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Adsorption of peptides to poly(D,L-lactide-co-glycolide): 1. Effect of physical factors on the adsorption

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Abstract

Four peptides, salmon calcitonin (sCT), the 8–22 amino acid portion of sCT (CT15), an LHRH superagonist, triptorelin (DP) and a somatostatin analogue (RC160) were studied for adsorption to poly(D,L-lactide-co-glycolide) (PLGA) as a function of peptide and polymer concentration. The adsorption of sCT and DP to the polymer showed a primary transient-equilibrium followed by a more rapid and extensive adsorption without reaching a concentration plateau for sCT which occurred earlier with higher peptide concentration. The amount of adsorption for CT15 was much lower than that for sCT and DP and RC160 showed no adsorption. Adsorption isotherms of sCT and DP were fitted to a Langmuir-type of relationship and the monolayer concentration was close to that observed in the kinetic studies or that calculated by the theoretical approach.

Keywords: Peptide-polymer interaction; Poly(D,L-lactide-co-glycolide); Langmuir isotherms

1. Introduction

Enhancing stability, bioavailability and providing prolonged delivery of peptides by incorporation into polymeric devices is influenced and often complicated by the resulting association of peptide and polymer. Peptides are potentially surface active and can non-specifically adsorb to various surfaces and may result in saturation of the peptide at the interface. This interaction can be of such a nature which would increase incorporation into the delivery system and influence release in a controllable manner, or it can also lead to inactivation of the peptide or influence release in a negative manner. Assessment of adsorption as a function of interaction time and adsorbate concentration is usually the first step in understanding the surface interaction (Horbett et al., 1986). While adsorption of plasma proteins onto biomaterials and containers has been extensively investigated (Bohnert et al., 1986; Brash and Thibodeau, 1986; Norde, 1986; Young et al., 1988a; Young et al., 1988b), there have been limited adsorption studies on smaller polypeptides. Since the primary structures of proteins and

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peptides are constructed by polycondensation of the amino acids (Stryer, 1981), the behaviour of proteins at interfaces can be a very useful reference for studying the behaviour of peptides.

It has been shown that an adsorption process may be divided into five steps (Norde, 1986): (1) transport to the surface; (2) surface association; (3) structural rearrangements; (4) detachment; and (5) transport away from the interface. Most likely, the adsorption rate will be controlled by steps 1 and 2. At very low bulk concentration, the transport of adsorbate to the surface is generally the rate-limiting step for adsorption and the adsorption could be similar to the diffusion mechanism. The total surface concentration of the adsorbate, especially the initial part of the adsorption, can be expressed as a function of time (Norde, 1986; Young et al., 1988a):

$$\Gamma_t = 2C_o\sqrt{\frac{(D)(t)}{\pi}} \tag{1}$$

where Γ_t, C_o, D and t are the surface adsorbate concentration, initial adsorbate concentration, diffusion coefficient (10^{-6}–10^{-7} cm^2/s) and time, respectively.

Young et al., 1988b have shown that as the surface is filled with adsorbed protein, the rate of adsorption becomes surface-reaction controlled which decreases to below the diffusion rate. No simple equation can be used to express such adsorption mechanism but the point at which the adsorption deviates from the diffusion-controlled system can serve as a relative ranking of the surface-reaction rate. There are also other studies showing that the surface reaction can be the rate determining step regardless of the extent of the surface coverage (Young et al., 1988a).

Unlike gas adsorption, protein adsorption does not necessarily form an easily identified monolayer on the adsorbent. There have been several attempts to model protein adsorption. Two models that have been frequently used are the Langmuir and the Freundlich adsorption isotherms (Langmuir, 1918; Freundlich, 1926). The monolayer coverage is generally calculated from the Langmuir isotherm and a simplified treatment has been proposed by Young et al., 1988a:

$$\frac{C_b}{C_s} = \frac{1}{K_1 C_{m1}} + \frac{C_b}{C_{m1}} \tag{2}$$

where, C_b is the equilibrium bulk protein concentration in solution, C_s is the measured surface concentration of protein, K_1 is the monolayer binding constant, and C_{m1} is the monolayer concentration on the adsorbent.

Controversy exists as to whether these models should be applied to protein adsorption that is often irreversible. Views opposing the treatment are based on the premise that the physical interpretation holds only if the associated model is designed for reversible adsorption (Norde, 1986; Bohnert et al., 1986; Brash and Thibodeau, 1986). However, it was interesting to find that in many cases the monolayer adsorption calculated from the Langmuir equation happened to be very close to that of a close packed surface coverage (Norde, 1986; Young et al., 1988a; Young et al., 1988b). The monolayer adsorption is normally within 0.1–1 $\mu g/cm^2$ and a general calculation based on the number of amino acid residues has been shown to be close to the experimental value (Norde, 1986). However, the plateau value obtained from the adsorption isotherm need not be the monolayer coverage. Robertson and Zydney compared a number of adsorption studies on bovine serum albumin (BSA) and showed that the adsorption plateau ranged from 0.05 to 270 $\mu g/cm^2$ (Robertson and Zydney, 1990). They showed that the values were significantly affected by the experimental design. For instance, in some cases where the process involved rinsing the adsorbent surface before and/or after the adsorption, the amount adsorbed to the surface could vary according to the properties of the rinsing medium (such as the composition and ionic strength) (Matthiasson, 1983; Aimar et al., 1986); direct measurement on the adsorption achieved by using the mixture of ^{125}I-BSA and non-labelled BSA could overestimate the extent of adsorption due to the preferential and stronger binding of ^{125}I-BSA over the non-labelled BSA (Aimar et al., 1986).

In this study, the adsorption of four peptides to PLGA powder was evaluated as a function of time, adsorbate concentration and adsorbent concentration. The rates of the adsorption were compared with the rates of the diffusion-controlled

mechanism. The amounts of peptide adsorbed at equilibrium, as well as at a given time, are presented and analyzed.

2. Materials and methods

2.1. Materials

Polymer: Poly(D,L-lactide-co-glycolide; 50:50) (PLGA) 34 000 MW (Boehringer Ingelheim, Germany).

Peptide: The purity of the peptides were all > 97%, therefore the peptides were used without further purification. Salmon Calcitonin (sCT) (Bachem Inc., Torrance, CA), Salmon Calcitonin 8–22 residues (CT15) (MSAF, University of Kentucky, Lexington, KY), LHRH analogue Decapeptyl (DP) and somatostatin analogue RC160 (Debiopharm, Lausanne, Switzerland).

Buffer solution: Phosphate buffer was prepared with sodium phosphate dibasic heptahydrate and sodium phosphate monobasic monohydrate and, if necessary, the pH was adjusted with dilute phosphoric acid and sodium hydroxide (all from Aldrich Chemical Co., Milwaukee, WI).

Other materials: Polypropylene vials (1.5 ml capacity) and HPLC grade acetonitrile were from Fisher Scientific Co. (Fair Lawn, NJ). Ultrafiltered water from a Milli-Q water system was used (Millipore, Bedford, MA).

2.2. Methods

Preparation and analysis of peptide solution: the peptide solutions of sCT and CT15 were prepared in 0.1 M phosphate buffer, pH 7.4. Decapeptyl was difficult to dissolve directly in the buffer, therefore the solution was prepared by first dissolving the peptide in ultrafiltered water and then adding 0.2 M phosphate buffer to form a final peptide solution in 0.1 M phosphate buffer. RC160 was not soluble in the buffer, therefore the peptide was prepared in water.

The analysis of peptides were by reversed phase High Performance Liquid Chromatography (HPLC) using a 25 cm C_{18} column and a mobile phase consisting of acetonitrile and water with 0.1% trifluoroacetic acid. The injection volume was 20–30 μl. The HPLC system included two Shimadzu pumps (LC-6A), a system controller (SCL-6B), an autoinjector (SIL-6B), a UV-VIS detector (SPD-6AV) and a data processor (Chromatopac CR601).

Adsorption study: PLGA was micronized to provide a larger surface area for the adsorption. Unless otherwise stated, 10 mg of PLGA powder (surface area = 718 cm^2) were placed in the vials and 1 ml of peptide solution was added. The vials were mounted on a rotary wheel vertically spinning at a speed of 18 cycles/min. At scheduled times, adsorption was terminated by phase separation. The vials were centrifuged at 12400 rpm for 10 min and the solution part was analyzed by HPLC for free peptide concentration. Bound peptide was calculated from the difference between the initial (total) and remaining (free) peptide concentrations. The effect of adsorbate concentration was studied with at least three peptide concentrations in the range of 50–1000 μg/ml. The study was set for a duration of 6 h for searching the equilibrium time. The effect of adsorbent concentration was studied on four polymer concentrations: 1, 5, 10 and 20 mg/ml for 4 h. The experiments were carried out at room temperature in triplicate. Two controls were carried out along with the adsorption samples: peptide control and polymer control. The peptide control was prepared by adding 1 ml of the peptide solution in a vial without PLGA followed by the same treatment as the samples. The polymer control was prepared by adding 1 ml of the peptide-free medium and subjected to the same treatment as the samples.

3. Results and discussion

3.1. Adsorption kinetics

The adsorption of sCT to PLGA powder plotted as the adsorbed peptide concentration (C_s in $\mu g/cm^2$) versus time (h) is shown in Fig. 1a. A reduced scale on the y-axis for the same data is given as Fig. 1b to emphasize the initial part of the adsorption. Except for the lowest concentration of sCT solution, about 30 μg of sCT (C_s =

0.04 $\mu g/cm^2$) were adsorbed in 30 min. The adsorption seemed to reach a transient-equilibrium state in < 90 min where the adsorption was maintained at a fairly constant level. Adsorption of about 0.05 ± 0.02 $\mu g/cm^2$ occurred during the transient-equilibrium state. This was relatively short-lived for the 1000 and 500 μg peptide levels.

Adsorption of sCT continued with time after the transient-equilibrium state and the starting time of continuing adsorption was concentration dependent. The continuous adsorption was faster and greater with higher sCT concentration. sCT concentration of 1000 $\mu g/ml$ showed a change of 0.06 $\mu g/cm^2$ between 1 to 4 h of adsorption time while the extent of change for sCT concentration of 50 $\mu g/ml$ was < 0.01 $\mu g/cm^2$. This adsorption phase continued for 1.5 h for C_o = 1000 $\mu g/ml$, 2 h for C_o = 500 $\mu g/ml$ and 3 h for C_o = 200 $\mu g/ml$. C_o of 50 and 100 $\mu g/ml$ maintained a steady state for a much longer time and the actual

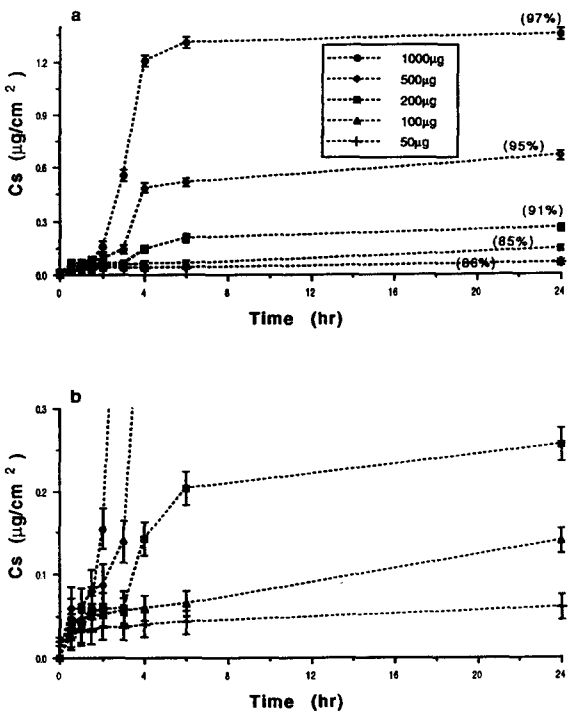


Fig. 1. Adsorption profiles of sCT to PLGA in (a) full scale and (b) expanded scale of Y-axis. Initial peptide in 1 ml is shown in the legend and depletion of sCT after 24 h is given in the parenthesis above each curve.

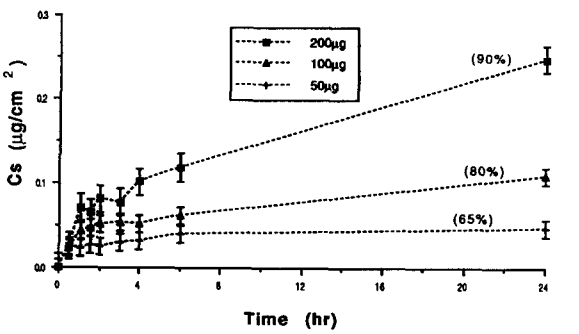


Fig. 2. Adsorption profiles of DP to PLGA. Initial peptide in 1 ml is shown in the legend and depletion of DP after 24 h is given in the parenthesis above each curve.

starting time for continuing adsorption is not clearly discerned from the figure. After 24 h depletion of peptide from the bulk exceeded 85% at all concentrations with the trend being more complete at the higher peptide levels suggesting that once a critical solution concentration is reached, more binding occurs.

Fig. 2 shows DP adsorption to PLGA. The adsorption increased steadily with time without forming a true equilibrium state. Almost complete depletion of DP from the solution occurred when the experiments were continued for 24 h and about 20 μg of DP remained in the bulk at the end of the study. DP concentrations higher than 200 $\mu g/ml$ in the buffer studied were not stable over 6 h and, therefore, higher C_o was not included in this study. Failing to attain a steady equilibrium state is not unusual since there have been reports that the adsorption of proteins to solid surfaces does not always result in an equilibrium and continuous adsorption with time has been found under certain adsorption conditions (Norde, 1986; Young et al., 1988a; Young et al., 1988b). Adsorption of albumin to four types of hydrophobic surfaces with protein concentrations > 5 $\mu g/ml$ did not reach a plateau, and the adsorption of α2-macroglobulin never reached an equilibrium even with concentrations as low as 2.5 $\mu g/ml$ (Young et al., 1988b).

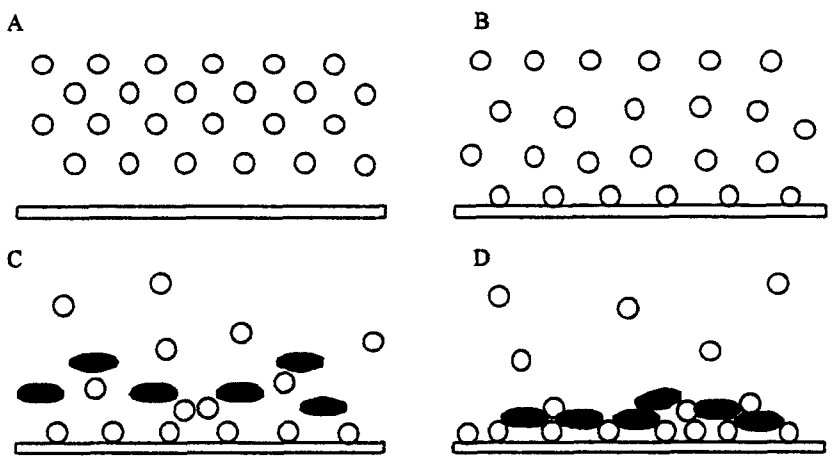
Adsorption results of sCT and DP in Figs. 1 and 2 suggest that the adsorption of these two peptides to PLGA should be multilayer in nature. The adsorption phenomenon, especially for sCT

where the study was carried out over a wider concentration range, showed that the adsorption did not follow a layer by layer formation where repetitive plateau with similar equilibrium duration between layers should be observed. Instead, sCT adsorption with a primary transient-equilibrium was followed by a more rapid and extensive adsorption without reaching plateau adsorption until near depletion. Since peptide molecules have the potential of forming aggregates in aqueous medium, adsorption of aggregated molecules is possible.

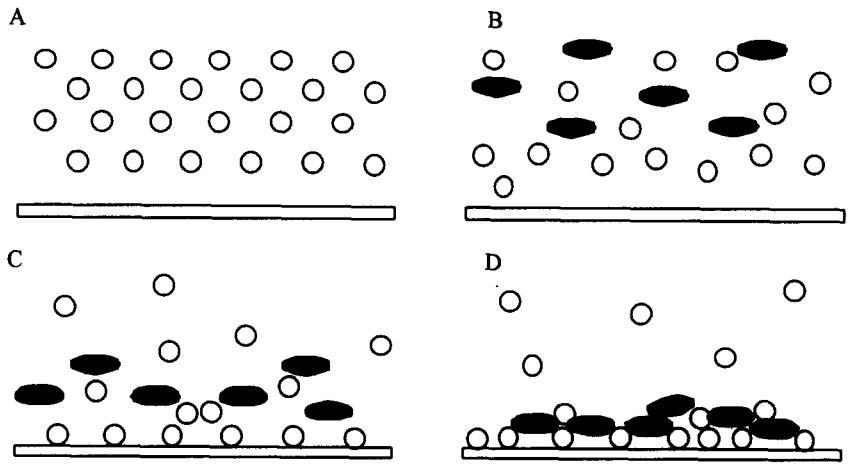
Scheme 1 illustrates the adsorption phenomenon observed in the depletion of sCT and DP. Peptide molecules are represented in circle and oval forms for the monomers and aggregates, respectively. Adsorption is divided into four stages according to time. In the beginning stage, (A), peptide molecules are distributed in the medium as monomers. When some peptide molecules are transported to the polymer surface, adsorption starts as shown in stage B. The peptide-polymer association then induces peptide molecules approaching the surface to form aggregates in the bulk as shown in stage C. The aggregates can form faster at higher peptide concentration in the bulk due to increased chance of 'collision'. The aggregates start to adsorb towards a depletion as shown in stage D while the remaining monomeric forms distribute accordingly in the bulk.

As depicted from the scheme, adsorption could not take place at the very beginning because adsorption of the peptide to the polymer surface requires transport of peptide. Since the adsorption was carried out in aqueous medium with dissolved peptide, transportation of peptide molecules should be very fast. After a short period of time, most likely within a few minutes, adsorption started with peptide molecules directly associated with the polymeric surface. At this stage, the concentration of the peptide in solution would not affect the result as long as enough peptide molecules are brought closer to the polymeric surface. This could correspond to the formation of the transient-equilibrium state. The presence of the polymeric surface with or without the adsorbed peptide molecules probably induces the approaching peptide molecules in solution to form aggregates with stronger affinity for the surface. The formation rate of peptide aggregates could be highly concentration dependent since the chance of peptide molecules in bulk to cluster together would be increased by increasing the peptide concentration. This may correspond to the relatively short-lived equilibrium state at the higher peptide concentrations. The ultimate near depletion of peptide suggests there is no limit for the adsorption of the peptides.

It has been reported that the presence of a hydrophobic surface, Teflon, induced the aggregation of insulin in solution and the rate of aggrega-



Scheme 1. Hypothetical scheme of peptide adsorption to PLGA: concentration and time dependent aggregation of peptide.



Scheme 2. Hypothetical scheme of peptide adsorption to PLGA: hydrophobic surface induces aggregation of peptide.

tion increased with increased in the area of the hydrophobic surface (Sluzky et al., 1991). Therefore, a second illustration based on the assumption that sCT forms aggregates in the presence of PLGA surface was also used to explain the experimental finding and is presented in Scheme 2. In the beginning stage, (A), peptide molecules are evenly distributed in the medium as monomers. Due to the presence of the hydrophobic surface, some peptide molecules started forming aggregates as shown in stage B. The amount of aggregates is greater with higher peptide concentration in the bulk. The transport of peptide monomers is faster than that of the aggregates, therefore, the initial association with the polymer surface is predominantly by the monomers as shown in stage C. It is likely that after the initial layer, the monomers no longer have the ability to associate with the bound peptide layer and the subsequent adsorption is dominated by the peptide aggregates. Before the aggregates approach to the surface, the amount adsorbed to the surface is constant, corresponding to the transient-equilibrium state. The aggregates are then transported to the surface. When there is higher peptide concentration in the bulk, the amount of aggregates formed in the solution is greater. Since the interaction between the aggregates and the surface depends on the time when the two can be associated, greater amount of the aggregates will allow greater chance of the aggregates to interact with the surface. Therefore, the time it takes to form the subsequent adsorption after the transient-equilibrium is shorter with higher peptide concentration in the bulk. As time increases, the aggregates start to adsorb towards a depletion as shown in stage D while some remaining monomers distribute accordingly in the bulk.

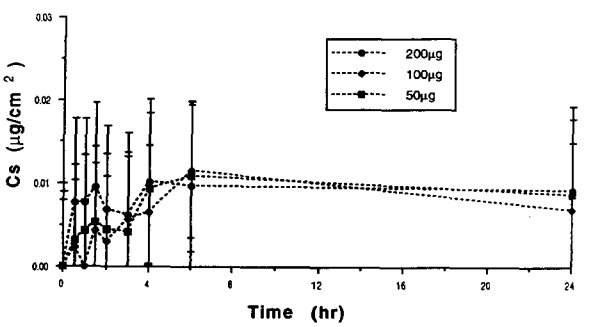


Fig. 3. Adsorption profiles of CT15 to PLGA. The Y-axis is an order of magnitude less than that seen with sCT and DP adsorption. Initial peptide in 1 ml is shown in the legend.

The adsorption of CT15 is shown in Fig. 3. CT15 adsorption differed from that of sCT and DP in the extent of adsorption to PLGA. CT15 adsorption was much lower even up to 24 h (C_s = 0.01 $\mu g/cm^2$) and the adsorption appeared to be maintained at an equilibrium state.

In order to relate the experimental data with diffusion-controlled adsorption, the amount of

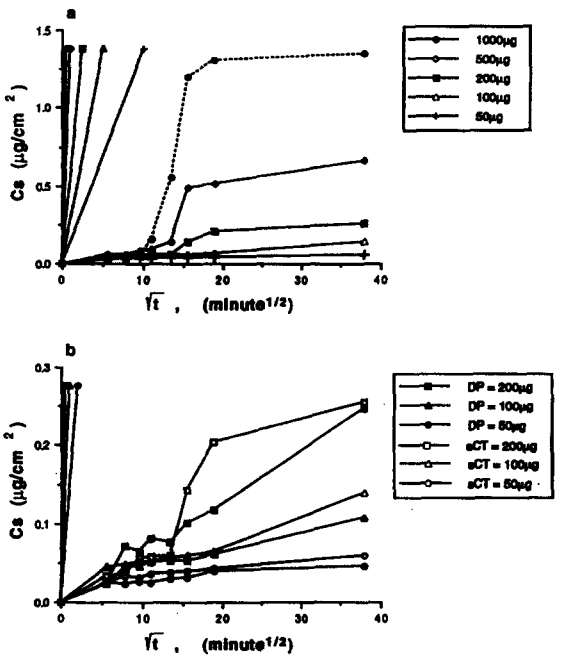


Fig. 4. Amount of adsorbed peptide versus square root of time ($min^{1/2}$) for (a) sCT and (b) comparison of sCT and DP. The solid lines close to the Y-axis represent diffusion-controlled adsorption predicted by assuming $D = 10^{-7}$ cm^2/s.

peptide adsorbed was plotted against the square root of time ($\sqrt{t}$) as shown in Fig. 4. Such a plot would be linear through the origin when the adsorption is determined by diffusion. It is apparent that the adsorption of sCT with C_o higher than 200 μg/ml could not be diffusion-controlled beyond 90 min of adsorption since the data were not linear. For $C_o < 200$ μg/ml, the adsorption deviated from the solid lines predicted by a diffusion controlled model given a diffusion coefficient, $D = 10^{-7}$ cm^2/s, generally reported for proteins (Norde, 1986). The explanation for lower adsorption than the predicted value can be found in the systems for adsorption of albumin and other large proteins (Norde, 1986; Young et al., 1988a; Young et al., 1988b). The adsorption of peptides to surfaces involves transport to the surface and adsorption on that surface (the latter referred to as the intrinsic adsorption rate). At very dilute peptide concentration, it is more likely that the adsorption is limited to the rate of transport to the surface since the number of peptide molecules are less. It is shown in Fig. 4a that the adsorption kinetics of sCT from higher peptide concentrations ($C_o \geq 200$ μg/ml) were very different from lower concentrations ($C_o \leq 200$ μg/ml) up to 24 h. As the peptide concentration increases, the adsorption is limited to the rate of association with the surface. This is due to an increased number of peptide molecules transported to the surface, probably exceeding that required to cover the entire surface, and the adsorption is thus limited to the rate of peptide association with the surface. The slower adsorption rate when compared to theoretical diffusion controlled transport could be due to stronger intermolecular peptide association in solution resulting in lower diffusion rate of peptide molecules to the polymer surface. Within 2–4 h of the interaction (corresponding to ($\sqrt{t}$) in the range of 10–15 $min^{1/2}$), sCT appeared to adsorb at a faster rate with C_o greater than 200 μg/ml. For C_o of 50 and 100 μg/ml, most sCT were already adsorbed in the first 2 h and, hence no rapid increase in the adsorption could be observed. Beyond 4 h, the adsorption rate was lower mostly due to the absence of enough free peptide molecules in the solution. Fig. 4b includes the data for DP and sCT. Unlike sCT, DP did not show a rapid increase in adsorption in the 2–4 h range even with $C_o = 200$ μg/ml.

3.2. Isotherm

An adsorption isotherm is generally obtained through a series of studies with different adsorbate concentrations and the data obtained at the equilibrium state are then plotted as surface adsorbate concentration versus bulk adsorbate concentration. To obtain the adsorption isotherm, the amount adsorbed at the surface, (C_s in $\mu g/cm^2$), was plotted as a function of the bulk peptide concentration, (C_b in μg/ml), during the transient-equilibrium state for sCT and in 2 h for DP. These data are listed in Table 1. C_s values obtained from the transient-equilibrium state were < 0.1 $\mu g/cm^2$, which is the lowest value suggested for monolayer coverage by a native protein (Heya et al., 1991). Due to the huge difference in size between these two peptides and the commonly

Table 1
Amount of peptide adsorption versus concentration in first 2 h of kinetic study

C_o (μg/ml)	sCT		DP	
	C_s (μg/cm^2)	C_b (μg/ml)	C_s (μg/cm^2)	C_b (μg/ml)
50	0.033 ± 0.001	26.2 ± 0.73	0.023 ± 0.002	33.8 ± 1.73
100	0.048 ± 0.003	66.0 ± 2.20	0.044 ± 0	68.4 ± 0.01
200	0.036 ± 0.010	174 ± 6.83	0.071 ± 0	150 ± 0.04
500	0.059 ± 0.004	458 ± 2.72	—	—
1000	0.041 ± 0.005	971 ± 3.84	—	—

Data represent average of 3 samples ± S.D.

studied proteins, the suggested monolayer coverage criteria for proteins may not be suitable for sCT and DP.

Fig. 5 shows the adsorption isotherms from the plotting of C_s versus C_b using the values listed in Table 1. The sCT surface concentration was around 0.045 μg/cm^2 over the range studied. Since the adsorption of sCT did not maintain an equilibrium between the bound and the free peptide, standard concaved-plateau plotting of C_s versus C_b need not apply. If the adsorption was slow in covering the surface, a plotting of C_s versus C_b would give a sigmoidal curve, gradually reaching a plateau.

Fig. 6 shows the plotting of C_s versus C_b for DP. A proportional increase of C_s with increase of C_b was observed indicating that the surface coverage increased as the peptide concentration in the bulk increased. Since the kinetic data had shown that the equilibrium state of DP lasted for a longer duration, a classical type of C_s-C_b relationship was expected.

Fig. 7a and Fig. 7b show the fitting of sCT and DP adsorption data by the Langmuir adsorption Eq. (2) using the transient-equilibrium values. Assuming these data were obtained before or around the monolayer formation, the results of the plotting should provide the value of the monolayer concentration, C_{ml} which is the reciprocal of the slope and the binding constant, K_l from the reciprocal of the intercept. The experimental and calculated monolayer concentrations and binding constants are listed in Table 2. The first theoretical value was calculated assuming the surface area occupied by an average amino acid residue of 15 Å^2 based on the study of proteins at interfaces

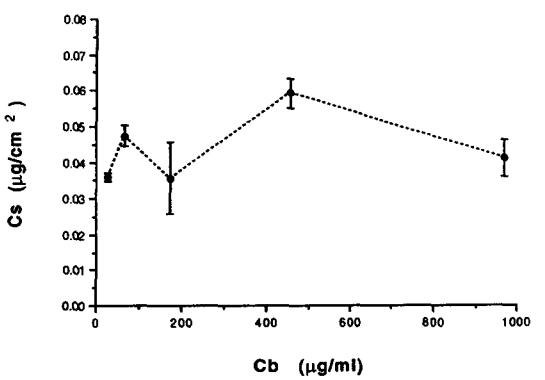


Fig. 5. Adsorption isotherm of sCT. Adsorption to PLGA was performed in 0.1 M phosphate buffer, pH 7.4. Data obtained during the transition-equilibrium within 2 h of adsorption.

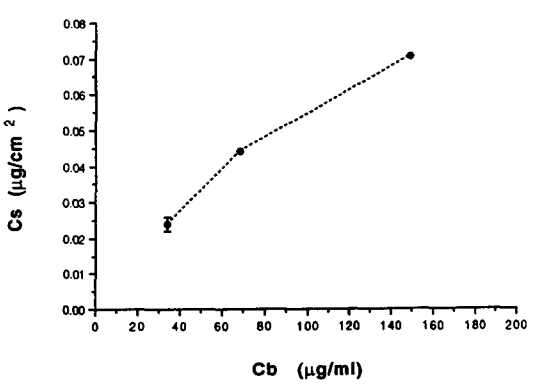


Fig. 6. Adsorption isotherm of DP. Adsorption to PLGA was performed in 0.1 M phosphate buffer, pH 7.4. Data obtained during the transition-equilibrium within 2 h of adsorption.

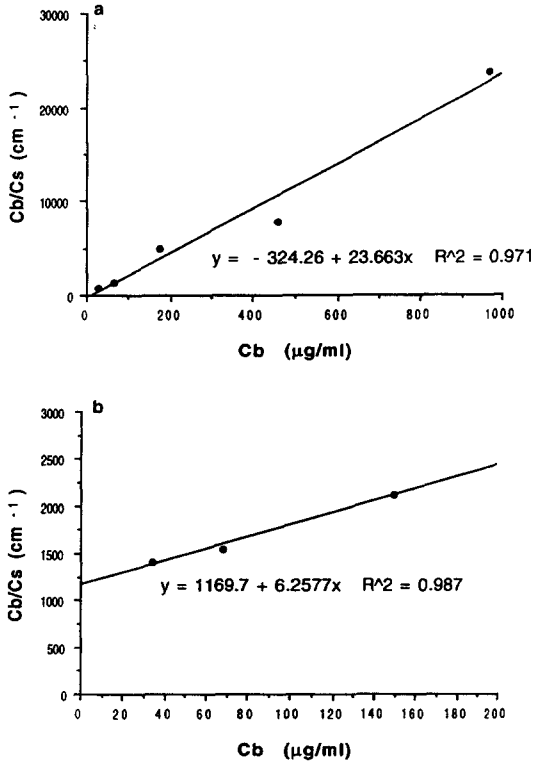


Fig. 7. Plot of adsorption isotherm in the form of C_b/C_s versus C_b for (a) sCt and (b) DP. Data were transformed from Table 1.

(McRitchie, 1981). The second and third theoretical calculations were achieved assuming the peptide molecules were of uniform shape, spherical or cylindrical, on the polymer surface.

The monolayer surface concentration of sCT from the model fitting was much smaller than that based on the theoretical calculations but was very close to what was observed in Fig. 5. Such smaller value of the monolayer concentration could be due to the extension of peptide molecules on the polymer surface upon adsorption which is favored due to increase in the contact points between the peptide and the polymer surface.

sCT has a very flexible structure at its tail region with an amphiphilic helical central region which could easily adapt itself in a variety of conformations favored for the surface interaction. DP, a relatively short peptide with a predominate β-II structure would probably find it difficult to adjust its conformation upon adsorption. The monolayer concentration from the model fitting was equal to the packing calculated by assuming a cylindrical shape for DP molecules with the base of the cylinder being the adsorption site. The binding constant (K_1) derived from Langmuir model fitting has been of questionable value in making comparisons between adsorption studies (Heya et al., 1991). The reason is believed to be the heterogeneity between different peptides and proteins, and the adsorbent surfaces. The equation was developed for gaseous molecules which are of a size similar to that of the binding site on the adsorbent. Therefore, the assumptions of the equation may not be realistic when dealing with macromolecules.

For very dilute solutions, adsorption isotherms have also been described by the Freundlich equation:

$$C_s = kC_b^{1/n} \tag{3}$$

where k and n are empirically determined constants. The Freundlich equation can be linearized by taking the logarithm of Eq. (3).

$$\log C_s = \log k + \frac{1}{n}\log C_b \tag{4}$$

A plotting of log C_s versus log C_b will provide the values of log k and 1/n from the intercept and slope after linear regression analysis. Modeling the data with the Freundlich equation would suggest a continuous increase in adsorption as the bulk peptide concentration is increased, whereas adsorption reaches a plateau with the Langmuir model. Because the isotherm shown in Fig. 5 did not show an increase in adsorption with increased bulk concentration of the peptide, sCT adsorption data would not follow the Freundlich model. On the other hand, the DP data were able to fit into the Freundlich equation as shown in Fig. 8. Since the Freundlich model is used to treat adsorption with multilayer formation, DP adsorption data that fitted into the equation suggest that if higher concentration of DP were used, greater adsorption could be obtained.

It has been suggested that protein adsorption isotherms which develop well-established plateau values are generally in the range between 0.1–0.5 $\mu g/cm^2$ (Young et al., 1988a). The peptides inves-

Table 2
Adsorption constant and monolayer surface concentration for sCT and DP derived from eq. 2 and theoretical calculations

Peptide	Langmuir constants		Calculated C_{m1}		
	K_1	C_{m1}	By no. of residues	(A) Packing	(B) Packing
sCT	−0.076	0.042	0.119	0.116	0.121 & 0.241
DP	0.005	0.175	0.145	0.084	0.088 & 0.175

(A) and (B) referred to the peptide molecules in spherical and cylindrical shapes, respectively. The unit of K_1 is ml/μg and the unit for C_{m1} is μg/cm^2. The upper value of (B) packing assumed the adsorption site is the longer side of the cylinder. The lower value of (B) packing assumed the adsorption site being the base of the cylinder.

tigated in this study are much smaller than those proteins used for adsorption studies and therefore may have a different range for the plateau values. Lack of references for peptide adsorption makes this comparison difficult. Smaller peptides could have a better chance to get loose and cover a larger surface than bigger proteins since the latter ones have to overcome stronger intramolecular forces. The monolayer formation could involve peptide molecules in the loose form and protein molecules in a more compact form. Therefore, the monolayer concentration for peptide adsorption could be a smaller value than that for the proteins.

The adsorption of DP could be of a different mechanism from sCT due to its simple structure and conformational rigidity. Kinetics of the adsorption of sCT and DP suggest that the adsorption phenomenon other than that at the equilibrium state should be investigated. Therefore, the amount of peptide interacted with the polymer at a longer time frame was evaluated. Since transient-equilibrium for sCT adsorption occurred in 3 h, the adsorption for sCT and DP was extended to 4 h with peptide concentration ranging from 50 to 1000 μg/ml. The results are shown in Table 3. The adsorbed surface concentration after 4 h was plotted against the initial peptide concentration (C_o in μg/ml) for both sCT and DP in Fig. 9. A proportional increase in the amount of adsorption over initial peptide concentration of 200–1000 μg/ml range was found. The magnitude of adsorption in 4 h of adsorption clearly demonstrates multilayer formation on the surface. Above an initial peptide concentration of 200 μg/ml the bound peptide appeared to be linearly dependent on the initial peptide concentration. Increase in the association between peptide molecules in the solution as well as at the solid surface should be the reason for such relationship.

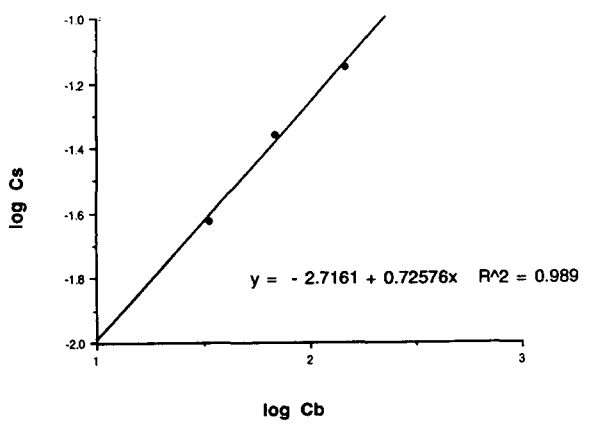


Fig. 8. Plot of adsorption isotherm in the form log C_s versus log C_b for DP. Data were transformed from Table 1.

Table 3
Adsorption of sCT and DP to PLGA (10 mg) after 4 h

C_o (μg/ml)	Bound peptide (μg/10 mg PLGA)	
	sCT	DP
50	33.3	17.0
100	44.7	37.1
200	102	72.5
400	286	278
500	351	426
800	658	752
1000	865	931

Data represent average of 3 samples.

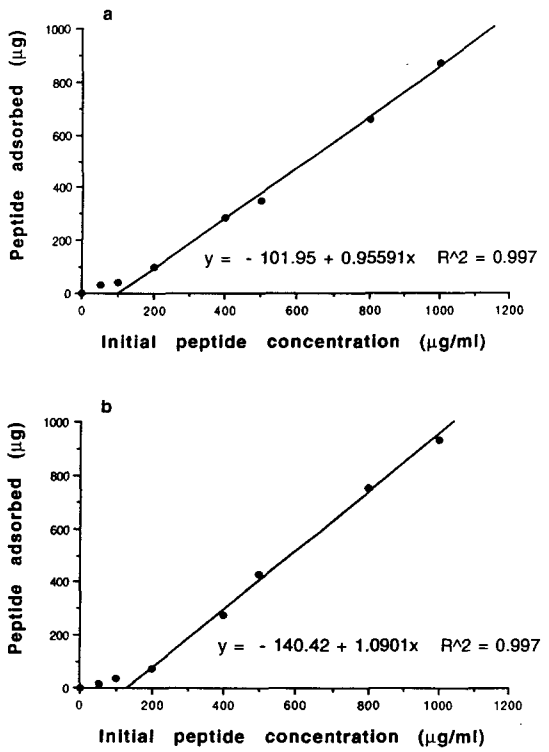


Fig. 9. Adsorption profile of (a) sCT and (b) DP to 10 mg PLGA after 4 h of interaction. Data for each point were averaged from at least triplicate samples.

Hydrophobic surfaces in an aqueous environment were found to exhibit attractive forces over distances as long as 140 Å and the forces decay exponentially with distance (Kaelble and Moacanin, 1977). These forces are much greater than those expected from van der Waals forces and arise primarily from the structuring of water molecules at the interface. Thus peptides such as sCT and DP whose dimensions are far less than 140 Å could be driven by hydrophobic forces and easily adsorb in multilayers.

Table 4
Effect of polymer concentration on the adsorption of peptides

PLGA (mg)	% Peptide bound			
	sCT*	sCT**	DP**	CT15*
1	22.61	14.47	5.02	0.27
5	70.94	20.76	16.42	0.59
10	86.94	50.96	36.24	3.36
20	92.74	68.59	57.00	2.10

Experiments were carried out for 4 h in triplicate. Two levels of initial peptide concentration were used: *1000 µg/ml and **200 µg/ml.

3.3. Effect of adsorbent concentration

If a higher adsorbent surface was available, adsorption would be expected to be enhanced due to the availability of surface sites for the adsorbate. Results of varying PLGA concentration for the adsorption of peptides are given in Table 4. As the polymer concentration was increased, adsorption of the peptides increased.

The increase in adsorption was not linearly correlated with the increase in PLGA. An interesting observation with these data of sCT and DP is that as the polymer amount increased, the amount of free peptide left in the bulk decreased. This observation supports the mechanism proposed in Schemes 1 and 2. As the polymer amount increased, direct adsorption of peptides to the surface increased. Therefore, the inducing effect on aggregate formation was enhanced resulting in a significant movement towards the polymer surface. With less amount of polymer to induce the subsequent adsorption, greater amount of free peptide would be left in the bulk.

4. Summary and conclusion

The adsorption of sCT and DP to the polymer did not follow the classical multilayer model which suggests a stepwise adsorption profile exhibiting transitory equilibrium times of similar length. Instead, a primary transient-equilibrium was followed by a more rapid and extensive adsorption without reaching plateau adsorption for sCT until near depletion. The resumed adsorption occurred earlier with higher bulk peptide levels suggesting that once a critical solution concentration was reached, more binding occurred. The adsorption of sCT with C_0 higher than 200 µg/ml was not entirely controlled by a diffusion process. This could be due to stronger intermolecular peptide association in solution resulting in slower diffusion of peptide molecules but a greater asso-

ciation of the peptide-peptide molecules with the polymer. The amount of adsorption for CT15 was much lower than that for sCT and DP and RC160 showed no adsorption unless in a very high salt (20% NaCl) medium. This difference in adsorption phenomenon is believed to be due to the nature of the peptides for the adsorbent surfaces.

Adsorption isotherms of sCT and DP to PLGA were fitted to a Langmuir type of relationship. Monolayer coverage derived from the Langmuir model of sCT was close to the concentration observed in the kinetic studies and the monolayer coverage of DP was close to that calculated by a theoretical approach. Even though the adsorption of sCT appears to result in multilayer formation, the transient-equilibrium data did not fit the Freundlich isotherm. However, DP adsorption data were able to fit into the Freundlich equation. The adsorption of DP could be of a different mechanism from sCT due to the differences in structure and conformational rigidity.

In aqueous systems where secondary structures of peptides could be diminished, changes on the conformation of peptides upon adsorption or induced by the adsorbent to form peptides with greater affinity for adsorption can happen. Since peptide molecules are generally susceptible to the environment, adsorption of peptides could be greatly affected by the environmental condition. Changing the environmental conditions for peptide adsorption will be discussed in a separate paper (Tsai et al., 1996).

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