Effect of Proteolytic Enzymes on the Binding of Cobalamin to R Protein and Intrinsic Factor

IN VITRO EVIDENCE THAT A FAILURE TO PARTIALLY DEGRADE R PROTEIN IS RESPONSIBLE FOR COBALAMIN MALABSORPTION IN PANCREATIC INSUFFICIENCY

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ABSTRACT Cobalamin (Cbl; vitamin B_{12}) malabsorption in pancreatic insufficiency can be partially corrected by bicarbonate and completely corrected by pancreatic proteases but the mechanisms involved are unknown. Because saliva contains enough R-type Cbl-binding protein (R protein) to bind all of the dietary and biliary Cbl, it is possible that R protein acts as an inhibitor of Cbl absorption and that pancreatic proteases are required to alter R protein and prevent such inhibition. To test this hypothesis we studied the ability of R protein and intrinsic factor (IF) to compete for Cbl binding and ability of pancreatic proteases to alter this competition.

Human salivary R protein bound Cbl with affinities that were 50- and 3-fold higher than those of human IF at pH 2 and 8, respectively. Cbl bound to IF was transferred to an equal amount of R protein with $t_{1/2}$'s of 2 and 90 min at pH 2 and 8, respectively, and within several hours respective ratios of R protein-Cbl/IF-Cbl of 50 and 2 were observed. Cbl bound to R protein was not transferred to IF at either pH 2 or 8. Incubation of R protein with pancreatic proteases at pH 8 led to a 150-fold decrease in its affinity for Cbl. Incubation of R protein-Cbl with pancreatic proteases led to complete transfer of Cbl to IF within 10 min. Gel filtration studies with R protein-[^{57}Co]Cbl and ^{125}I-R protein showed that pancreatic proteases partially degraded R protein. Pancreatic proteases differed in their ability to effect these changes with trypsin > chymotrypsin > elastase. Pancreatic proteases did not alter IF in any of the parameters mentioned above. Pepsin failed to alter either R protein or IF.

These studies suggest the following: (*a*) that Cbl is bound almost exclusively to R protein in the acid milieu of the stomach, rather than to IF as has been assumed previously; (*b*) that Cbl remains bound to R protein in the slightly alkaline environment of the intestine until pancreatic proteases partially degrade R protein and enable Cbl to become bound exclusively to IF; and (*c*) that the primary defect in Cbl absorption in pancreatic insufficiency is a lack of pancreatic proteases and a failure to alter R protein and effect the transfer of Cbl to IF. These studies also suggest that the partial correction of Cbl malabsorption observed with bicarbonate is due to neutralization of gastric HCl, since at slightly alkaline, pH IF can partially compete with R protein for the initial binding and retention of Cbl.

INTRODUCTION

Approximately 50% of patients with pancreatic insufficiency malabsorb crystalline cobalamin (Cbl; vitamin B_{12})[1] (1–4) and in some cases actually develop Cbl deficiency (5). The malabsorption can be partially corrected with oral bicarbonate (2, 6) and completely corrected with oral pancreatic extract (2, 4, 6, 7), trypsin

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Received for publication 30 June 1977 and in revised form 19 August 1977.

[1] *Abbreviations used in this paper*: Cbl, cobalamin; DFP, diisopropyl fluorphosphate; IF, intrinsic factor; R protein, R-type cobalamin-binding protein; TLCK, *N*-*α*-*p*-tosyl-L-lysine chloromethyl ketone HCl; TPCK, L-1-tosylamide-2-phenylethylchloromethyl ketone.

(7, 8), or chymotrypsin (8) but the mechanisms involved have not been defined.

Determinations of ileal pH have found values near neutrality in patients with pancreatic insufficiency and differences have not been observed between those with normal and those with abnormal Cbl absorption (3). These observations, together with the fully corrective effects of pancreatic proteases, rule out the unlikely possibility that the primary defect in those patients with Cbl malabsorption is an inability of the pancreas to secrete enough bicarbonate to neutralize gastric HCl and raise the ileal pH to values (pH 5.6–10) that will enable intrinsic factor (IF)-Cbl to bind to its ileal receptors.

Studies in patients have shown that IF is produced in normal amounts (3) and that this IF functions normally in terms of correcting Cbl malabsorption in patients with pernicious anemia (2). Studies in partially pancreatectomized rats (9) have shown that ileal receptors for IF-Cbl are present in normal amounts and that they function normally in terms of binding the IF-Cbl complex. Cbl malabsorption in humans with pancreatic insufficiency is not corrected by the oral administration of antibiotics nor is it corrected by binding Cbl to human gastric juice or crude hog IF in vitro and then administering it either orally or in the latter case directly into the distal ileum (3).

Toskes et al. (8) have shown that Cbl is absorbed normally in pancreatic insufficiency when it is given bound to human gastric juice or crude hog IF that have been incubated previously with insolubilized trypsin or chymotrypsin. This has suggested that pancreatic proteases might alter the structure of IF and that this might be required for IF to facilitate the actual absorption of Cbl. Attempts to demonstrate that pancreatic proteases do alter the structure of IF in terms of its molecular weight, electrophoretic mobility, or affinity for Cbl have, however, been unsuccessful (8).

It has also been suggested (4, 8, 10–13) that pancreatic proteases might inactivate an endogenous inhibitor of Cbl absorption and several investigators (10–13) have suggested that the R-type Cbl-binding protein[2] (R protein) might act as such an inhibitor. This possibility is supported by the presence of R protein in saliva, milk, gastric juice, bile, and crude hog IF (14, 15) and by the fact that the 25 nmol/24 h of R protein in saliva alone (16, 17) is capable of binding all of the Cbl present in a normal diet (4–12 nmol/24 h) and in bile (2–6 nmol/24 h) (14). Because little is known about the abilities of R protein and IF to compete for binding Cbl, we have investigated this phenomenon at pH 2 and 8, to simulate conditions in the stomach and intestine, respectively and have also investigated the effects of pepsin and a number of pancreatic proteases.

[2] The term "R protein" was originally devised to denote a B_{12}-binding protein in human gastric juice that was devoid of intrinsic factor activity. It was designated as protein "R" because of its rapid mobility on electrophoresis. Subsequently, immunologically related proteins have been observed in a variety of human tissues and body fluids and have been referred to as the R proteins.

METHODS

Cbl-binding proteins and antisera. Human gastric juice was collected as described previously (18) and 10 ml was applied to a 2 × 90-cm column of Sephadex G-150 (Sigma Chemical Co., St. Louis, Mo.) equilibrate at 4°C with 0.01 M Tris-HCl, pH 8.0, containing 0.15 M NaCl and 50 μg/ml of bovine serum albumin (Sigma Chemical Co.). 5-ml fractions were collected and assayed for total Cbl-binding ability (18) and Cbl-binding ability due to IF (18). Fractions in which IF represented >98% of the Cbl-binding ability were pooled and utilized as the source of IF. Human saliva (16) was dialyzed against 100 vol of 0.01 M Tris-HCl, pH 8.0, containing 0.15 M NaCl, for 24 h at 4°C and utilized as the source of R protein unless otherwise indicated. Homogeneous human saliva R protein was isolated (16) and labeled with ^{125}I and ^{131}I by the method of Bolton and Hunter (19) as previously described (20). Rabbit anti-human IF (21) and anti-human R protein (16) sera were obtained as previously described. Association constants for Cbl-binding proteins and Cbl were determined at 37°C in standard incubation buffer using the charcoal adsorption technique as described (22).

Assay of the distribution and transfer of Cbl between R protein and IF. The standard assay contained the following components in buffer consisting of 0.01 M Tris-HCl, pH 8.0, 0.15 M NaCl: (*a*) 25 μl of IF containing 1 pmol of Cbl-binding ability; (*b*) 25 μl of R protein containing 1 pmol of Cbl-binding ability; (*c*) 25 μl containing 0.5 pmol of CN-[^{57}Co]Cbl, (10–15 μCi/nmol, Amersham/Searle Corp., Arlington Heights, Ill.); and (*d*) 425 μl of buffer. After 15 min at 37°C the assay tubes were incubated in a 4°C water bath for 10 min and 100 μl of either rabbit anti-human IF serum, rabbit anti-human R protein serum, or rabbit control serum was added. After 30 min at 4°C 500 μl of H_2O saturated with $(NH_4)_2SO_4$ at pH 8.0 was added and 15 min later the tubes were centrifuged at 10,000 *g* for 20 min and 500 μl of the supernatant fraction was assayed for [^{57}Co]Cbl. The time-course of transfer of [^{57}Co]Cbl from one Cbl-binding protein to the other was determined in the same way except that only one of the Cbl-binding proteins was present during the initial 15-min incubation at 37°C; the second Cbl-binding protein was added subsequently followed by a further incubation at 37°C.

The ability of pancreatic proteolytic enzymes to alter the distribution and transfer of [^{57}Co]Cbl between R protein and IF was studied by performing a 60-min preliminary incubation at 37°C with enzyme and various components of the standard assay. This was followed by the addition of 1 μl (5.5 mmol) of diisopropyl fluorophosphate (DFP) (Sigma Chemical Co.) and after 15 min at 37°C the remaining components of the assay were added.

Studies performed at acid pH employed 0.01 M potassium phosphate pH 2.0 in place of Tris-HCl. The volume of the initial incubation was reduced to 450 and 50 μl of 0.5 M Tris base was added to bring the pH to 8.0 immediately before the addition of rabbit antiserum.

Proteolytic enzymes, inhibitors, and assays. Bovine pan-

creatic trypsin, trypsin treated with L-1-tosylamide-2-phenylethylchloromethyl ketone (TPCK), (TPCK-trypsin), and bovine pancreatic α-chymotrypsin were obtained from Worthington Biochemical Corp., Freehold, N. J., and had stated activities of 180, 190, and 61 U/mg dry wt, respectively. Viokase (4 National Formulary pancreatin) was obtained from VioBin Corp., Monticello, Ill. Orcein-coated elastin and human pancreatic elastase were generous gifts from Dr. John Pierce, Washington University School of Medicine, St. Louis, Mo. Hog gastric pepsin, 2,650 U/mg dry wt, TPCK, and *N*-α-*p*-tosyl-L-lysine chloromethyl ketone HCl (TLCK) were obtained from Sigma Chemical Co.

TLCK-chymotrypsin was prepared immediately before use by incubating chymotrypsin with TLCK (50 μg/mg chymotrypsin) in 0.01 M Tris-HCl, pH 8.0, 0.15 M NaCl for 15 min at 37°C. TLCK-TPCK-elastase was prepared in the same manner with both TLCK and TPCK.

Trypsin and chymotrypsin were assayed by the method of Hummel (23) using *p*-toluenesulfonyl-L-arginine methyl ester and benzoyl-L-tyrosine ethyl ester (both from Sigma Chemical Co.) as the respective substrates. Elastase was assayed by the method of Sacher et al. (24) using orcein-coated elastin as substrate. Pepsin was assayed by the method of Anson (25) using hemoglobin as substrate. Using these assays and the units defined therein the various enzyme preparations were found to have the following activities, expressed as units per milligram of trypsin, chymotrypsin, and elastase, respectively: trypsin, 172, <0.1, and 1.4; TPCK-trypsin, 156, <0.1, and 1.0; chymotrypsin, 50, 31, and 7.9; TLCK-chymotrypsin, <0.1, 30, and 1; elastase, <0.1, 0.5, and 68; TLCK-TPCK-elastase, <0.1, 0.1, and 62; and Viokase, 1.4, 5.1, and 5.8.

RESULTS

Distribution and transfer of Cbl between R protein and IF. When 0.5 pmol of [^{57}Co]Cbl was added to a mixture of 1 pmol of IF and 1 pmol of R protein at pH 8 and incubated for 60 min at 37°C, 30% of the [^{57}Co]Cbl was bound to IF and 70% to R protein. When the same experiment was performed at pH 2, 2% of the [^{57}Co]Cbl was bound to IF and 98% was bound to R protein. The results of experiments designed to determine the transfer of [^{57}Co]Cbl between R protein and IF are shown in Fig. 1 and show that [^{57}Co]Cbl bound to IF was transferred to R protein with $t_{1/2}$'s of 90 and 2 min at pH 8 and 2, respectively. In contrast, [^{57}Co]Cbl bound to R protein was not transferred to IF in significant amounts at either pH 8 or 2.

In another experiment, [^{57}Co]Cbl was bound to IF at pH 8 followed by the lowering of the pH to 2 and the addition of R protein. At the end of 60 min >95% of the [^{57}Co]Cbl was bound to R protein indicating that the formation of IF-Cbl at pH 8 does not prevent the transfer of Cbl to R protein at acid pH.

Association constants for Cbl and R protein and IF. At pH 8, R protein bound Cbl with an association constant of 1.6/pM which is approximately threefold higher than the value of 0.6/pM determined for IF and Cbl under the same conditions. At pH 2, the association constant for R protein and Cbl was found to be 1.0/pM which is 50-fold higher than the value of 0.02/pM found for IF and Cbl. These differences resemble the relative abilities of these proteins to compete with each other for binding Cbl with the exception that Cbl bound to R protein was not transferred to IF at pH 8 during the 4-h time period studied.

Ability of proteolytic enzymes to alter the transfer of Cbl between R protein and IF. R protein alone was incubated with a variety of pancreatic proteolytic enzymes at pH 8 with the subsequent sequential additions of DFP and [^{57}Co]Cbl. IF was then added and after 60 min the distribution of [^{57}Co]Cbl between IF and R protein was determined. The results are shown in Fig. 2 and show that 50% of the [^{57}Co]Cbl was transferred to IF at the following concentrations of enzyme: TPCK-trypsin, 8 μg/ml; Viokase, 24 μg/ml; TLCK-chymotrypsin, 300 μg/ml; and TLCK-TPCK-elastase, 600 μg/ml. With an equal mixture of untreated trypsin, chymotrypsin, and elastase, 50% transfer oc-

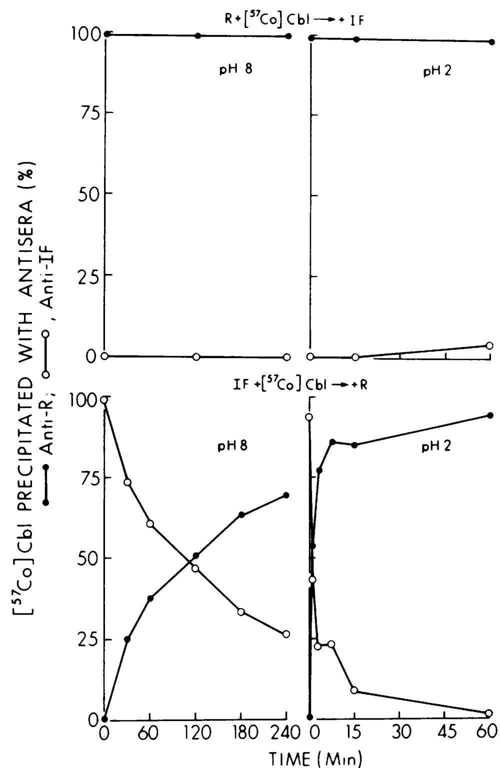

FIGURE 1 Transfer of [^{57}Co]Cbl from R protein to IF (top) and from IF to R protein (bottom) at pH 8 (left) and pH 2 (right). Initial incubations contained 0.5 pmol of [^{57}Co]Cbl and 1.0 pmol of either IF or R protein. The second Cbl-binding protein was then added, the incubations were continued for the times indicated on the abscissa, and the amount of [^{57}Co]Cbl bound to R protein and IF was assayed by adding anti-R and anti-IF to separate samples that had been prepared in duplicate. Only R protein or IF was added for the zero time points. Additional details are contained under Methods.

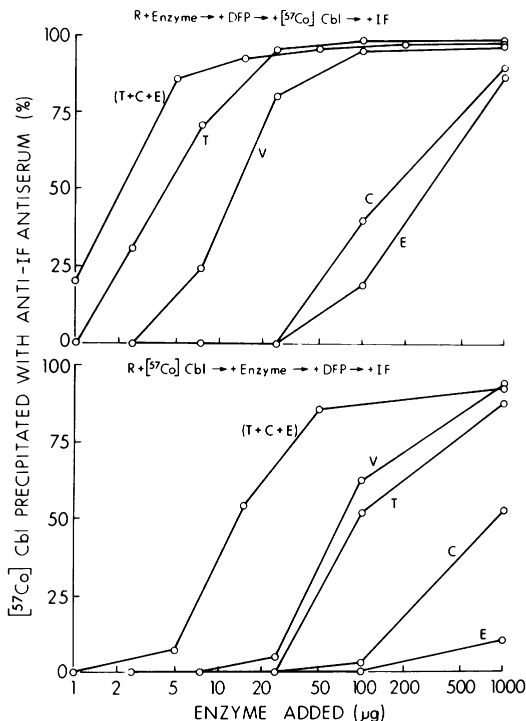

FIGURE 2 Effect of pancreatic proteolytic enzymes on the transfer of [^{57}Co]Cbl from R protein to IF at pH 8. Enzyme incubations were performed at 37°C in a 0.5-ml volume. The components and incubation times were as follows: (top) 1.0 pmol R protein + enzyme, 60 min; +DFP, 15 min; +0.5 pmol [^{57}Co]Cbl, 30 min; and +1.0 pmol IF, 60 min. (Bottom) 1.0 pmol R protein + 0.5 pmol [^{57}Co]Cbl, 30 min; +enzyme, 60 min; +DFP, 15 min; +1.0 pmol IF, 60 min. Additional details are described under Methods. The enzymes employed were: T, TPCK-trypsin; C, TLCK-chymotrypsin; V, Viokase; E, TLCK-TPCK-elastase; and (T + C + E), an equal mixture of untreated trypsin, chymotrypsin, and elastase. The amount of enzyme noted with the mixture refers to total enzyme and not to the amount of each individual enzyme.

curred at a total protein concentration of only 4 μg/ml which indicates that levels of these enzymes well within the physiologic range (26) act synergistically in their ability to effect the transfer of Cbl from R protein to IF. Additional experiments at the highest enzyme concentrations showed that the observed transfer of [^{57}Co]Cbl from treated R protein to IF was maximal within 10 min of adding the IF.

Similar experiments were performed in which R protein-[^{57}Co]Cbl was incubated with the same proteolytic enzymes. The results are also presented in Fig. 2 and show that 50% of the Cbl was transferred to IF at the following concentrations of enzymes: Viokase, 140 μg/ml; TPCK-trypsin, 200 μg/ml; TLCK-chymotrypsin, 2000 μg/ml; TLCK-TPCK-elastase, >2,000 μg/ml; and an equal mixture of untreated trypsin, chymotrypsin, and elastase, 26 μg/ml. These results indicate that physiologic levels of these enzymes also act synergistically on the R protein-Cbl complex to effect the subsequent transfer of Cbl to IF although the required concentrations of enzymes are 6- to 25-fold higher than when these enzymes act on R protein alone. Additional experiments performed at the highest enzyme concentrations showed that transfer of [^{57}Co]Cbl from treated R protein-[^{57}Co]Cbl to IF was maximal within 10 min of adding the IF.

Treatment of TPCK-trypsin and TLCK-chymotrypsin with TLCK and TPCK, respectively completely abolished their respective enzyme activities (>99%) and also completely abolished their ability to act on R protein and R protein-[^{57}Co]Cbl and effect the subsequent transfer of [^{57}Co]Cbl from R protein to IF. Similar inhibitions of enzyme and transfer activity were observed when TPCK-trypsin, TLCK-chymotrypsin, TLCK-TPCK-elastase, and Viokase were treated with the serine protease inhibitor DFP. No changes in the transfer of Cbl in either direction between IF and R protein at pH 8 were observed when IF, IF-[^{57}Co]Cbl, or [^{57}Co]Cbl were incubated with the pancreatic proteolytic enzymes either singly or in combination at enzyme concentrations ranging from 10 to 2,000 μg/ml. No changes in transfer at pH 2 or 8 were observed when pepsin was incubated with IF, IF-[^{57}Co]Cbl, R protein, R protein-[^{57}Co]Cbl,

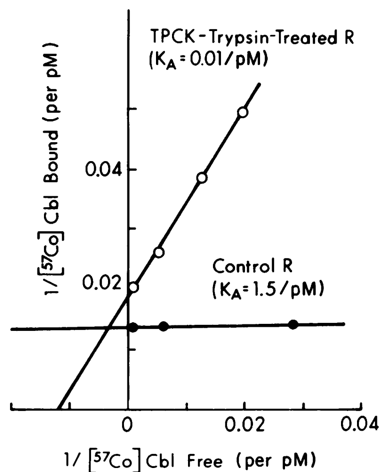

FIGURE 3 Determination of the association constant (K_A) of TPCK–trypsin-treated R protein and control R protein at pH 8. TPCK–trypsin-treated R protein was prepared by incubating 10 pmol/ml of R protein with 2,000 μg/ml of TPCK-trypsin for 60 min at 37°C followed by the addition of 2 μl/ml of DFP and an additional 15-min incubation. Control R protein was prepared simultaneously in the same way except that TPCK-trypsin was omitted. Equal volumes of trypsin-treated and control R protein were used for the Cbl-binding determinations which were performed at the same time.

or [^{57}Co]Cbl at pH 2 at enzyme concentrations ranging from 10 to 2,000 μg/ml.

Association constants for Cbl of R protein and IF treated with proteolytic enzymes. As shown in Fig. 3 the association constant for R protein and [^{57}Co]Cbl at pH 8 decreased from 1.5 to 0.01/pM when R protein was incubated with TPCK-trypsin, at an enzyme concentration of 2,000 μg/ml followed by the addition of DFP. The maximal Cbl-binding capacity of R protein decreased by ≅25% indicating that the Cbl-binding ability of some of the R protein molecules was destroyed by incubation with TPCK-trypsin. Similar results (data not shown) were obtained with R protein treated with TLCK-chymotrypsin, TLCK-TPCK-elastase, and Viokase. Enzyme incubations performed with IF did not lead to any change in its association constant or maximal binding capacity for [^{57}Co]Cbl. No changes were observed at either pH 8 or 2 when R protein or IF were incubated at pH 2 with pepsin at an enzyme concentration of 2,000 μg/ml.

Effect of proteolytic enzymes on the apparent molecular weights of R protein and IF. The data in Fig. 4 indicate that incubation of R protein with 2,000 μg/ml of TPCK-trypsin, TLCK-chymotrypsin, TLCK-TPCK-elastase, or Viokase, followed by the sequential addition of DFP and [^{58}Co]Cbl, led to a decrease in the apparent molecular weight of the R protein-[^{58}Co]Cbl complex from 150,000 to ≅70,000. Similar changes were observed when R protein-[^{58}Co]Cbl was treated with TPCK-trypsin or Viokase. After treatment of R protein-[^{58}Co]Cbl with TLCK-chymotrypsin, only 50% of the R protein-[^{58}Co]Cbl was converted to the 70,000 apparent molecular weight form. Treatment with elastase caused no change in the apparent molecular weight of R protein-[^{58}Co]Cbl. The changes in apparent molecular weight obtained with these enzymes correlate well with their abilities to effect the transfer of Cbl from R protein to IF (cf. Figs. 2 and 4) and indicates that these enzymes effect the transfer of Cbl from R protein to IF by limited proteolysis of R protein. Changes in apparent molecular weight were not observed when IF or IF-Cbl were treated similarly with TPCK-trypsin, TLCK-

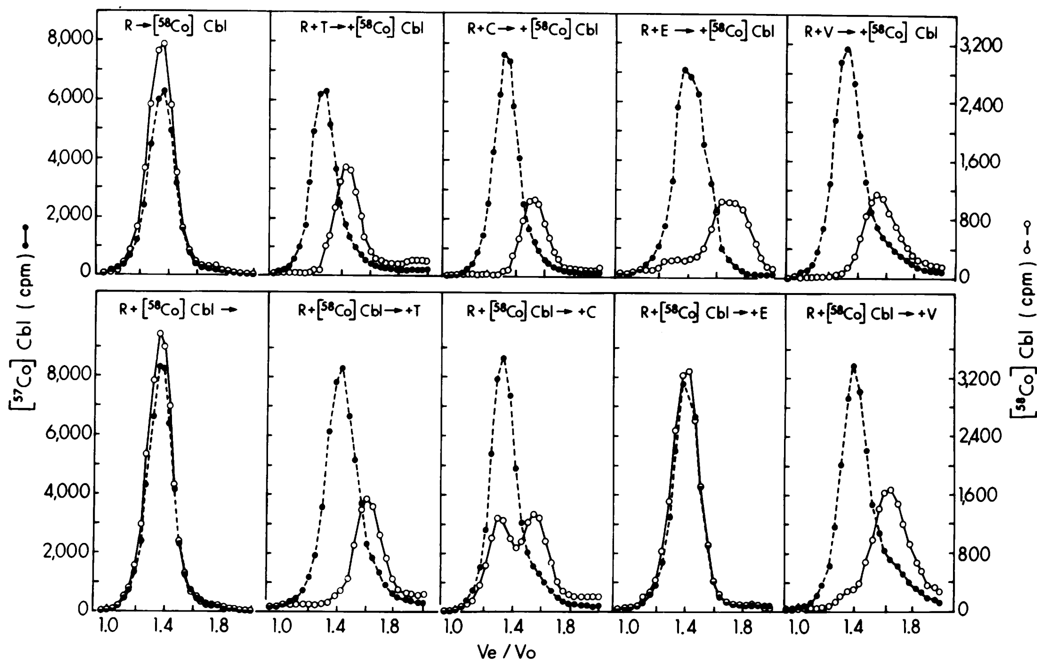

FIGURE 4 Effect of pancreatic proteases on the gel filtration elution profile of R protein-[^{58}Co]Cbl. (Top) The sequence of adding components and incubation times at 37°C were as follows: 50 pmol of R protein in 2.7 ml of buffer consisting of 0.01 M Tris-HCl pH 8, 0.15 M NaCl, 15 min; 6 mg of enzyme in 0.3 ml of buffer, 60 min; 6 μl of DFP, 15 min; and 100 pmol of [^{85}Co]Cbl in 20 μl of H_2O, 30 min. (Bottom) Same as above except that the [^{58}Co]Cbl was present during the initial incubation. The enzymes employed were: T, TPCK-trypsin; C, TLCK-chymotrypsin; E, TLCK-TPCK-elastase; and V, Viokase. After the final incubation, all samples were stored frozen at −20°C until just before they were applied to 2 × 90-cm columns of Sephadex G-150 equilibrated with incubation buffer at 4°C. Immediately before application, 3 pmol of R protein-[^{57}Co]Cbl and 4 mg of blue dextran in 0.3 ml of buffer were added to each sample.

chymotrypsin, TLCK-elastase, or Viokase at pH 8 nor were changes observed when IF, IF-Cbl, R protein, or R protein-Cbl were treated with 2,000 μg/ml of pepsin at pH 2.

Additional evidence that pancreatic enzymes cause proteolysis of R protein was obtained from experiments employing ^{125}I-R protein. The gel filtration pattern obtained after incubating ^{125}I-R protein with 2,000 μg/ml of TLCK-chymotrypsin is shown in Fig. 5 and shows that the apparent molecular weight of the ^{125}I shifts from 150,000 to a new distribution in which ~30 and 70% of the ^{125}I have apparent molecular weights of 70,000 and <5,000, respectively. Similar results were obtained after incubations with 2,000 μg/ml of TPCK-trypsin, TLCK-TPCK-elastase, and Viokase.

DISCUSSION

It has been assumed that orally administered Cbl is bound by IF in the stomach and that the IF-Cbl complex remains intact until sometime after it becomes bound to its ileal receptor. The first assumption appears incorrect, however, since our studies indicate that orally administered Cbl, and even Cbl bound to IF, would be bound almost exclusively by R protein in the stomach under normal conditions of acid pH. It also appears that Cbl would remain bound to R protein at the nearly neutral pH of the small intestine and would not become bound to IF until the R protein moiety is partially degraded by the pancreatic serine proteases, trypsin and chymotrypsin, which act synergistically in this regard together possibly with elastase. Free R protein is even more susceptible to partial degradation by these proteases and this process appears to be important physiologically since some but not all of the Cbl bound to IF would otherwise become bound to intact R protein during the several hours required for Cbl to reach the terminal ileum. Human bile contains significant amounts of R protein and R protein-Cbl and it appears likely that this R protein is also partially degraded by pancreatic proteases especially since most of the biliary Cbl appears to be reabsorbed via the IF mechanism (14).

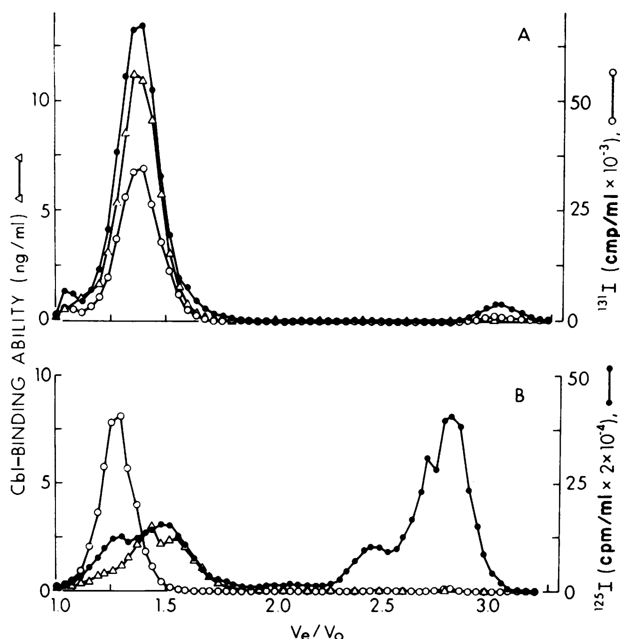

FIGURE 5 Effect of TLCK-chymotrypsin on the gel filtration elution profile of ^{125}I-R protein. Samples contained 300 pmol of ^{125}I-R protein in 3.0 ml of 0.01 M Tris-HCl pH 8, 0.15 M NaCl and were incubated at 37°C for 60 min. DFP, 6 μl, was added and after an additional 15 min the samples were frozen at −20°C. No enzyme was present in sample A (top) and 6 mg of TLCK-chymotrypsin was present in sample B (bottom). Immediately before the samples were subjected to gel filtration, which was performed as described in the legend to Fig. 4, 1 pmol of ^{131}I-R protein and 4 mg of blue dextran in 0.3 ml of buffer were added to each sample.

Our studies indicate that the primary defect in Cbl absorption in pancreatic insufficiency is an inability to partially degrade R protein and R protein-Cbl due to a deficiency of pancreatic proteases. Our studies also provide an explanation for the partially corrective effect of bicarbonate in Schilling tests (2, 6) since in the absence of low pH in the stomach much but not all of the radioactive Cbl would become bound to IF, and only a portion of this would be transferred to R protein during the several hours required to reach the ileum. Such transfer might be even lower than expected since endogenous pancreatic proteases may be present at levels capable of degrading free R protein although not R protein-Cbl. Superimposed achlorhydria would have the same effect as oral bicarbonate and may be responsible for the fact that some patients with total pancreatectomies have normal Schilling tests and for the observation that levels of pancreatic proteases in the intestine do not always correlate with Schilling test results in pancreatic insufficiency (4). The correlation of achlorhydria with alcohol abuse and its common occurrence with increasing age (27) could explain why Schilling tests are abnormal in only 50% of adults with pancreatic insufficiency (1–4) from a variety of causes, many of whom are alcoholics, and yet are invariably abnormal in children and young adults with pancreatic insufficiency due to cystic fibrosis (6).

Our model of Cbl absorption is consistent with the results of previous experiments showing that Cbl was not absorbed when it was bound to crude IF before its administration to patients with pancreatic insufficiency. The addition of Cbl to crude gastric juice before its oral administration (3) would not be expected to correct absorption since gastric juice usually contains significant amounts of R protein (11,

18, 21) and even in its absence the Cbl would be transferred to endogenous R protein in the stomach. The addition of Cbl to crude hog IF before either its oral or intra-ileal administration (3) should also fail to correct absorption since crude hog IF contains approximately four times more non-IF Cbl-binding protein than IF (15) and since this non-IF has many of the properties of human R protein including its superiority in competing with IF for binding Cbl and its susceptibility to degradation by pancreatic proteases.[3] It appears likely that the correction in Cbl malabsorption observed when gastric juice or crude hog IF were treated with insoluble proteases (8) was due to one or more of the following: (*a*) partial degradation of R protein in gastric juice and crude hog IF, (*b*) solubilization and administration of enough protease to partially degrade endogenous free R protein, and (*c*) the possible ability of partially degraded R protein to compete for Cbl binding with intact R protein at acid pH. We did not detect any effect of pancreatic proteases on free IF or IF-Cbl although our studies do not exclude the possibility that pancreatic proteases also cause an alteration in IF that is required for Cbl absorption.

Although our model can account for a number of in vivo observations regarding Cbl malabsorption in pancreatic insufficiency, it is important to note that it is based primarily on experiments performed in vitro. We have recently studied a nonradioactive Cbl analogue that is bound with high affinity by R protein but not by IF and have shown that this analogue is capable of inhibiting the ability of R protein to compete with IF for [^{57}Co]Cbl binding in vitro. The oral administration of this analogue also corrects [^{57}Co]-Cbl malabsorption in patients with pancreatic insufficiency[4] and provides additional in vivo support for our hypothesis that the primary defect in pancreatic insufficiency is an inability to partially degrade R protein.

ACKNOWLEDGMENTS

We thank Mrs. Carol Mehlman Margolis for her expert technical assistance.

This work was supported by grants AM 16668, AM 14038, and AM 05280 from the National Institutes of Health and Faculty Research Award FRA-122 from the American Cancer Society.

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[4] Manuscript in preparation.

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