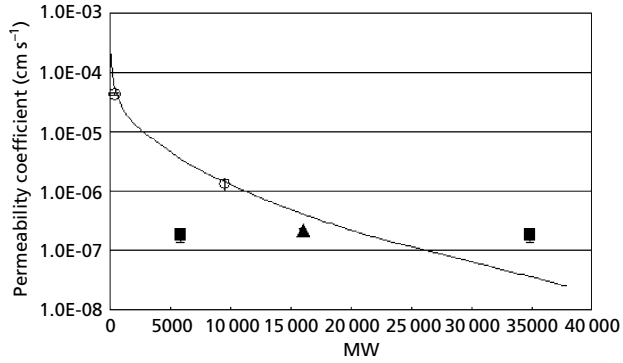

Figure 2 Relationship between the molecular weight and the permeability coefficient through a porous membrane. The plots show the observed permeability coefficient (○, CF and FD10; ▲, poly-lysine; ■, insulin). The observed permeability coefficient of insulin was plotted versus the equivalent molecular weight of insulin monomer and hexamer. Each plot is the mean ± s.e. (n = 4). The line is a simulation curve based on log D = −0.434 log MW − 4.059 (Seki et al 2003a) and equation 3. The P_i values of FD10 (P_{FD10}) and CF (P_{CF}) were used for the calculation of r_p and ε/L in equation 3. The values of r_p and ε/L of the membrane are 4.39 nm and 13.3 cm^{-1}, respectively.

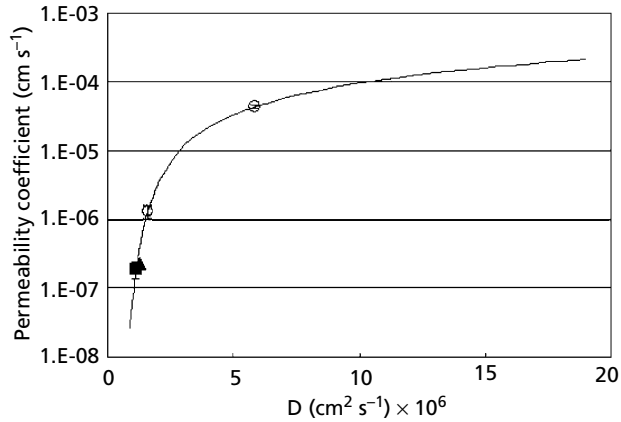

Figure 3 Relationship between the observed D and the permeability coefficient through a porous membrane. The plots show the observed permeability coefficient (○, CF and FD10; ▲, poly-lysine; ■, insulin). Each plot is the mean ± s.d. (n = 4). The line is a simulation curve based on equation 3. The P_i values of FD10 (P_{FD10}) and CF (P_{CF}) were used for the calculation of r_p and ε/L in equation 3. The values of r_p and ε/L of the membrane are 4.39 nm and 13.3 cm^{-1}, respectively.

For insulin, the observed P_{ins} was considerably different from the calculated values based on the equivalent molecular weight of insulin monomer and hexamer (Figure 2). Figure 3 shows the simulation curve of D_i vs P_i based on equation 3, and Figure 4 shows the simulation curve of r_i, which is calculated from D_i, vs P_i. When the P_{Ins} is plotted against the observed D of insulin and the hydrodynamic radius of insulin, calculated from the observed D, the plots are close to the lines in Figures 3 and 4, respectively. For peptides and proteins, which are capable of association or have specific conformations, the

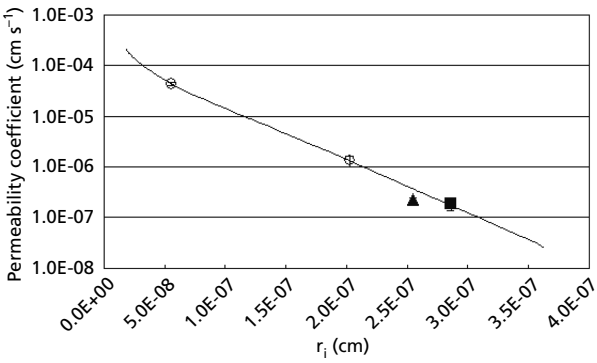

Figure 4 Relationship between the hydrodynamic radius (r_i) and the permeability coefficient through a porous membrane. The plots show the observed permeability coefficient (○, CF and FD10; ▲, poly-lysine; ■, insulin). The hydrodynamic radii were calculated from the observed D using equation 4. Each plot is the mean ± s.d. (n = 4). The line is a simulation curve based on equation 3. The P_i values of FD10 (P_{FD10}) and CF (P_{CF}) were used for the calculation of r_p and ε/L in equation 3. The values of r_p and ε/L of the membrane are 4.39 nm and 13.3 cm^{-1}, respectively.

direct determination of D could be useful for predicting the permeability through such porous membranes.

Chittchang reported a relationship between the D of poly-lysine in different media and its permeability through a porous polyester membrane, and showed the effect of a conformation change in poly-lysine on its permeability (Chittchang et al 2002). The pore size of the membrane used, however, was large (1 μm diameter) compared with the size of the penetrants. Therefore, friction between the penetrants and the pore wall did not affect the results. The pore size of the membrane used in this study was small to affect the mobility of the penetrants in the pore. There are at least two different sized paracellular pathways in mucous membranes (Kim & Crandall 1983; Tomita et al 1992). The bigger one could be for high-molecular-weight hydrophilic drugs, such as peptide and protein drugs, and the size of the pathways may be similar to that of the membrane used in this study.

There are many barriers to the absorption of peptide and protein drugs through biological membranes, such as mucous membranes. One of them is a permeation barrier to high-molecular-weight and hydrophilic compounds. Another important one is a proteolytic enzyme barrier associated with hydrolytic degradation. As far as the prediction of permeability through membranes using equation 3 is concerned, only a permeation barrier is taken into account. Therefore, when the methodology is applied to the permeation of peptide and protein drugs through mucous membranes, the predicted permeability is higher than the observed permeability, since enzyme barriers cannot be ignored in many cases (Salama et al 2004). This is the reason why proteolytic enzyme inhibitors can be used as absorption enhancers for peptide and protein drugs. If the permeability coefficients of peptide and protein drugs through biological membranes are predicted correctly by the methodologies discussed in this study, the effect of enzyme inhibitors can be evaluated more quantitatively by comparing predicted and observed values.

Conclusions

In this study, the D values of FDs and peptides were determined and the permeability of poly-lysine and insulin through a porous membrane was discussed. The observed D values of FDs and peptides were similar to those calculated based on a relationship previously reported between the molecular weight and D of lower-molecular-weight compounds, although the molecular weight range was completely different. The membrane permeability of poly-lysine and insulin predicted by equations 3–5 was compared with the observed value. Although molecular weight is a useful parameter for predicting the permeability through a porous membrane, for insulin the determination of D will be needed to estimate the permeability because of the irregular relationship between molecular weight and D. The methodology described in this study will be useful for obtaining a more quantitative evaluation of the absorption of peptide and protein drugs applied to mucous membranes.

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