Adsorption of Pepsin by Aluminum Hydroxide I: Adsorption Mechanism

ROBERT J. SEPELYAK *, JOSEPH R. FELDKAMP ‡, TIMOTHY E. MOODY ‡, JOE L. WHITE ‡, and STANLEY L. HEM *x

Received July 18, 1983 from the *Departments of *Industrial and Physical Pharmacy and ‡Agronomy, Purdue University, West Lafayette, IN 47907.* Accepted for publication February 9, 1984.

Abstract □ Adsorption of pepsin by gibbsite and boehmite, non-acid-reactive forms of aluminum hydroxide, was observed and related to the surface area of the adsorbent. Adsorption was pH dependent, with maximum adsorption occurring between pH 2.7–3.3 for gibbsite and pH 2.7–4.3 for boehmite. Electrostatic attraction was an important adsorption mechanism at the pH conditions encountered in the GI tract; the isoelectric point of pepsin was ~1, giving it a negative charge, and the point of zero charge for the adsorbents was >9, giving them a positive charge. However, the pH–adsorption profile can not be fully explained by electrostatic considerations. Desorption studies indicate the importance of specific adsorption because pepsin was not desorbed by washing with acidified water, but was partly desorbed by exchange with phosphate. The IR spectrum of adsorbed pepsin also suggested that specific adsorption of pepsin occurred through anionic ligand exchange involving carboxylate groups of pepsin and surface aluminum ions.

Keyphrases □ Aluminum hydroxide—adsorption, pepsin □ Pepsin—adsorption by aluminum hydroxide □ Adsorption—pepsin by aluminum hydroxide

Aluminum hydroxide is widely used in peptic ulcer therapy. Recent clinical studies, which have shown that aluminum hydroxide-containing antacid products are effective in healing ulcers (1–3), have stimulated interest in the mechanism by which antacids act to heal ulcers. The Food and Drug Administration's OTC Panel on Antacids suggested that the neutralization of gastric acid was the only mechanism of action (4). Because the activity of pepsin is pH dependent, the neutralization of gastric acid will affect the activity of pepsin. Piper and Fenton (5) concluded that one mechanism by which aluminum hydroxide reduced pepsin activity was to raise the pH of the gastric fluid. Kuruvilla (6) concluded that antacids in general reduce pepsin activity by raising the gastric pH. Piper and Fenton (7) also stated that antacids which raise the gastric pH to 4–5, *i.e.*, aluminum hydroxide, only temporarily inhibit pepsin activity while antacids such as sodium bicarbonate, which raise the pH above 8, will denature pepsin and permanently inhibit its activity.

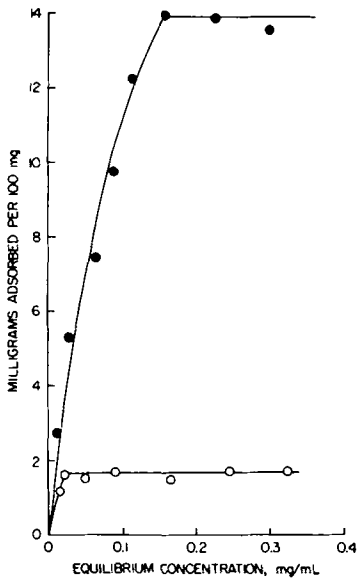

Figure 1—*Adsorption isotherms of pepsin on gibbsite (○) or boehmite (●).*

Other reports have speculated that antacids reduce pepsin activity by precipitating pepsin from solution (8, 9) or by adsorbing pepsin (5, 10–16). The adsorption of bile acids is believed to aid in ulcer therapy (17–19). Earlier studies suggested that antacids act by coating the mucosal lining and protecting it from the digestive action of gastric acid and pepsin (20–22). The reports of ulcer coating by antacids have been contradicted (23, 24), and the recent trend in formulating antacid products has been to incorporate only acid-reactive antacid materials.

This study was undertaken to investigate the interaction of aluminum hydroxide and pepsin with special emphasis on any specific mechanism by which aluminum hydroxide reduces the proteolytic effect of pepsin on the gastrointestinal mucosa.

BACKGROUND

Pepsin, a globular proteolytic enzyme, is secreted by the chief cells of the gastric mucosa as its zymogen, pepsinogen. At ≤pH 5, pepsinogen is converted to pepsin by the cleavage of six peptide segments from the *N*-terminal end of pepsinogen. Pepsin and pepsinogen have molecular weights of ~35,000 and 42,000, respectively (25).

The primary structure of pepsin consists of 327 amino acids including 43 acidic amino acids and 4 basic amino acids (26). The isoelectric point of pepsin is ~1, so pepsin is a negatively charged protein in the GI tract (27). Pepsin is composed of a single polypeptide chain which has folded and packed to form a globular protein. The calculated molecular diameter of pepsin is 4.38 nm (28). The three-dimensional structure is stabilized by three disulfide bonds. Pepsin has only one active site which is believed to include the carboxyl groups from two aspartic acids (29).

The role of pepsin in the body is to digest proteins, and it is most active in hydrolyzing bonds involving phenylalanine, tyrosine, and leucine (30). Bonds involving glutamic acid are hydrolyzed, but to a lesser extent (30). The proteolytic activity of pepsin is dependent on pH. Pepsin A, which has also been termed pepsin III, is the predominant pepsin and exhibits an optimal pH range for activity between 1.5 and 3.0 (31).

The adsorption of pepsin by aluminum hydroxide is well documented, but the mechanism of adsorption has not been elucidated. Pawelczak determined the extent of pepsin adsorption by a number of antacid preparations (32). Wenger and Sundy determined the adsorption of pepsin by various colloidal materials at different pH conditions and suggested that the extent of adsorption was dependent on particle size and the chemical nature of the colloidal material (13). Piper and Fenton determined that aluminum hydroxide and charcoal were the best adsorbers of pepsin among a group of materials (5). Anderson and Harthill found pepsin to be completely adsorbed by an acid-reactive aluminum hydroxide gel as well as by an acid-insoluble aluminum hydroxide gel (14). Liebman also found that pepsin was adsorbed by aluminum hydroxide (15).

EXPERIMENTAL SECTION

Materials—Porcine pepsin[1], gibbsite[2], and boehmite[2] were obtained commercially. The surface area of gibbsite and boehmite was determined by

[1] Worthington Biochemical Corp., Freehold, N.J.
[2] Reynolds Metals Co., Bauxite, Ark.

0022-3549/84/1100-1514$01.00/0

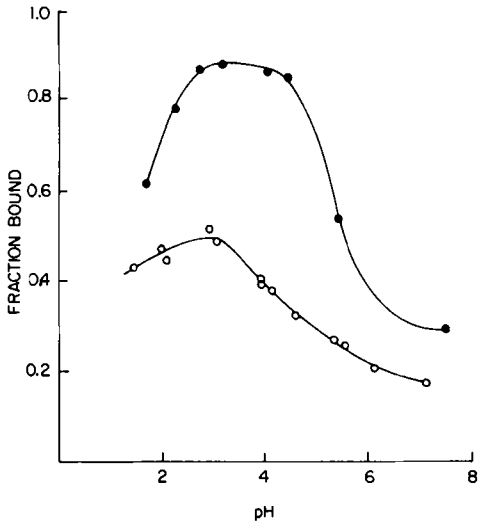

Figure 2—*pH profile of the percent pepsin bound on gibbsite (○) or boehmite (●).*

the triple-point gas adsorption method[3]. The point of zero charge was determined using a continuous titration procedure (33).

Adsorption Isotherms—Adsorption isotherms for pepsin on gibbsite or boehmite were determined at pH 2.3. Pepsin stock solutions were prepared at 2 and 3 mg/mL in 0.01 M HCl for gibbsite and boehmite, respectively. Gibbsite and boehmite stock suspensions (1%) were prepared and sonicated to break up aggregates. Ten milliliters of the stock suspension was transferred into a 25-mL volumetric flask. The suspension was adjusted to pH 2.3 by adding a total of 0.26 or 0.27 mmol of HCl to the gibbsite or boehmite suspensions, respectively. The actual amount of 0.1 M HCl added depended on the amount of pepsin required, since 1 mL of the pepsin solution provided 0.01 mmol of HCl. After the addition of the appropriate amount of 0.1 M HCl, the required amount of pepsin was added. The final volume of the suspension was brought to 25 mL by the addition of distilled water. The suspensions were shaken for a 5-min adsorption period, after which the pH was recorded. The suspensions were centrifuged at 10,000 rpm for 30 min and the supernatants were filtered through a 0.45-μm filter. The absorbance of the supernatants was read at 280 nm (34, 35); the equilibrium pepsin concentration was determined by use of a Beer's law plot, and the amount adsorbed was calculated by difference.

Fraction Bound Study—The percentages of pepsin bound on gibbsite and boehmite were determined at various pH conditions. Pepsin solutions (1 and 2 mg/mL for gibbsite and boehmite, respectively) were prepared in 0.001 M HCl. Gibbsite or boehmite stock suspensions (1%) were prepared and sonicated to break up aggregates. Ten milliliters of the stock suspension was transferred into 25-mL volumetric flasks and adjusted to the desired pH with appropriate quantities of HCl or NaOH. Five milliliters of the appropriate pepsin solution was added, and the final volume was adjusted to 25 mL with distilled water. The suspensions were shaken for 5 min, and the pH was recorded. The suspensions were centrifuged at 10,000 rpm for 25 min, and the supernatants were filtered through a 0.45-μm filter. The absorbance of the supernatants was read at 280 nm. Blanks were prepared and treated in the same manner except that 0.001 M HCl was used instead of the pepsin solution. Also, another pepsin solution was prepared in the absence of gibbsite or boehmite. The percent pepsin bound was calculated by:

$$B = \frac{A_0 - (A_s - A_b)}{A_0} \times 100$$

where B is the percent pepsin bound; A_0 is the absorbance of the pepsin solution without gibbsite or boehmite; A_s is the absorbance of the supernatant from the pepsin-adsorbent suspension; and A_b is the absorbance of the supernatant of the blank.

Desorption by Acid Wash—Pepsin solutions in 0.001 M HCl having a concentration of 1 and 2.5 mg/mL were prepared for gibbsite and boehmite, respectively. Gibbsite and boehmite stock suspensions (1%) were prepared and sonicated to break up aggregates. Ten milliliters of the stock suspension was transferred into 25-mL volumetric flasks and adjusted to pH 2.4 with 0.1 M HCl. The appropriate pepsin solution (5 and 8 mL, respectively) was added to the gibbsite or boehmite suspensions. The suspensions were adjusted to 25 mL with distilled water. A blank was prepared in the same manner, except

[3] Quantasorb; Quantachrome Corp., Greenvale, N.Y.

Table I—Desorption of Pepsin by Washing Pepsin–Adsorbent Complex with Acidified Water (pH 2.4)

Wash	Pepsin Adsorbed, mg/100 mg of Gibbsite	Cumulative Percent Desorbed	Pepsin Adsorbed, mg/100 mg of Boehmite	Cumulative Percent Desorbed
0	1.938		14.413	
1	1.884	2.8	13.137	8.8
2	1.811	6.6	12.977	10.0
3	1.810	6.6	12.926	10.3
4	—		12.861	10.8
5	—		12.829	10.9

0.001 M HCl replaced the pepsin solution. The suspensions were shaken for 5 min, and the pH was recorded. The suspensions were centrifuged at 10,000 rpm for 15 min. The supernatant was decanted, and its volume was recorded. The absorbance of the supernatant was read at 280 nm, and the pepsin concentration determined by use of a Beer's law plot. If the absorbance of the supernatant was >0.001, the solids were washed with acidified water at pH 2.4. The volume of the acidified water added to the solids was the same as the volume of the supernatant that was decanted. The suspension was shaken for 5 min, the pH was recorded, and the procedure was repeated until the absorbance of the supernatant was ≤0.001. The solid phase was resuspended in 25 mL of water at pH 2.4 and lyophilized. The carbon content of the solid phase was determined by microanalysis[4]. The amount of pepsin adsorbed was calculated from the carbon content of pepsin.

Desorption by Exchange with Phosphate—Pepsin solutions having concentrations of 0.5 mg/mL and 2.5 mg/mL in 0.01 M HCl were prepared for gibbsite and boehmite, respectively. Gibbsite and boehmite stock suspensions (1%) were prepared and sonicated to break up aggregates. Ten milliliters of the stock suspension was adjusted to pH 2.4 with 0.01 M HCl. Five milliliters of the appropriate pepsin solution was added, and the volume was adjusted to 20 mL. The suspensions were shaken for 20 min (control). Similar suspensions were prepared, and 5 mL of a 0.12-g/mL phosphate solution was added to 20 mL of suspension after the 20-min shaking period. The control and phosphate-containing suspensions were centrifuged at 10,000 rpm for 15 min, and the supernatants were filtered through a 0.45-μm filter. The absorbance of the supernatants was read at 280 nm; the equilibrium pepsin concentration was determined by use of a Beer's law plot, and the amount adsorbed was calculated by difference.

Infrared Spectroscopy—Samples were lyophilized and prepared as potassium bromide pellets (1 mg of lyophilized sample/300 mg of KBr). The IR spectrum was recorded between 1800–1000 cm^{-1}. The IR spectrophotometer[5] was interfaced with a computer to provide subtraction of different spectra.

RESULTS AND DISCUSSION

Acid-reactive aluminum hydroxide gels, which are known as carbonate-containing aluminum hydroxide or aluminum hydroxycarbonate (36) and which are used in antacid products, usually react completely within 15–20 min at the pH and temperature of the stomach. Although the adsorption of pepsin by acid-reactive aluminum hydroxide has been demonstrated (5, 12–15), elucidation of the mechanism of adsorption requires a stable surface. To obtain a stable aluminum hydroxide surface, gibbsite and boehmite were used as models because they are closely related structurally to acid-reactive aluminum hydroxide, but they react very slowly with acid, *i.e.*, <2% reacts in 90 min at pH 3 and 37°C. Gibbsite is the most stable polymorph of aluminum hydroxide and has the molecular formula $Al(OH)_3$ (37). Boehmite is an aluminum oxyhydroxide which has the molecular formula AlO(OH) (37). Hydroxyl is the only surface group of gibbsite, while hydroxyl and oxide surface groups are present in boehmite. The samples of gibbsite and boehmite had surface areas of 5 and 250 m^2/g, respectively.

The adsorption isotherms of pepsin on gibbsite and boehmite were determined at pH 2.3, the pH of maximum proteolytic activity (31). As seen in Fig. 1, the adsorptive capacity was 1.7 mg of pepsin/100 mg of gibbsite and 13.9 mg of pepsin/100 mg of boehmite. Thus, the adsorptive capacity was related to the surface area of the adsorbent.

The percent pepsin bound by gibbsite and boehmite was determined at various pH values (Fig. 2). Adsorption of pepsin by both gibbsite and boehmite was observed over the pH range of the GI tract. The greater adsorptive capacity of boehmite was again apparent. Maximum pepsin adsorption occurred at pH 2.7–3.3 for gibbsite, while the range was pH 2.7–4.3 for boehmite. The broader range of maximum adsorption observed for boehmite may be due to

[4] Model 240C; Perkin-Elmer Co., Norwalk, Conn.
[5] Model 180; Perkin-Elmer Co., Norwalk, Conn.

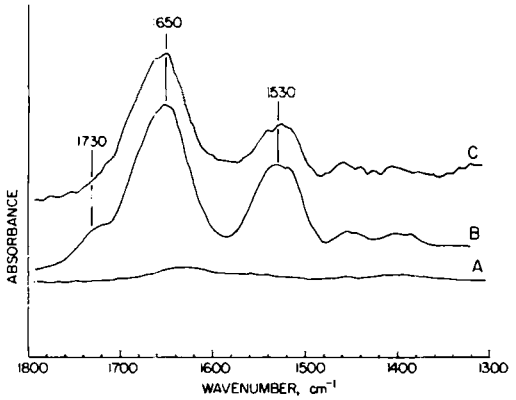

Figure 3—*IR spectrum of boehmite (A), pepsin (B), and pepsin adsorbed on boehmite (C).*

a difference in the pH of maximum adsorption for hydroxyl surface groups when compared with oxide surface groups.

The adsorption of pepsin by gibbsite and boehmite can be partially explained on the basis of electrostatic attraction; the isoelectric point of pepsin was ~1 (38), and the points of zero charge of gibbsite and boehmite were 9.2 and 9.1, respectively. Thus, in the pH region studied, *i.e.*, pH <8, the adsorbent will have a positive surface charge while pepsin will have a negative charge which will decrease as the pH approaches 1. The decreased adsorption at very acidic conditions seen in Fig. 2 may be related to a reduced electrostatic attraction. However, the adsorbent should retain a strong positive charge until the pH was within 1 unit of the point of zero charge, *i.e.*, pH ~8 (39). Thus, the decreased adsorption above pH 3.3 or 4.3 for gibbsite or boehmite, respectively, was not expected based solely on electrostatic attraction.

The pH-adsorption profile seen in Fig. 2 suggests that specific adsorption was also important. Hingston *et al.* (40) and Bowden *et al.* (41) found that anions of incompletely dissociated acids, such as phosphate, are specifically adsorbed by metal oxides and that maximum adsorption occurs when the pH equals the pK_a of the weak acid. The pK_a values of the acidic amino acids in pepsin, such as aspartic acid and glutamic acid, are 3.86 and 4.25, respectively (42), and are in the pH region where maximum adsorption occurred. In addition, the general shape of the pH-adsorption profile is similar to that observed for specific adsorption of anions by metal oxides (40, 41, 43). Bowden *et al.* (41) indicated that the increase in specific adsorption as the pH increases toward the pK_a is due to a more rapid increase in the number of adsorbing species than in the rate of decrease of the positive charge of the surface. The extent of specific adsorption decreases as the pH increases above the pK_a because the surface becomes less positive at a rate greater than the rate of dissociation.

A study of pepsin desorption from gibbsite or boehmite was performed to determine if specific adsorption was an important mechanism. Once pepsin was adsorbed by gibbsite or boehmite at pH 2.4, the pepsin-adsorbent complexes were washed repeatedly with acidified water at pH 2.4. Analysis of the supernatant (Table I) following each wash showed that little pepsin was desorbed by acidic washing. The amount of pepsin adsorbed following five washes was also determined by carbon analysis of the solid phase. The analysis of duplicate samples yielded the following results: 1.80 and 1.74 mg of pepsin adsorbed/100 mg of gibbsite and 12.7 and 13.2 mg of pepsin adsorbed/100 mg of boehmite. It is interesting to note that the adsorptive capacity determined from the plateau of the adsorption isotherm (Fig. 1) also agrees with the results of the desorption experiment. Desorption was expected if the only mechanism of adsorption was electrostatic attraction, as a chloride anion would replace pepsin, or protons would neutralize the negative charge of pepsin, and facilitate pepsin desorption.

Phosphate is specifically adsorbed by aluminum hydroxide by anionic ligand exchange (40, 44). Therefore, washing with phosphate should desorb pepsin if pepsin is also adsorbed by specific adsorption through anionic ligand exchange. The pepsin-gibbsite or pepsin-boehmite complexes were washed with a phosphate solution at 8 times the phosphate concentration of intestinal fluid at pH 2.4. Analysis of the supernatant showed that 95 and 79% of the adsorbed pepsin was desorbed from gibbsite and boehmite, respectively. Since phosphate desorbed pepsin, it was concluded that pepsin was also specifically adsorbed by anion ligand exchange. Phosphate is known to complex with gibbsite by a mono- or bidentate ligand mechanism (40, 44). Desorption studies of monodentate ligands show that binding is reversible, whereas multidentate ligands favor irreversible binding (44). Since phosphate did not totally desorb pepsin, pepsin is believed to form multidentate ligands with gibbsite and boehmite.

The mechanism of adsorption was further investigated by IR spectroscopy. The IR spectrum of pepsin in the 1800–1300-cm^{-1} region is shown in Fig. 3 (line B). The major absorption bands occur at 1530 and 1650 cm^{-1}. The band at 1530 cm^{-1}, known as the amide II band, is due to the N–H deformation vibrations of the main chain of pepsin (45). The band at 1650 cm^{-1}, termed the amide I band, results from the C=O stretching vibrations of the main chain (45). A shoulder present at 1730 cm^{-1} is believed to be due to the C=O stretching vibrations from the undissociated carboxylic acid groups of glutamic and aspartic acids (46).

The IR spectrum of pepsin adsorbed on gibbsite could not be determined because the small amount of adsorbed pepsin could not be detected. However, the degree of adsorption of pepsin on boehmite was sufficient to permit measurement of the IR spectrum of adsorbed pepsin. Boehmite has minimal absorbance in the 1800–1300-cm^{-1} region of the IR spectrum (Fig. 3A). However, to eliminate the contribution of boehmite, the spectrum of the adsorbent was subtracted by computer from the pepsin-boehmite spectrum to produce the IR spectrum of adsorbed pepsin (Fig. 3, line C). The subtraction was performed based on a boehmite band at 1070 cm^{-1}. Before subtracting, a multiplication factor was used on the pepsin-boehmite spectrum to cancel the 1070 cm^{-1} band, *i.e.*, the contribution of boehmite to the spectrum. A multiplication factor of 2.2 was used on the spectrum to produce the same relative absorbance of the amide I band as in pepsin.

As seen in Fig. 3 line C, the amide II band of pepsin at 1530 cm^{-1} was unaffected by adsorption, but the amide I band was shifted to a slightly higher frequency. A slight deformation of structure of pepsin due to adsorption might cause this frequency shift (47). The shoulder at 1730 cm^{-1} was not present in the IR spectrum of adsorbed pepsin, indicating that the carboxyl groups are ionized. The absence of the band at 1730 cm^{-1} supports the ligand exchange adsorption mechanism, since the carboxylic acid groups involved in ligand exchange would have to be in their ionized form to interact by ligand exchange with surface aluminum ions. Thus, ligand exchange would be expected to cause the decrease in absorbance at 1730 cm^{-1} observed for adsorbed pepsin.

Pepsin is adsorbed by aluminum hydroxide in the pH range of the GI tract by specific adsorption involving anionic ligand exchange as well as by electrostatic attraction. Some carboxylate groups of pepsin will displace hydroxyl or water groups from the surface of aluminum hydroxide and covalently bond with surface aluminum. Other carboxylate groups of pepsin which are not physically able to specifically adsorb will interact with the positive surface of aluminum hydroxide by electrostatic attraction. This combination of mechanisms results in strong adsorption which is not reversed by simple washing.

REFERENCES

(1) T. Morris and J. Rhodes, *Gut*, **20**, 538 (1979).

(2) S. K. Lam, K. C. Lam, C. L. Lai, C. K. Yeung, L. Y. C. Yam, and W. S. Wong, *Gastroenterology*, **76**, 315 (1979).

(3) H. J. Priebe, J. J. Skillman, L. S. Bushnell, P. C. Long, and W. Silen, *N. Engl. J. Med.*, **302**, 426 (1980).

(4) *Fed. Regist.*, **39**, 19875 (1974).

(5) D. Piper and B. Fenton, *Am. J. Dig. Dis.*, **NS6**, 134 (1961).

(6) L. Kuruvilla, *Gut*, **12**, 897 (1971).

(7) D. Piper and B. Fenton, *Gut*, **6**, 506 (1965).

(8) M. Schiffrin and S. Komarov, *Am. J. Dig. Dis.*, **8**, 215 (1941).

(9) S. Komarov and O. Komarov, *Am. J. Dig. Dis.*, **7**, 166 (1940).

(10) N. Mutch, *Lancet*, **1**, 859 (1949).

(11) P. Bateson, *J. Pharm. Pharmacol.*, **10**, 123 (1958).

(12) A. Berstad, H. Holm, and E. Kittang, *Scand. J. Gastroenterol.*, **14**, 121 (1979).

(13) J. Wenger and M. Sundy, *J. Clin. Pharmacol.*, **12**, 136 (1972).

(14) W. Anderson and J. Harthill, *J. Pharm. Pharmacol.*, **24**, 166P (1972).

(15) W. Liebman, *IRCS Med. Sci.: Library Compend.*, **8**, 114 (1980).

(16) A. C. Playle, S. R. Gunning, and A. F. Llewellyn, *Pharm. Acta Helv.*, **49**, 298 (1974).

(17) J. Wenger and S. Heymsfield, *J. Clin. Pharmacol.*, **14**, 163 (1974).

(18) J. Wenger and S. Heymsfield, *Gastroenterology*, **64**, 821 (1973).

(19) F. Begeman, V. Schumpelick, and G. Bandomer, *Scand. J. Gastroenterol.*, **16**, 191 (1981).

(20) L. Boyd, W. Russ, and H. Barowsky, *Rev. Gastroenterol.*, **9**, 20 (1942).

(21) G. McHardy and H. Van Rossen, *Curr. Ther. Res. Clin. Exp.*, **4**, 421 (1962).

(22) R. Nelson, *Curr. Ther. Res. Clin. Exp.*, **6**, 83 (1964).

(23) J. Hoon, *Arch. Surg.*, **92**, 467 (1966).

(24) J. Morrissey, T. Honda, Y. Tanaka, and G. Perna, *Arch. Intern. Med.*, **119**, 510 (1967).

(25) W. Taylor, in "Handbook of Physiology," Vol. 5, C. Code, Ed., American Physiology Society, Washington, D.C., 1968, pp. 2568, 2569.
(26) P. Sepulveda, J. Marciniszyn, D. Liu, and J. Tang, *J. Biol. Chem.*, **250**, 5082 (1975).
(27) A. Lehninger, "Biochemistry," 2nd ed., Worth, New York, N.Y., 1975, p. 162.
(28) A. McLaren and G. Peterson, *Nature*, **192**, 960 (1961).
(29) J. Hartsuck and J. Tang, *J. Biol. Chem.*, **247**, 2575 (1972).
(30) W. Taylor, in "Handbook of Physiology," Vol. 5, C. Code, Ed., American Physiology Society, Washington, D.C., 1968, p. 2577.
(31) M. Scijffers, L. Miller, and H. Segal, *Biochemistry*, **3**, 1203 (1964).
(32) M. Pawelczak, *Acta Pol. Pharm.*, **23**, 380 (1966).
(33) J. R. Feldkamp, D. N. Shah, S. L. Meyer, J. L. White, and S. L. Hem, *J. Pharm. Sci.*, **70**, 638 (1981).
(34) A. Lehninger, "Biochemistry," 2nd ed., Worth, New York, N.Y., 1975, p. 83.
(35) J. Tang, *J. Biol. Chem.*, **246**, 4510 (1971).
(36) C. J. Serna, J. L. White, and S. L. Hem, *J. Pharm. Sci.*, **67**, 1144 (1978).
(37) P. H. Hsu, in "Minerals in Soil Environments," J. B. Dixon and S. B. Weed, Eds., Soil Science Society of America, Madison, Wis., 1977, pp. 99-105.
(38) R. Messing, *J. Am. Chem. Soc.*, **91**, 2370 (1969).
(39) D. N. Shah, J. R. Feldkamp, J. L. White, and S. L. Hem, *J. Pharm. Sci.*, **71**, 266 (1982).
(40) F. Hingston, A. Posner, and J. Quirk, *J. Soil Sci.*, **23**, 177 (1972).
(41) J. Bowden, M. Bolland, A. Posner, and J. Quirk, *Nature (London), Phys. Sci.*, **245**, 81 (1973).
(42) A. Lehninger, "Biochemistry," 2nd ed., Worth, New York, N.Y., 1975, p. 79.
(43) F. J. Hingston, R. J. Atkinson, A. M. Posner, and J. P. Quirk, *Nature (London)*, **215**, 1459 (1967).
(44) F. J. Hingston, A. Posner, and J. P. Quirk, *J. Soil Sci.*, **25**, 16 (1974).
(45) F. Parker, "Applications of Infrared Spectroscopy in Biochemistry, Biology and Medicine," Plenum, New York, N.Y., 1971, p. 188.
(46) R. D. B. Fraser and E. Suzuki, in "Physical Principles and Techniques of Protein Chemistry, Part B," S. J. Leach, Ed., Academic, New York, N.Y., 1970, p. 265.
(47) B. Morrissey and R. Stromberg, *J. Colloid Interface Sci.*, **46**, 152 (1974).

ACKNOWLEDGMENTS

Supported in part by the American Foundation for Pharmaceutical Education, through the 1982 AFPE Manufacturing/Industrial Pharmacy Fellowship (R.J.S.), and William H. Rorer, Inc. This report is Journal Paper Number 9490, Purdue University Agricultural Experiment Station, West Lafayette, IN 47907.

Adsorption of Pepsin by Aluminum Hydroxide II: Pepsin Inactivation

ROBERT J. SEPELYAK *, JOSEPH R. FELDKAMP ‡, FRED E. REGNIER §, JOE L. WHITE ‡ and STANLEY L. HEM *x

Received July 18, 1983, from the *Departments of *Industrial and Physical Pharmacy, ‡Agronomy, and §Biochemistry, Purdue University, West Lafayette, IN 47907.* Accepted for publication February 9, 1984.

Abstract □ Pepsin adsorbed on gibbsite or boehmite, non-acid-reactive forms of aluminum hydroxide, had a significantly lower activity than pepsin in solution. IR and desorbed pepsin activity studies showed that the reduced activity of adsorbed pepsin was not due to denaturation of pepsin on adsorption. Steric occlusion of the active site, following pepsin adsorption, was responsible for the lower activity of pepsin adsorbed on gibbsite. The porous morphology of boehmite caused diffusional resistance and steric exclusion, contributing to the decreased activity of adsorbed pepsin. The specific inactivation of pepsin by adsorption on aluminum hydroxide may be important in ulcer therapy.

Keyphrases □ Aluminum hydroxide—adsorption of pepsin, pepsin inactivation □ Pepsin—adsorption by aluminum hydroxide, inactivation □ Adsorption—pepsin adsorption by aluminum hydroxide, pepsin inactivation

The first phase of this study demonstrated that aluminum hydroxide adsorbs pepsin by anionic ligand exchange and electrostatic attractive forces (1). The effect of adsorption on the activity of pepsin and the elucidation of the mechanism responsible for any specific antipepsin effect is the focus of this study.

BACKGROUND

The reduction of pepsin activity by colloidal antacid materials was initially hypothesized to be due to the precipitation of pepsin (2, 3). Berstad (4) has recently concluded that aluminum hydroxide has powerful adsorbent and precipitation effects on pepsin in human gastric juice. Aluminum hydroxide was reported to precipitate pepsin in an inactive form (2). Mutch (5) indicated that pepsin was adsorbed on kaolin, silica gel, calcium phosphate, and magnesium phosphate. Pepsin adsorbed on kaolin or silica gel remained active, but pepsin adsorbed on calcium phosphate or magnesium phosphate was inactive due to denaturation by the high local pH of the surface. Bismuth aluminate, magnesium oxide, and magnesium carbonate were effective in inhibiting pepsin following adsorption (6). Berstad *et al.* (7) assumed that pepsin adsorbed by aluminum hydroxide was inactive. Piper and Fenton (8) stated that the effect of adsorption on pepsin activity was just as important as the pH effect for aluminum hydroxide. Anderson and Harthill (9) found that pepsin adsorbed on an acid-reactive aluminum hydroxide exhibited only 21% of the theoretical activity. Liebman (10) reported that the activity of pepsin was 22% of the control following adsorption on aluminum hydroxide, 29% of the control following adsorption by magnesium hydroxide, and 46% of the control following adsorption by cholestyramine. It was concluded that pepsin was still active while adsorbed because pepsin activity, following dissolution of the three adsorbents, was 88-91% of the control.

EXPERIMENTAL SECTION

Gibbsite[1], boehmite[1], aluminum hydroxycarbonate gel[2], aluminum chlorohydrate[3], porcine pepsin[4], and bovine hemoglobin[4] were obtained commercially. The proteolytic activity of pepsin was determined by either the Anson method (11, 12), using hemoglobin as the substrate, or the dipeptide method (13), which uses the dipeptide *N*-acetyl-L-phenylalanyl-L-3,5-diiodotyrosine as the substrate. The activity of pepsin in the presence of a series of soluble cations was determined using a pepsin solution (20 μg/mL) which was 0.14 mM with respect to the appropriate cation. Likewise, the activity of pepsin in the presence of gibbsite or boehmite was determined using a pepsin solution (20 μg/mL) which contained 0.5 mg/mL of gibbsite or boehmite.

Pepsin solutions (20 μg/mL), or pepsin (20 μg/mL) and gibbsite (0.5 mg/mL) or boehmite (0.5 mg/mL) suspensions, were lyophilized and pre-

[1] Reynolds Metal Co., Bauxite, Ark.
[2] Chattem Chemical Co., Chattanooga, Tenn.
[3] Wicken Products, Huguenot, N.Y.
[4] Worthington Biochemical Corp., Freehold, N.J.

0022-3549/84/1100-1517$01.00/0