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Adsorption of Secretin on Glass Surfaces

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Adsorption of secretin on a silicone-coated glass surface was compared with that onto a non-coated surface using controlled pore glass beads. The silicone-coated glass beads adsorbed 20 times more secretin than the non-coated beads. The adsorption was slightly affected by pH and amino acids, but was reduced to an insignificant amount in the presence of bovine serum albumin. It is suggested that the preferential adsorption of this peptide hormone onto a silicone-coated glass surface is probably the result of hydrophobic attraction between non-polar moieties of the secretin molecule and non-polar portions of the silicone membrane.

Keywords——secretin; adsorption; controlled pore glass; glass surfaces; silicone-coated glass surfaces; pH; amino acids; bovine serum albumin

In the quantitative treatment of biological and chemical substances such as hormones, enzymes, and radioisotopes at micro levels, their adsorption on the inner surfaces of glassware or other materials must be prevented. To date, there have been several investigations[2–7] on the adsorption of proteins and certain drugs onto glass surfaces. It has been suggested that the major forces operating for the adsorption of proteins, amino acids and basic drugs are ionic amino-silanol bonding and a cooperative cohesive force between the substance and the glass.[2]

In order to prevent the adsorption of biological material onto glass surfaces or other substances present in the medium, bovine or human serum albumin is routinely added.[4,7] Silicone-coated containers are also often used to prevent adsorption, especially during the treatment of hydrophilic substances such as proteins, peptides and drugs which dissolve readily in aqueous solutions. However, there is no proof that the silicone membrane does not adsorb these substances. In addition, there may be other undesirable effects such as hydrophobic interactions between non-polar moieties of proteins and the silicone membrane.

This communication describes the preferential adsorption of secretin onto silicone-coated glass surfaces as compared to non-coated surfaces, presumably due to hydrophobic interaction.

Materials and Methods

Secretin——Eisai standard secretin (100 Crick-Haper and Rapper (CHR) units plus 5 mg of cysteine HCl, lyophilized in a silicone-coated glass ampule), batch No. 510603, prepared from a highly purified porcine secretin (5400 CHR units/mg), was used throughout these experiments.

Silicone-coating of Glass Beads——Controlled pore glass beads, CPG 10-700 (mesh size 80—120, mean pore dia. 677Å, pore vol. 0.89 ml/g) were purchased from Electro Nucleonic Inc., Fairfield, U.S.A. After washing with 1 N NaOH followed by 1 N HCl and rinsing thoroughly with distilled water to neutrality, the glass beads were dried at 100° for 8 hr. The beads were then coated as follows: 5 g of glass beads was dipped into about 100 ml of an aqueous solution of dimethyl-polysiloxane (Siliconizer®, Fuji Kobunshi Kogyo, Inc.,

1) Location: *Koishikawa-4, Bunkyo-ku, Tokyo 112, Japan.*
2) T. Mizutani and A. Mizutani, *J. Pharm. Sci.*, **67**, 1102 (1978).
3) F.L. Pearce, D.V. Banthorpe, J.M. Cook, and C.A. Vernon, *Eur. J. Biochem.*, **32**, 576 (1973).
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5) R.L. Trouy and J.N. Etteldorf, *Clin. Res.*, **24**, 74A (1976).
6) K.D. Thakker, *Dissertation Abstr. Intern. B.*, **37**, 3975 (1977).
7) P.Q. Barrett and W.F. Neuman, *Biochim. Biophys. Acta*, **541**, 223 (1978).

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Tokyo, Japan) consisting of one ampule, 2 ml, dissolved in 100 ml of distilled water. The mixture was deaerated *in vacuo* under vibration for 2 min. The supernatant was discarded by decantation and the beads were then washed twice with 100 ml of distilled water and collected on filter paper. After drying *in vacuo*, the silicone-coated beads were heated for 4 hr at 100° to set the membrane.

Adsorption of Secretin on the Glass Beads——Physiological saline (2.5 ml) was added to each ampule of Eisai standard secretin, the resulting concentration of secretin being 40 CHR units/ml. Because of the presence of 5 mg of cysteine HCl in each ampule, the pH of the solutions was approximately 3. Various amounts of non-coated or silicone-coated glass beads were added to the solution, which was then vibrated and stored at 4° overnight. The residual secretin activity was determined by bioassay in rats as described below.

To determine the effect of pH on secretin adsorption, the secretin was dissolved in 2.5 ml of 0.4 M Na_2CO_3, bringing the pH to 9.5. To examine the effects of bovine serum albumin and amino acids on the adsorption, the secretin was dissolved in 2.5 ml of saline containing bovine serum albumin (0.1—10 mg/ml), phenylalanine (0.1 M) or leucine (0.05 M).

Assay of Secretin Activity——Secretin activity was determined in rats by the twin-crossover method described by Tachibana.[8] Eisai standard secretin (100 CHR units/ampule) dissolved in 2.5 ml of saline containing bovine serum albumin (1 mg/ml) was used as a standard throughout the experiments. In order to avoid the injection of a large volume of acidic solution, the high and low doses of the test sample (TH=2TL) were set at 100 and 50 μl, respectively. The doses of the standard (SH=2SL), on the other hand, were varied and were chosen in order to obtain responses of the pancreas comparable to those obtained with the test samples. The secretin activity of each test sample was calculated using the equation given in the Pharmacopoeia of Japan.[9]

In assays of secretin dissolved in alkaline solution, which produces marked effects on pancreatic response to secretin, the high test dosage was reduced to 50 μl; 0.2 ml of phosphate-saline buffer, pH 7.5, was injected soon after test sample administration in order to neutralize the pH.

Results

Secretin Adsorption on Glass Beads under Acidic Conditions

Various amounts of non-coated and silicone-coated glass beads were added to secretin dissolved in saline, and the residual activity of secretin in the supernatant was determined. The results are shown in Fig. 1 as the percentage of secretin adsorbed. Silicone-coated glass beads adsorbed much greater quantities than non-coated glass beads, as shown in Fig. 1 (shaded circles and shaded triangles, respectively). The amount of secretin adsorbed on 5 mg of coated beads was approximately equal to that on 100 mg of non-coated beads. Furthermore, it was apparent that secretin was also adsorbed on the inner surface of the silicone-coated glassware; a 20% decrease of secretin activity in a control ampule was observed without the addition of glass beads. The experimental results on the stability of secretin under the test conditions and the inhibitory effect of albumin (described later) on secretin adsorption support the conclusion that this decrease was due to adsorption and not to loss of activity.

Secretin Adsorption on Glass Beads under Alkaline Conditions

The adsorption of secretin on silicone-coated and non-coated glass beads under alkaline conditions (pH 9.5) is shown in Fig. 1 (half-shaded circles and triangles, respectively). The adsorption of secretin on non-coated and coated beads at pH 9.5 was 10% greater than that under acidic conditions at all points. This difference, considered to be insignificant, was thought to be due to the instability of secretin under alkaline conditions.[8]

Effect of Bovine Serum Albumin on Secretin Adsorption onto Glass Beads

The adsorption of secretin onto silicone-coated and non-coated glass beads in the presence of bovine serum albumin is shown in Fig. 1 (open circles and triangles, respectively). The secretin dissolved in saline containing bovine serum albumin (10 mg/ml) was adsorbed neither

8) S. Tachibana, Secretin and Cholecystkinin-Pancreozymin; Their Bioassay and Purification. "Gastro-Entero-Pancreatic Endocrine System," ed. by T. Fujita, Igaku Shoin Ltd., Tokyo, 1973, p. 174.
9) The Pharmacopoeia of Japan, 8th Edition, D-230 (1971).

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Table I. Effects of Bovine Serum Albumin and Amino Acids on Secretin Adsorption on Silicone-coated Glass Beads

Solvent	Amount of silicone-coated glass beads		
	0 mg	5 mg	100 mg
	Percent adsorption of secretin		
BSA 0 mg/ml	20%	82%	100%
0.1	0	40	100
1.0	—	0	86
10.0	0	0	42
Phe 0.1 M	—	80	—
Leu 0.05	—	81	—

Secretin (100 CHR units) was dissolved in 2.5 ml of saline containing bovine serum albumin or amino acids. Various amounts of silicone-coated glass beads were added and the residual activity of secretin in the supernatant was determined as described in the text.
Each experiment was performed 2—3 times and the results were averaged.

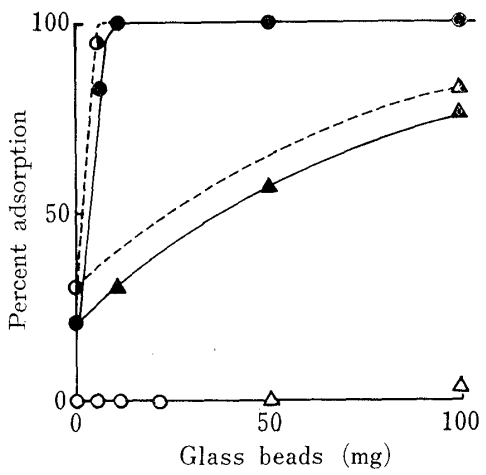

Fig. 1. Adsorption of Secretin on Non-coated and Silicone-coated Glass Beads

Secretin (100 CHR units) was dissolved in 2.5 ml of saline, saline containing bovine serum albumin (10 mg/ml), or 0.4M Na_2CO_3, and non-coated (triangles) and silicone-coated (circles) glass beads were added to each solution. The residual activity of secretin in the supernatant was determined by bioassay as described in the text. Each experiment was performed 3—4 times and the results were averaged.
▲: saline-noncoated. ●: saline-coated.
△: saline-BSA-noncoated. ○: saline-BSA-coated.
▲: Na_2CO_3-noncoated. ◑: Na_2CO_3-coated.

by the coated (5—10 mg) nor the non-coated glass beads (50—100 mg); the original secretin activity was completely recovered from the supernatant.

In order to clarify the inhibitory effect of albumin, the relation between albumin concentration and the adsorption of secretin onto silicone-coated glass beads was investigated. As shown in Table I, the concentration of albumin required to prevent secretin adsorption increased as the amount of beads increased.

Effect of Amino Acids on Secretin Adsorption onto Silicone-coated Glass Beads

The effect of amino acids on secretin adsorption onto coated glass beads was also examined. Phenylalanine and leucine, which are comparatively hydrophobic amino acid constituents of the secretin molecule, are known to be adsorbed onto glass surfaces.[10] However neither phenylalanine nor leucine at the concentrations shown in Table I had an inhibitory effect comparable to that of albumin.

Discussion

It has previously been reported that proteins, amino acids and some amine drugs are adsorbed on glass surfaces, the adsorption being a result of ionic-silanol bonding or a cooperative cohesive force between the material and the glass. Therefore, there may be some uncertainty involved as to the actual dosages of biological compounds used at low concentrations.

In order to prevent the adsorption of biological materials on the inner surfaces of containers, containers are sometimes coated with silicone. To date, there is no evidence

10) T. Mizuno and K. Mizuno, *Analy. Biochem.*, **83**, 216 (1977).

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that such coated surfaces do not adsorb materials. In this communication we report the preferential adsorption of secretin onto silicone-coated glass beads rather than onto non-coated ones.

The silicone-coated glass surface adsorbed 20 times more secretin than the non-coated surface. The adsorption of secretin onto silicone-coated and non-coated glass beads was only slightly affected by pH and the addition of amino acids, whereas the adsorption of protein on glass surfaces as a result of ionic bonding is markedly affected by pH[2] and is reduced to some extent by amino acids.[11] Therefore, instead of ionic or cooperative cohesive bonding, the force resulting in secretin adsorption is thought to be the hydrophobic attraction between non-polar moieties of the secretin molecule and non-polar portions of the silicone membrane and glass surface.

The adsorption of secretin onto glass surfaces was effectively prevented by bovine serum albumin. This albumin contains a total of 28 mole per cent of hydrophobic amino acids (Leu, Ileu ,Val, Met, Tyr, and Phe).[12] The adsorption reaction with glass surfaces was suggested to depend not only on ionic bonding, but also on intermolecular forces, hydrogen bonding and/or hydrophobic bonding.[2] Therefore, the preventive effect of albumin on secretin adsorption onto glass surfaces is thought to be due to competitive adsorption of albumin. As stated in previous communications[2-7] and in this communication, when performing assays and manipulations of biological materials in micro-scale quantities, it is important to consider the amount of the material that has been adsorbed on the surface of the container, instrument or injector being used.

Hence for the assay of peptide hormones such as secretin or insulin, it is recommended that a protein, such as bovine serum albumin, which has no effect on the hormonal activity be added to the medium to prevent loss by adsorption. It is also important to select a suitable container surface, non-coated or silicone-coated, depending on the compound being tested.

11) K. Mizutani and A. Mizutani, *J. Chromatography*, **111**, 214 (1975).
12) "Handbook of Biochemistry-Selected Data for Molecular Biology," Second Edition, ed. by H.A. Sober, The Chemical Rubber Co., Cleveland, Ohio, 1970, p. C-281.

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