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COMMUNICATION

Adsorption of Calcitonin to Glass

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ABSTRACT

Surface adsorption of calcitonin on soda lime silica glass was investigated. An attempt was also made to examine the effect of additives on the inhibition of calcitonin adsorption. Results showed that the adsorption isotherms were of the Langmuir and Freundlich type, depending on pH. Less adsorption was found for calcitonin at pH 4.3. The addition of nonionic surfactants such as Pluronic F68 and Tween 80 to the calcitonin solutions demonstrated inhibition of adsorption and reduction of adsorption rate. The addition of chlorobutanol also showed the effect of minimizing adsorption.

INTRODUCTION

Surface adsorption of peptide drug to a container always occurs and may cause a significant loss of concentration. In the formulation study of peptide drugs, adsorption is one of the important parameters to characterize. Also, methods to prevent adsorption need to be developed.

Calcitonin, a major regulating factor in mineral and skeletal metabolism, is a 32-amino-acid polypeptide hormone. It is available in glass ampoules, vials, and bottles (1). Therefore, it is of particular interest to study the adsorption of calcitonin on glass. The objective of this study was to investigate the effect of concentration of calcitonin, temperature, pH of the medium, and preservatives on the adsorption characteristics of calcitonin to a soda lime silica glass surface. An attempt was also made to examine the effect of additives of surface-active agents on the inhibition of calcitonin adsorption.

MATERIALS AND METHODS

Materials

Salmon calcitonin was obtained from Penta Biotech, Incorporated (United States). Soda lime silica glass beads (unwashed), Tween 80, and chlorobutanol were purchased from Sigma Company (United States). The glass beads were washed by vortex with water six times and dried in an oven at 100°C overnight before use. Pluronic F68 was obtained from Fluka Chemie AG (Switzerland).

Adsorption Measurements

For the adsorption measurements, 500 mg of glass beads were vortexed with 0.5 ml of buffer and then were equilibrated with 0.5 ml of calcitonin stock solution in a rotary shaker at a rate of 20 rpm. The concentration of calcitonin used for adsorption was in the range 1 to 20 mg/ml. The adsorption media were pH 3.4 acetate buffer (0.1 M acetic acid and sodium acetate), pH 4.3 acetate buffer (0.04 M acetic acid and 0.02 M sodium acetate), pH 7.4 phosphate buffer (0.067 M monopotassium phosphate and disodium phosphate), and pH 9.3 carbonate buffer (1.0 M sodium hydrogen carbonate and sodium carbonate), and the tonicity was adjusted by sodium chloride to a value equivalent to that of 0.9% sodium chloride. The equilibrium time for adsorption to be carried out was 5 hr. After equilibration, the glass beads were separated by centrifugation at 5000 rpm. The supernatant was obtained to analyze the concentration loss for adsorption.

Effect of Additives on Adsorption

The additives were formulated in the calcitonin stock solution, added to the glass beads, then diluted with buffers before incubation. The surface-active agents used were nonionic surface-active agents of Pluronic F68 and Tween 80 at concentrations of 0.0025%, 0.05%, and 0.5% w/w. The chlorobutanol used was 0.125 mg/ml, which is usually used as a preservative concentration in peptide preparations (2); a higher concentration of 0.25 mg/ml was also studied to examine the concentration effect.

Analysis of Calcitonin

Calcitonin concentration was determined by HPLC. The column employed for analysis was a Cosmosil packed column (5C18-AR, 4.6 × 250 mm; Nacalai Tesque, Inc., Japan). The mobile phase was acetonitrile 0.1% (v/v) and trifluoroacetic acid (31:69, v/v). The detection wavelength was 220 nm. The flow rate was 1.0 ml/min, and the temperature was ambient. Propyl paraben was used as an internal standard.

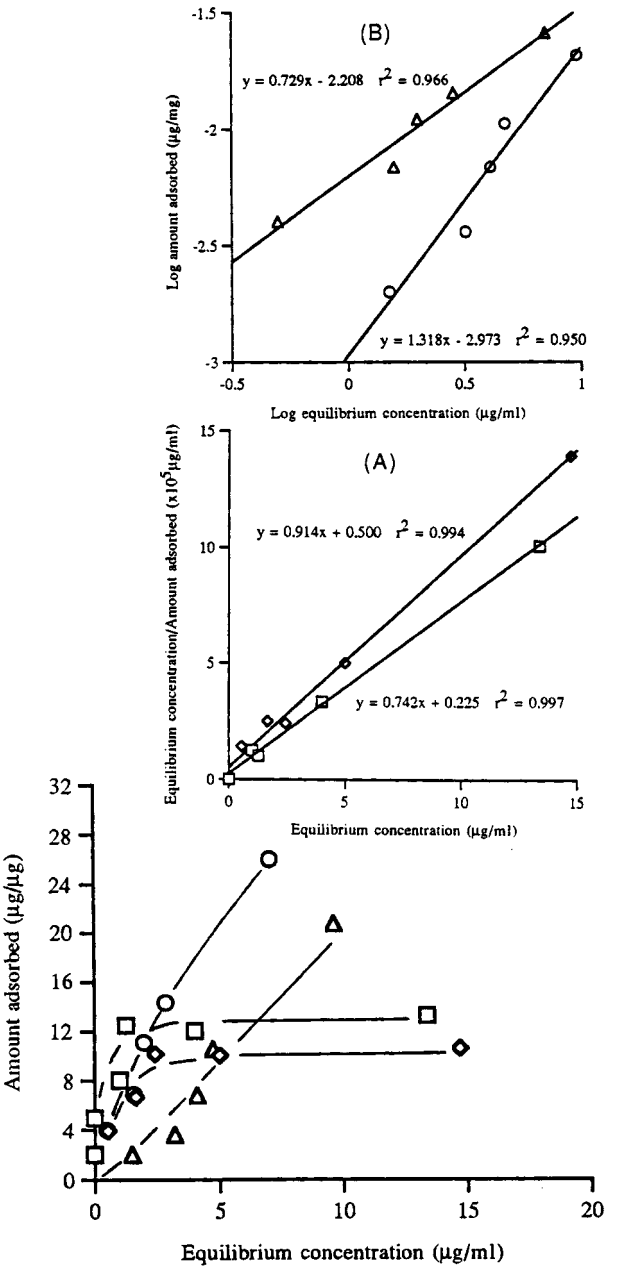

Figure 1. Adsorption of calcitonin on soda lime silica glass surface at 25°C (□, pH 3.4; ◇, pH 4.3; ○, pH 7.4; △, pH 9.3): (A) Langmuir isotherms; (B) Freundlich isotherms.

RESULTS AND DISCUSSION

Two types of adsorption isotherm were shown over the calcitonin concentration range studied, depending on the pH of the adsorption medium (Fig. 1). With the effect of pH 3.4 and 4.3, at low equilibrium calcitonin concentrations, the amount adsorbed increased with increasing calcitonin concentration; at high equilibrium concentrations, it attained a plateau. Less adsorption was shown

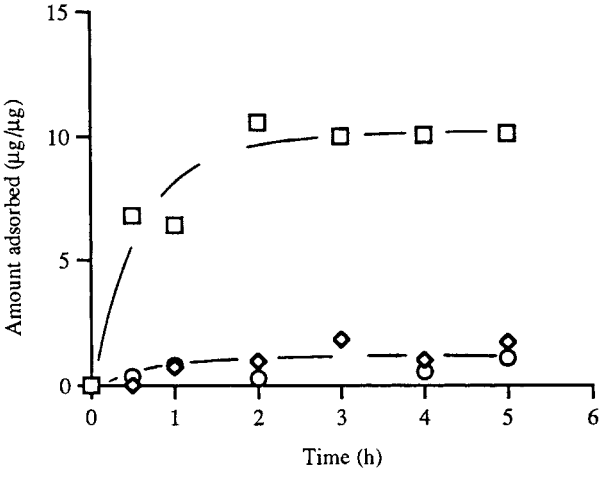

Figure 2. Rate of adsorption of calcitonin on soda lime silica glass at 25°C and pH 4.3: □, without additive; ◇, with 0.5% Pluronic F68; ○, with 0.5% Tween 80.

from calcitonin solution at pH 4. 3. Using the Langmuir equation (3) to evaluate the adsorption characteristics, a straight line was found (Fig. 1A). This suggested that, at pH 3.4 and 4.3, calcitonin adsorption formed a monolayer on the surface of glass. In the case of pH 7.4 and 9.3, the adsorption of calcitonin from solution onto glass beads demonstrated an increased amount adsorbed with increasing calcitonin concentration, which was found to fit the Freundlich equation (Fig. 1B) (3). This indicated a multilayer formation for the adsorption of calcitonin on glass. Since calcitonin has a tendency to aggregate in solution (4,5), the multilayer formation may be due to aggregation of calcitonin at the solid-liquid interface or adsorption of calcitonin aggregates on the solid surface. From the above results, it is apparent that pH 4.3 may be the best pH for the formulation of calcitonin solution.

The rate of calcitonin adsorption on the glass surface is shown in Fig. 2. In the first 2 hr, calcitonin adsorption increased as incubation time increased; then, after 2 hr, an equilibrium was attained. In this experiment, an incubation time of 5 hr was used. This is the time sufficient for calcitonin to reach an equilibrium adsorption.

Most of the protein or peptide adsorption is dependent on temperature, and adsorption usually increases with increasing temperature (6,7). Therefore, the storage of protein or peptide preparations is preferably done at a low temperature to prevent adsorption. However, in the case here, the adsorption of calcitonin on the glass surface did not change when the temperature was increased from 4°C to 37°C (Fig. 3).

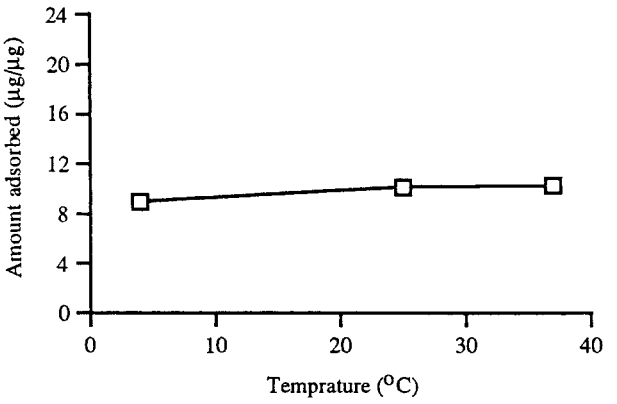

Figure 3. Effect of temperature on the adsorption of calcitonin on soda lime silica glass at pH 4.3.

The effect of nonionic surface-active agents of Pluronic F68 and Tween 80 on calcitonin adsorption to the glass surface is shown in Fig. 4. For both Pluronic F68 and Tween 80, an inhibition of adsorption was observed. The degree of inhibition of adsorption depended on the concentration of nonionic surface-active agents. Concentration at 0.5% demonstrated a stronger inhibition effect on adsorption than at 0.05%. No effect on the decrease of adsorption was shown at 0.0025%. With the addition

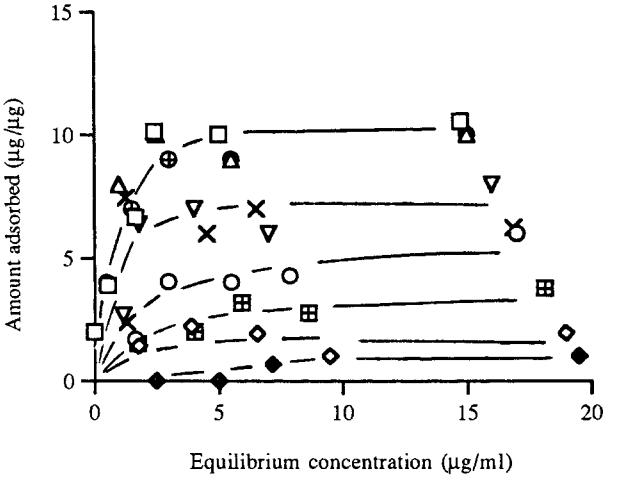

Figure 4. Adsorption of calcitonin on soda lime silica glass at 25°C and pH 4.3: □, without additive; ◇, with 0.5% Pluronic F68; ○, with 0.5% Pluronic F68; △, with 0.0025% Pluronic F68; ◆, with 0.5% Tween 80; ⊞, with 0.05% Tween 80; ⊕, with 0.0025% Tween 80; ▽, with 0.25 mg/ml chlorobutanol; ×, with 0.125 mg/ml chlorobutanol.

of Pluronic F68 and Tween 80 at a concentration of 0.5%, a dramatic decrease of adsorption rate was also shown (Fig. 2). Surface-active agents have been found to be an effective inhibitor for the adsorption of peptides on glass and polymer surfaces (6,8). The inhibition of peptide adsorption by a surface-active agent may be due to the interaction of molecules of the surface-active agent and peptide reducing the binding sites for the peptide to interact with the surface and the strong competition of the surface-active agent molecules with the peptide molecules on the surface. Here, it is proposed that the nonionic surface-active agents Pluronic F68 and Tween 80 at the concentrations of 0.5% or 0.05% can be used in the formulation to prevent loss of calcitonin by adsorption on glass.

Chlorobutanol is commonly used as a preservative in the preparation of proteins and peptides (2). The stability of chlorobutanol is pH dependent, and the preservative activity is optimal in low pH conditions. Therefore, chlorobutanol may be a suitable preservative for calcitonin solution at pH 4.3. The effect of chlorobutanol on the adsorption of calcitonin on glass is shown in Fig. 4. The addition of chlorobutanol to the calcitonin solution reduced the amount adsorbed on glass. However, this reduction of calcitonin adsorption was not affected by increasing the concentration of chlorobutanol from 0.125 to 0.25 mg/ml. Although the mechanism of chlorobutanol on the inhibition of adsorption is not clear, it is likely that, in the calcitonin formulation, chlorobutanol may be considered as an adsorption inhibitor as well as a preservative.

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