Inhibition and promotion of cholesterol crystallization by protein fractions from normal human gallbladder bile

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Abstract Pooled, normal human gallbladder biles were initially separated on a molecular sieving chromatography column to remove soluble mucin glycoproteins as well as high molecular weight proteins (>200,000). The remaining lower molecular weight proteins and other bile components were then examined by lectin affinity chromatography with four different types of lectin. The separated bound fractions were compared for inhibiting and promoting activities with a newly devised sensitive cholesterol crystal growth assay and for differences in electrophoretic patterns on SDS-gels. Protein factors (presumably glycoproteins) were found to have both inhibiting and promoting activities, even in the absence of cholesterol gallstone disease. The promoting effect was indicated by shortened crystal detection times and increases in crystal growth rate; whereas the inhibiting effect was indicated by decreases in crystal growth rate and reductions in the final crystal concentration as determined by the growth assay. Affinity chromatography mitigated the major problems of removing both lipids and pigment from the glycoproteins. In addition, partial purification of bound fractions with potent cholesterol crystal nucleation-altering activity can be obtained by this technique.—**Busch, N., N. Matiuck, S. Sahlin, S. P. Pillay, and R. T. Holzbach.** Inhibition and promotion of cholesterol crystallization by protein fractions from normal human gallbladder bile. *J. Lipid Res.* 1991. **32:** 695–702.

Supplementary key words human gallstone pathogenesis • inhibiting factors • promoting factors • lectin affinity chromatography • glycoproteins • cholesterol crystal growth assay

Recent studies support the hypothesis that supersaturated human bile contains proteins than can either promote or inhibit the formation of cholesterol crystals (1–9). Based on the nucleation time assay, these proteins were designated as pro- or anti-nucleating (4–9). Evidence for the existence of a protein-derived inhibitory activity has been based solely on studies of a crude and heterogenous fraction of proteins obtained from normal human bile by low-yield and cumbersome methods (10). These methods included lipid composition matching, reconstitution, and protease ablation. The difficulties presented by these methods for obtaining sufficient biliary protein for further purification have thus far proved insurmountable.

Lectin affinity chromatography using concanavalin A (CA) lectin was introduced by Groen and coworkers to identify the presence of a promoting activity in bile samples (6, 7). This finding has recently been independently confirmed (8). The implication of these reports is that the promoting activity is attributable to a specific type of glycoprotein in bile. On the other hand, an inhibitory activity has also been reported in the unbound CA fraction (6). This finding suggests that the coexistent inhibitory factor is not a glycoprotein. To support or refute this possibility, we observed and compared the promoting and inhibiting effects of lectin-bound fractions using a spectrum of four types of lectins. These effects were measured using a new crystal growth assay (11).

We attempted to determine whether nucleation-inhibiting activity, like nucleation-promoting activity, occurred in the same bile glycoprotein fraction. Our results indicate the presence of protein glycoprotein-related inhibiting activity when promoting activity is also present. We also attempted to determine whether the balance of coexistent glycoprotein-related promoting and inhibitory activities is lectin-specific and possibly concentration-dependent. The balance of these activities was found to be lectin-specific and concentration-dependent, with a trend toward greater promoting activity in the presence of higher biliary glycoprotein concentrations.

Abbreviations: CA, concanavalin A; STC, sodium taurocholate; STDC, sodium taurodeoxycholate; PL, egg lecithin; CH, cholesterol; CSI, cholesterol saturation index; LL, lentil lectin-Sepharose 4B; WG, wheat germ lectin-Sepharose 6MB; HP, *Helix pomatia* lectin-Sepharose 6MB; TBS, Tris-buffered saline.

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MATERIALS AND METHODS

Sodium salts of taurocholic acid (STC) and taurodeoxycholic acid (STDC) (Calbiochem, San Diego, CA) were twice recrystallized according to the method of Pope to achieve greater than 99% purity (12). Egg lecithin (PL), grade I (Lipid Products, S. Nutfield, Surrey, UK) was proven to be greater than 99% pure by high-performance liquid chromatography (13), and cholesterol (CH) (Eastman Kodak Co., Rochester, NY) was 99.8% pure by differential scanning calorimetry (14). Concanavalin A-Sepharose 4B (CA), lentil lectin-Sepharose 4B (LL), wheat germ lectin-Sepharose 6MB (WG) and *Helix pomatia* lectin-Sepharose 6MB (HP) were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden), and D-methylpyranoside, N-acetyl-D-glucosamine, and N-acetyl-D-galactosamine were obtained from Sigma Chemical Co. (St. Louis, MO). Purified lectins were purchased from either Pharmacia or Sigma. Sodium dodecyl sulfate (SDS), acrylamide, bisacrylamide, ammonium persulfate, and TEMED were purchased from Bio-Rad Laboratories (Richmond, CA).

All other chemicals used were ACS or reagent grade. Organic solvents were HPLC grade (Fisher Scientific Co., Pittsburgh, PA or Sigma Chemical Co.). Glassware was acid-washed and thoroughly rinsed with purified water prior to drying. The water was filtered, ion-exchanged, and glass-distilled (Corning Glass Works, Corning, NY). Solutions were filtered through 0.22-μgm filters (Millipore Corp., Bedford, MA) and degassed before their use in preparing model bile and subsequent growth assay measurements.

Growth assay measurements

Bile acid concentrations were measured enzymatically using 3-α-hydroxysteroid dehydrogenase (Sigma Chemical Co.) (15). Lecithin concentrations were determined by the method of Bartlett (16, 17), and cholesterol concentrations were assayed enzymatically based on cholesterol esterase and cholesterol oxidase using a commercially available assay kit (Boehringer Mannheim Corp., Indianapolis, IN) (18). Protein concentrations were measured fluorometrically relative to bovine serum albumin standard (10) using a Turner fluorometer (Sequoia-Turner Corp., Mountain View, CA) equipped with an excitation filter of 405 nm and an emission filter of 485 nm. Samples were assayed directly, or if necessary, after trichloroacetic acid (TCA) precipitation (10%, w/v) and twofold delipidation with ethyl ether–ethanol 2:1 (v/v) as described by Sewell (19) and validated by Yamazaki, Powers, and LaRusso (20).

Model biles

Cholesterol saturation indices (CSI) were calculated from Carey's critical tables (21). Concentrations of lipid stock solutions (STC in methanol, cholesterol in methanol, and phospholipid in chloroform–methanol 2:1) were determined as described above, taking into account the expected dilutions caused by adding seed crystals and effector solutions to the final model-bile test solution. Appropriate aliquots of lipid stock solutions were mixed, flushed with nitrogen, and shaken at 37°C for 2 h. Organic solvents in this mixture were then evaporated to dryness under a stream of nitrogen at 50°C with subsequent lyophilization for 6 h as previously described (22). The lyophilized lipid films were then resuspended in Tris-buffered saline (TBS) (25 mmol Tris, 150 mmol NaCl, pH 7.45) including 3 mmol NaN_3 as an antimicrobial agent. The suspension was then incubated in a G-24 Environmental Incubator/Shaker (New Brunswick Scientific, New Brunswick, NJ) at 100 rpm and maintained at 55°C until it was microscopically isotropic. After 6 h the clear solution was filtered through a preheated, 0.22-μm Swinney filter assembly (Millipore Corp.), flushed with nitrogen, and incubated at 37°C for 15 min before use in the crystal growth assay.

Crystal growth assay

Cholesterol crystal growth in model bile was measured as recently described and validated (11, 23). Before the start of each crystal growth experiment, aliquots of filtered (0.22 μm) aqueous solutions of the effectors of interest were inserted into vials equipped with Teflon-lined screw caps, lyophilized, and resolubilized in 40 μl TBS. Control vials contained only 40 μl TBS. Vials were pre-equilibrated at 37°C. Aliquots (400 to 500 μl) of model bile, equilibrated at 37°C, were distributed to each vial 15 min after model bile filtration. The crystal growth assay was carried out in two versions: seeded and unseeded (spontaneous). Seed crystals were prepared by sonifying cholesterol crystals that were grown in supersaturated solutions of cholesterol in ethanol and then filtered to retain only those crystals between 0.22 μm and 0.8 μm in diameter. At zero time, the crystal growth of one set of test solutions was initiated by adding a small amount of seed crystals (10 to 25 μl, to achieve 0.5 to 5 μg/ml of crystalline cholesterol in the final test solution).

The second unseeded set of test solutions was adjusted for the volume of seed crystals by adding the same volume (10 to 25 μl) of distilled water and allowing nucleation and crystal growth to initiate spontaneously.

Samples were flushed with N_2, incubated at 37°C, and shaken at 50 rpm. To determine the crystal concentration in the reaction mixture at a specific time, an aliquot (25 μl) of the model bile mixture was sampled and diluted with a TBS/10 mmol STDC solution (dilution factor 15 to 50). After 20 min, absorbance at a single wavelength within the visible range (400–900 nm), was measured using a DU-50 Spectrophotometer (Beckman Instruments, Inc., Fullerton, CA), equipped with a semimicro

quartz cell (10 mm pathway, 2 mm width). Measurements were made at different intervals between 1 and 24 h, depending on the crystal growth rate of the sample.

Protein separation procedure

Human gallbladder bile specimens were obtained at surgery as previously described (2) or were collected in a similar manner from legally dead living donors for renal and liver transplant programs. The protocol for the collection procedure was approved by the Clinical Research Projects and Institutional Review Committee regarding human studies. No differences were observed between the specimens from these two groups of patients. The absence of gallstones in the transplant donors was established by surgical palpation. Bile specimens were tested promptly for contamination by assessing the presence of hemoglobin (Hemoccult test, Smith Kline Diagnostics, Inc., San Jose, CA).

Gallbladder aspirates were either immediately fractionated or, after removal of aliquots for quantitative analysis, were frozen at −80°C under nitrogen for storage. Cryoprecipitates were not observed in thawed bile samples stored up to 4 months.

Pooled bile specimens were initially separated on a molecular sieving chromatography column (Bio-Gel A-5m, 5 × 100 cm; buffer: 10 mmol Tris, 150 mmol NaCl, 4 mmol STDC, 3 mmol NaN_3, pH 7.45) (Bio-Rad Laboratories, Richmond, CA) to remove soluble mucin-glycoproteins eluting in the void volume, as well as higher molecular weight proteins (>200,000) present in the initial fraction (Fraction I). Thus, these were mostly separated from lower molecular weight proteins present in the subsequently eluted main fraction (Fraction II) that also contained most of the biliary lipids. Both fractions were dialyzed against 10 mmol ammonium bicarbonate for 40 h at 4°C, lyophilized, and stored at −80°C. Only fraction II, however, was pooled and applied to the various lectin affinity chromatography columns.

The procedure outlined here for removing mucin-glycoproteins and other higher molecular weight glycoproteins differs from that of others who have studied concanavalin A-binding biliary glycoproteins without first removing mucin. Therefore our results may not be entirely comparable to theirs (6–9).

The chromatographic protocol used with the four different lectins is compared as summarized in **Table 1**. In general, lectin columns (10 ml in 1.5 × 15 cm column) were equilibrated with 5 column volumes of starting buffer (10 mmol Tris, 0.5 mol NaCl, 4 mmol STDC, 1.5 mmol NaN_3). Aliquots of Fraction II (about 8 mg protein) were resolubilized in 2 ml of the appropriate starting buffer, loaded to a given lectin affinity column (Table 1) and recycled overnight at 4°C. Columns were then washed (>7 ×) with starting buffer until the eluant was free of lipids and biliary pigment. After this washing, the residual lipid concentration in the washed bound-protein fraction was only less than 0.02% of the original lipid concentration. The bound-fraction was then eluted with 5 column volumes of eluting buffer that contained the lectin-specific sugar (Table 1). The eluant was then diafiltered with 10 mmol ammonium bicarbonate and concentrated to 3 ml using a YM5 ultrafiltration filter (Amicon Division, Danvers, MA) and stored at −80°C.

SDS-polyacrylamide electrophoresis

SDS-polyacrylamide gels (4–16%) were developed in the buffer system described by Laemmli (24). Aliquots (5 μg) of protein samples were lyophilized and resolubilized with sample buffer (60 mmol Tris, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, pH 6.8). Gels were fixed in a 40% methanol–10% acidic acid solution overnight and

TABLE 1. Summary of lectin affinity chromatography

Lectin	Concanavalin A (CA)	Lentil Lectin (LL)	Wheat Germ (WG)	*Helix pomatia* (HP)
Specificity	glucose mannose	mannose glucose	N-AC-glucosamine	N-AC-galactosamine
Starting buffer (washes, n>7)	TBS + 1 mM $Ca^{2+}/Mg^{2+}/Mn^{2+}$	TBS + 1 mM $Ca^{2+}/Mg^{2+}/Mn^{2+}$	TBS	TBS
Eluting buffer (washes, n = 5)	TBS + 0.25 M α-D-methylmannopyranoside	TBS + 0.25 M α-D-methylmannopyranoside	TBS + 0.5 M N-AC-glucosamine	TBS + 0.5 M N-AC-galactosamine
% Bound (direct assay)	9.5%	5%	10%	4.5%
% Bound (assay after TCA)	7%	1.25%	2%	0.5%
E^{280nm}/E^{450nm}	<3	35	36	30

TBS, 20 mM Tris, 0.5 M NaCl, pH 7.4.

stained with silver nitrate according to the method of Merril (25).

Statistical methods

Calculation of standard deviations in all control crystal growth experiments was by application of the standard Pearson method (root mean-square deviation). It represents the 95% confidence level of the mean for each set (n = 5) of control measurements.

RESULTS

Recovery of bound protein after affinity chromatography with the four different lectin columns was about 10% for CA and WG and only 5% for LL and HP of the loaded total protein, when assayed directly by the fluorescamine protein assay (Table 1). However, when aliquots were treated with TCA-precipitation and two ether–ethanol washes before the fluorescamine assay, the calculated percentage of bound protein was greatly lower (by 80%) for WG, LL, and HP, but only slightly lower for CA. This lower value suggested that for the first three lectins, recovery with TCA precipitation and ether–ethanol wash was relatively ineffective. The bile pigment concentration also seemed to be more associated with the CA-bound fraction than with the others because this fraction contained the highest amount of bile pigment relative to the amount of protein, as shown by the quotient of the absorbance at 280 nm and 450 nm (Table 1).

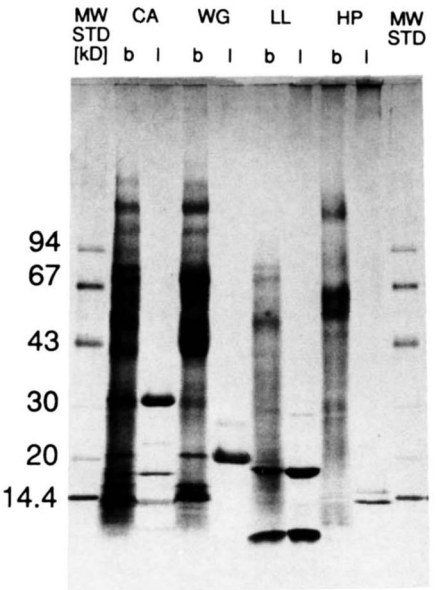

Fig. 1. SDS-gel electrophoresis of bile proteins separated from normal gallbladder bile using four different types of lectin columns: CA, Concanavalin A; WG, wheat germ; LL, lentil lectin; HP, *Helix pomatia*. Bound fraction, b; purified lectin, l; load, 10 μg.

The four lectin-bound fractions are compared by SDS-gel electrophoresis in **Fig. 1**. Many apparently overlapping bands can be identified for different bound fractions, despite differences in the principle of separation by using lectin columns of different binding specificities, that is CA, WG, LL, and HP. On the other hand, the pattern for fractions bound to columns with a similar specificity, that is, CA and LL, were unquestionably different, suggesting possible nonspecific binding of biliary proteins to the various lectin columns.

Also shown on the SDS gel are bands representing the purified lectins. A side-by-side comparison reveals bleeding of the coupled lectin in each of the columns. Such bleeding is highest for concanavalin-A and lentil lectins, intermediate with wheat germ, and lowest for *Helix pomatia* lectin. The bleeding led us to examine the effect of each of the four different purified lectins at 30 μg concentrations on the crystal growth assay. No evidence of either a promoting or inhibiting effect was observed with addition of any of the lectins.

The effects of the four lectin-bound protein fractions on cholesterol crystal growth in model bile were tested using the newly devised growth assay (11). For a more quantitative comparison, we derived from the growth curve three parameters that show the variation in crystal growth relative to a control: *1*) maximum growth rate (growth index I_g = maximal slope of experimental curve/maximum slope of control); *2*) final crystal concentration (crystal index I_C = final crystal concentration of experimental/final crystal concentration of control); and *3*) onset time of crystal detection (time index I_t = onset time of experimental/onset time of control).

The onset time is the intercept of an extrapolation of the maximal slope of the growth curve with the abscissa (baseline). The results are summarized in **Table 2**. The CA-bound protein fraction (**Fig. 2**) exerted an inhibiting effect at its lowest concentration (150 μg/ml) on onset time as well as on crystal concentration. With increasing effector concentration there was a continuous increase in growth rate for both seeded and unseeded experiments and in the final crystal concentration for spontaneous growth, whereas the onset time was continuously decreased. The shift in baseline with increasing protein concentration results from the presence of pigment, which is highest in the CA-bound fraction. At the highest protein concentration, the growth curve shows a rapid increase with an excess in crystal concentration, especially in the seeded experiment. This excess indicates a major difference between the kinetic effects of inhibiting and promoting factors. At high concentrations of the CA-bound fraction, the promoting effect (I_t = 0.36) is compatible with the pronucleating effect reported by Groen (6, 7, 9) for protein fractions separated from human bile. Evidence for the existence of an inhibiting factor in the CA-bound fraction, however, was not reported in these studies.

TABLE 2. Summary of crystal growth parameters for the various lectin bound fractions tested in a series of experiments depending on bound protein concentrations

Lectin-Bound Protein Concentration	Seeded		Not Seeded		
	I_g	I_C	I_g	I_C	I_t
μg/ml					
CA 600	1.08	0.70	1.14	1.21	0.36
CA 300	1.03	1.08	1.08	1.29	0.79
CA 150	0.95	0.96	0.97	0.87	1.5
LL 300	1.04	0.88	0.98	0.90	0.36
LL 150	0.96	0.86	0.83	0.89	0.57
LL 75	0.95	0.83	0.83	0.84	0.82
WG 600	0.88	0.96	0.92	1.02	0.05
WG 300	0.79	0.92	0.76	1.00	0.10
WG 150	0.74	0.91	0.60	0.82	0.18
HP 260	1.01	0.91	0.65	0.84	2.0
HP 130	0.74	0.88	0.35	0.50	1.6
HP 65	0.84	0.96	0.49	0.55	2.0

I_g, maximum crystal growth rate; I_C, final crystal concentration; I_t, onset time of crystal detection (refer to Results section). Abbreviations: CA, concanavalin A; LL, lentil lectin; WG, wheat germ; HP, *Helix pomatia*.

The effects seen with the LL-bound fraction were considerably different (**Fig. 3**) from those with the CA-bound fraction. The unseeded crystal growth curve showed a decrease in onset time with increasing bound-protein concentration. Also, both seeded and unseeded curves consistently showed a decrease in total crystal concentration to about 85–90% of control values as well as slight reduction in crystal growth rate at the lower bound-protein concentration.

The reduced growth rate for the WG-bound protein fraction (**Fig. 4**) was more striking with the lowest value (I_g = 0.6) observed at the lowest bound-protein concentration for unseeded growth. The seeded growth curves showed similar qualitative dependencies. The WG-bound fraction also showed the greatest effect on the crystal growth onset time, which was less than 20% of control time, within the observed range of bound-protein concentrations.

The most effective suppression of net crystal growth, however, was observed for the HP-bound fraction (**Fig. 5**). The qualitative pattern of the growth curve in dependence on total bound-protein concentrations was similar for both induced and spontaneous growth experiments. With increasing concentrations, the inhibiting effect was at first increased (to 35% of the control value for I_g). Further increases in total bound-protein concentrations, however, enhanced crystal growth rate, although the overall effect was still inhibiting. The onset times were prolonged and showed the greatest prolongation of values amongst the four different lectin-bound fractions studied in this report.

A consistent and general pattern was that, with increasing total bound-protein concentrations, the overall effect on crystal growth was enhancement of promoting activity with each of the lectin-bound fractions. On the other hand, with increasing total protein concentrations, the relative composition with respect to glycoprotein inhibiting or promoting activity in these fractions did not change. The results of the growth experiments, therefore, suggest that the inhibiting effect must be saturable, whereas the promoting effect does not show saturation in the range of total bound-protein concentrations examined in this study.

DISCUSSION

Although gel electrophoresis of bound normal gallbladder bile proteins shows some apparently identical bands for lectins with different sugar-binding specificities, this does not necessarily mean that glycoproteins producing

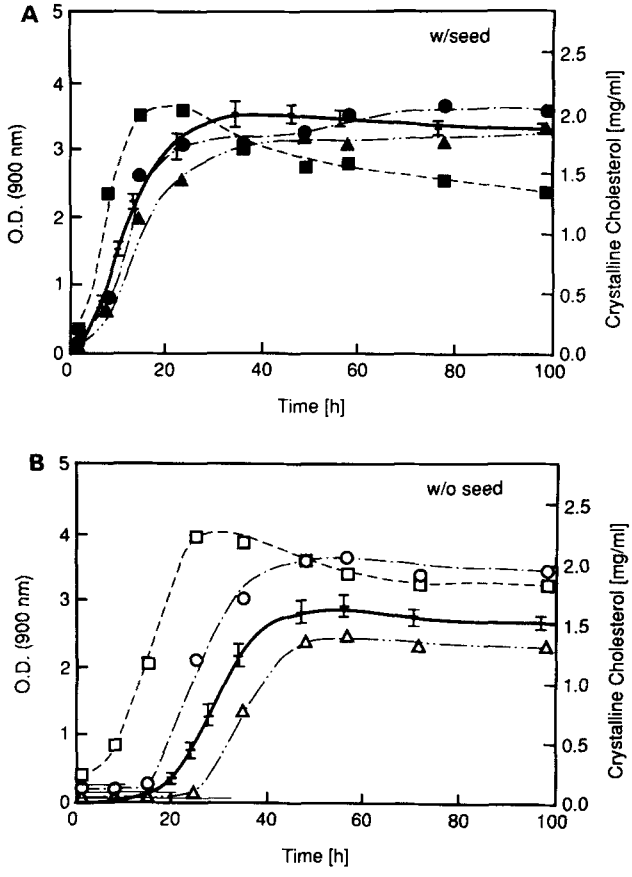

Fig. 2. Effect of concanavalin A lectin-bound fraction on cholesterol crystal growth in model bile (CSI = 1.5; total lipid = 100 g/l; BA/PL = 4.4). A: Solid symbols represent effects after seeding with 0.5 μg/ml. B: Open symbols represent effects without seeding. Concentrations of bound fraction: (■, □) 600 μg/ml; (●,○) 300 μg/ml; (▲, △) 150 μg/ml; (——) control. Mean control values ± SD (n = 5).

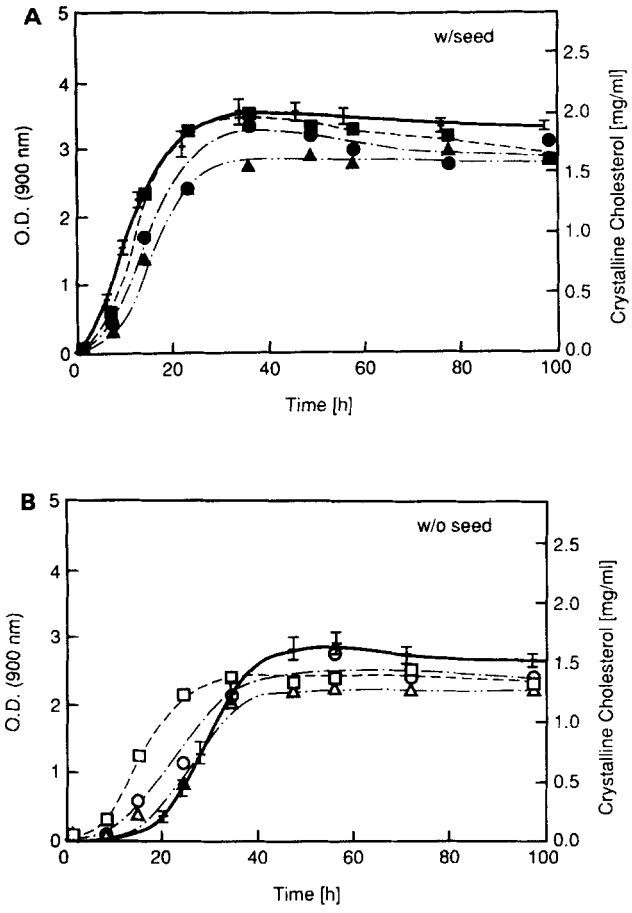

Fig. 3. Effect of lentil lectin-bound fraction on cholesterol crystal growth in model bile (CSI = 1.5; total lipid = 100 g/l; BA/PL = 4.4). A: Solid symbols represent effects after seeding with 0.5 μg/ml. B: Open symbols represent effects without seeding. Concentrations of bound fraction: (■, □) 300 μg/ml; (●, ○) 150 μg/ml; (▲, △) 75 μg/ml; (——) control. Mean control values ± SD (n = 5).

similar bands are identical. It is also possible that nonspecific binding has been variably superimposed on specific binding, either to the bound proteins or to the supporting chromatographic column gel, or both. Nonspecific binding is unlikely to contribute importantly, however, to the pattern of bound-fractions obtained with specific elution given the observed low baseline level of solute removal following the immediately prior column washing step. Another distinct possibility is that lectin binding for different glycoproteins with different terminal carbohydrate moieties may be variably influenced by identical carbohydrates, e.g., mannose, that are penultimately located and might therefore also be exposed to permit lectin binding.

Our findings clearly indicate that affinity columns with different specific lectins yield bound-protein fractions that show discernably different effects on cholesterol crystal growth. Information about the modulation of these various lectin-bound fractions in model bile was obtained by measuring effects with the growth assay using a series of total protein concentrations for each fraction. The results indicate that all lectin-bound fractions contain a mixture of promoting and inhibiting factors. The difference in net effect on crystal growth modulation indicates that various lectin columns yield bound-protein fractions having apparently different relative concentrations of these antagonistic factors. In general, the lowest protein concentrations of the lectin-bound fractions always showed inhibition. With increasing concentrations, however, the overall effect was to enhance the growth rate, the onset time for appearance of detectable crystals, or both. Only in the case of the HP-bound protein mixture was the inhibition increased with the initial increase in total protein concentration. But even in this instance, with a further increase in total protein concentration, crystal growth was then relatively enhanced, although the overall effect was still inhibiting. Because the relative composition of the protein mixtures under investigation

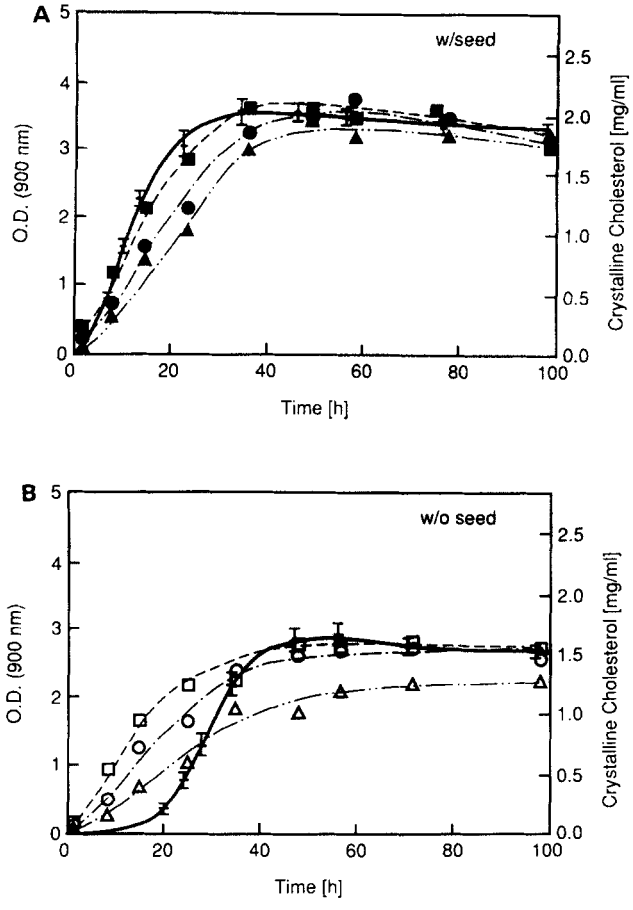

Fig. 4. Effect of wheat germ lectin-bound fraction on cholesterol crystal growth in model bile (CSI = 1.5; total lipid = 100 g/l; BA/PL = 4.4). A: Solid symbols represent effects after seeding with 0.5 μg/ml. B: Open symbols represent effects without seeding. Concentrations of bound fraction (■, □) 600 μg/ml; (●, ○) 300 μg/ml; (▲, △) 150 μg/ml; (——) control. Mean control values ± SD (n = 5).

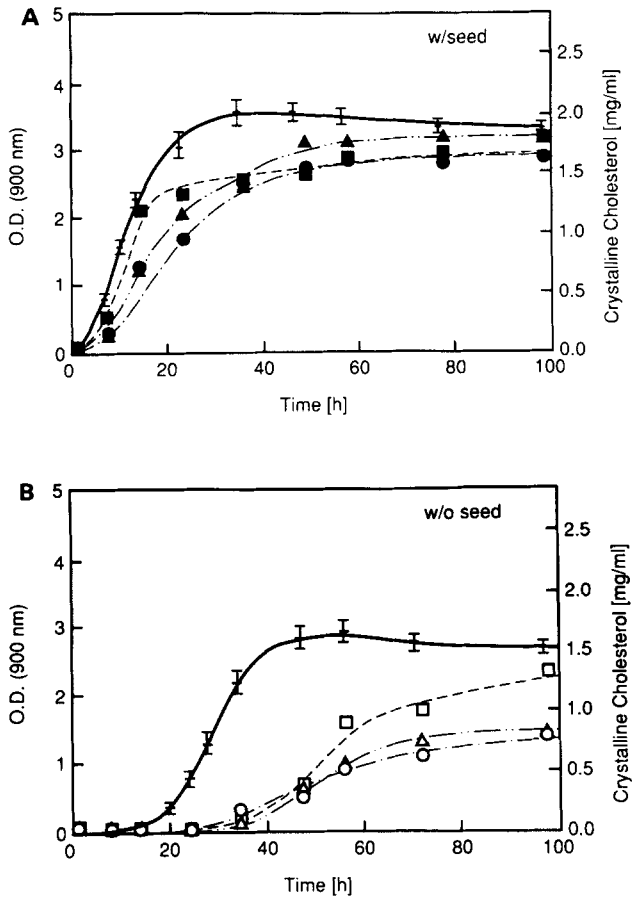

Fig. 5. Effect of *Helix pomatia* lectin-bound fraction on cholesterol crystal growth in model bile (CSI = 1.5; total lipid = 100 g/l; BA/PL = 4.4). A: Solid symbols represent effects after seeding with 0.5 μg/ml. B: Open symbols represent effects without seeding. Concentrations of bound fraction: (■, □) 260 μg/ml; (●, ○) 130 μg/ml; (▲, △) 65 μg/ml; (——) control. Mean control values ± SD (n = 5). 5).

was unchanged, this indicates that the inhibiting effect appears to be saturable, whereas the promoting effect within the range of total protein concentration studied does not show saturation.

A comparison of the patterns of seeded and unseeded crystal growth provides additional qualitative information about the probable mode of action of the putative protein factors. Promotor effects are primarily targeted toward the onset time of first detectable crystals (e.g., LL and WG) or toward both onset time and crystal growth rate (e.g., CA). In contrast, inhibiting factors primarily affect crystal growth rate and degree of final crystal concentration.

Our present report of nucleation-inhibiting activity in human bile differs from other reports in demonstrating that inhibition, like nucleation-promoting activity, can be found in glycoprotein fractions. The bound fractions obtained from the various lectins demonstrate a spectrum of comparative promoting activity-rich fractions to inhibiting activity-rich fractions.

Based on our findings, the best way to obtain a bound-protein fraction that is most enriched in promoting activity for further purification is to use concanavalin A lectin for affinity chromatography. This result provides a rationale and justification for the current use of this lectin by other groups for this purpose (6–9). Conversely, if the purpose of a study is to obtain a bound-protein fraction that is the most enriched in inhibiting activity as a step towards purification of the responsible glycoproteins, the use of *Helix pomatia* lectin has a definite advantage. The finding of coexisting opposing glycoprotein-relating activities in the same isolated fractions points to the idea that a balance of these coexistent effectors, especially in the presence of cholesterol supersaturation, may be of importance in defining health. Conversely, by favoring cholesterol crystal formation and growth, some form of imbalance of these glycoprotein kinetic factors having opposing effects is likely to be important in cholesterol gallstone pathogenesis.

This imbalance could arise in several different ways, such as overproduction of promoting factors or a deficiency of inhibiting activity somehow related to a quantitative loss of the inhibitor protein(s). Yet another possibility would be the formation of a defective inhibitor, as occurs in calcium oxalate nephrolithiasis (26). These hypotheses should be testable after further isolation and characterization of the specific kinetic factors.

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