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PURIFICATION AND CHARACTERIZATION OF BILE SALT HYDROLASE FROM *BACTEROIDES FRAGILIS* SUBSP. *FRAGILIS*

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Summary

A high-molecular-weight (250 000) bile salt hydrolase (cholylglycine hydrolase, EC 3.5.-.-) was isolated and purified 128-fold from the "spheroplast lysate" fraction prepared from *Bacteroides fragilis* subsp. *fragilis* ATCC 25285. The intact enzyme had a molecular weight of approx. 250 000 as determined by gel filtration chromatography. One major protein band, corresponding to a molecular weight of 32 500, was observed on 7% sodium dodecyl sulfate polyacrylamide gel electrophoresis of pooled fractions from DEAE-cellulose column chromatography (128-fold purified).

The pH optimum for the 64-fold purified enzyme isolated from Bio-Gel A 1.5 M chromatography was 4.2 and bile salt hydrolase activity measured in intact cell suspensions had a pH optimum of 4.5. Substrate specificity studies indicated that taurine and glycine conjugates of cholic acid, chenodeoxycholic acid and deoxycholic acid were readily hydrolyzed; however, lithocholic acid conjugates were not hydrolyzed. Substrate saturation kinetics were biphasic with an intermediate plateau (0.2—0.3 mM) and a complete loss of enzymatic activity was observed at high concentration for certain substrates. The presence or absence of 7-α-hydroxysteroid dehydrogenase was absolutely correlated with that of bile salt hydrolase activity in six to ten strains and subspecies of *B. fragilis*.

Introduction

In man, bile acids are formed by the liver as conjugates of glycine or taurine [1] and stored in the gall bladder. Upon stimulation, the gall bladder discharges the bile salts and other biliary constituents into the duodenal region of the small intestine. Bile salts are transported from the lumen of the small intestine via an active transport mechanism in the ileum [2] and returned to the liver by

the portal venous system. It has been estimated that approx. 500 mg bile salts per day escape the enterohepatic circucaltion and enter the large bowel [3]. The indigenous intestinal microflora of the large bowel in man contains bacteria which are capable of transforming bile salts into a variety of compounds [4,5] some of which have been implicated in the etiology of colon cancer in man [6].

One of the major biotransformation reactions carried out by human intestinal bacteria is the hydrolysis of bile salts to form free bile acids [7]. The hydrolysis of bile salts results in a decrease in the polarity of the side chain due to the hydrolysis of the peptide bond, an increase in the pK_a and decreased solubility of bile acid in acid solutions [8]. Bile salt deconjugation has been demonstrated with certain members of the genera *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Enterococcus* and *Veillonella* [9].

The enzyme responsible for the hydrolytic cleavage of bile salts, bile salt hydrolase (cholylglycine hydrolase, EC 3.5.-.-), was first isolated and partially purified from a strain of *Clostridium perfringens* by Nair et al. [10]. Properties of bile salt hydrolase also have been described in crude cell extracts prepared from strains of the genera *Bacteroides*, *Bifidobacterium*, *Clostridium* and *Enterococcus* [11]. Although various characteristics of bile salt hydrolase have been described using whole cells or crude cell extracts, there remains a paucity of published information regarding the molecular characteristics of the purified enzyme.

Materials and Methods

Strains of bacteria. Selected strains of *Bacteroides fragilis* were kindly donated by Drs. J.L. Johnson and W.E.C. Moore of the Anaerobe Laboratory, Virgina Polytechnic Institute (V.P.I.) and State University. Stock cultures of *B. fragilis* were maintained in chopped-meat medium under anaerobic conditions as described by Holdeman and Moore [12].

Growth media and cultural conditions. B. fragilis subsp. *fragilis* ATCC 25285 (NCTC 9343) utilized for enzyme isolation and characterization was cultured anaerobically as described previously [13]. Cells were harvested by centrifugation at 13 700 × *g* for 20 min at 25° C from a 6 l culture at the stationary growth phase.

Selected strains of *B. fragilis* investigated for the qualitative ability to deconjugate bile salts were cultured in a chemically defined growth medium containing 0.5 mM sodium glycocholate as described previously [14]. Growth of bacteria was determined by measuring the culture absorbance with a Bausch and Lomb Spectronic 20 at 660 nm.

Qualitative assay for bile salt hydrolase in whole cells. In qualitative assays for the presence of bile salt hydrolase from selected strains of *B. fragilis*, conjugated and free bile acids were extracted from 10 ml cell culture (72 h) by acidification to pH 1.0 with 6 M HCl followed by extraction of free and conjugated bile acids with 1 ml ethylacetate as described previously [14]. The cultures were then centrifuged at 10 000 × *g* for 20 min to sediment cell debris and promote phase separation. The ethylacetate phase was removed and portions were

spotted on thin-layer chromatograms (20 by 20 cm) of silica gel IB-F (Baker-Flex). Conjugated and free bile acids were separated in a solvent system consisting of chloroform/acetone/acetic acid/water (7 : 1 : 2 : 0.1, v/v) [7]. Conjugated and free bile acids were identified by comparing their mobilities to the mobility of known standards following development by spraying plates with phosphomolybdic acid (Baker Reagent) and heating at 150°C for 10 min.

Quantitative enzyme assay for bile salt hydrolase. Enzymatic cleavage of glycine- and taurine-conjugated bile acids was followed by measuring the initial rate of free glycine or taurine formed from the bile salt substrates. Glycine and taurine concentrations were determined spectrophotometrically at 570 nm by a modification of the ninhydrin reaction as described by Troll and Cannan [15]. Standard curves were prepared using free glycine or free taurine.

The standard reaction mixture (1.0 ml) contained in final concentrations, 1.0 mM bile acid conjugate, 50 mM sodium acetate buffer (pH 4.2) and an appropriate sample of enzyme. Reaction mixtures were incubated at 37°C for a time course of 30 min. The substrate conversion rate was linear over 15 min with deviations from linearity for longer time course depending on the substrate utilized. Enzymatic activity was terminated by the addition of 1.0 ml of 0.5 M trichloroacetic acid directly to the reaction mixture at various time intervals and the precipitated protein removed by centrifugation at 15 000 × *g* for 10 min. The supernatant fluid (0.2 ml) was assayed spectrophotometrically for glycine or taurine. A unit of enzyme activity was expressed as the amount of enzyme required for the formation of 1 μmol of glycine or taurine in 1 min per mg extract protein under standard assay conditions. The initial reaction velocity was directly proportional to protein concentration over a range of 25—400 μg/ml. Protein concentration was measured by the method of Lowry et al. [16].

Preparation of spheroplast fractions. Spheroplast fractions of *B. fragilis* were prepared as described previously [13]. The procedure for spheroplast fractionation is outlined in Fig. 1. This initial step for enzyme purification was employed because bile salt hydrolase activity in cell extracts obtained from sonicated whole cell suspensions was very unstable. Furthermore, this initial spheroplast fractionation procedure also removed approx. 10—20% of the total cell protein.

Enzyme chromatography. The enzyme preparation obtained from the "spheroplast lysate" fraction (Fig. 1) was chromatographed on Bio-Gel A 1.5-M. The column was equilibrated prior to applying sample using 20 mM potassium phosphate buffer (pH 7.0) which was 1 mM in sodium ethylenediamine-tetraacetate (EDTA) and 20 mM in 2-mercaptoethanol. The Bio-Gel A 1.5-M column was calibrated for molecular weight estimations using Dextran Blue 2000, beef liver catalase (250 000) aldolase (158 000) and ovalbumin (45 000) as molecular weight standards. Bile salt hydrolase was eluted from the column using equilibration buffer. Measurement of enzyme activity in eluted fractions allowed an approximation of the molecular weight of bile salt hydrolase. Fractions (6.0 ml) were eluted at a rate of 10 ml/h.

10 ml of the combined Bio-Gel A 1.5-M fractions (57—59) (Fig. 2) were applied to a column (1 by 15 cm) of DEAE-cellulose that had been equilibrated with 10 mM sodium phosphate buffer (pH 7.0). The protein on the column was

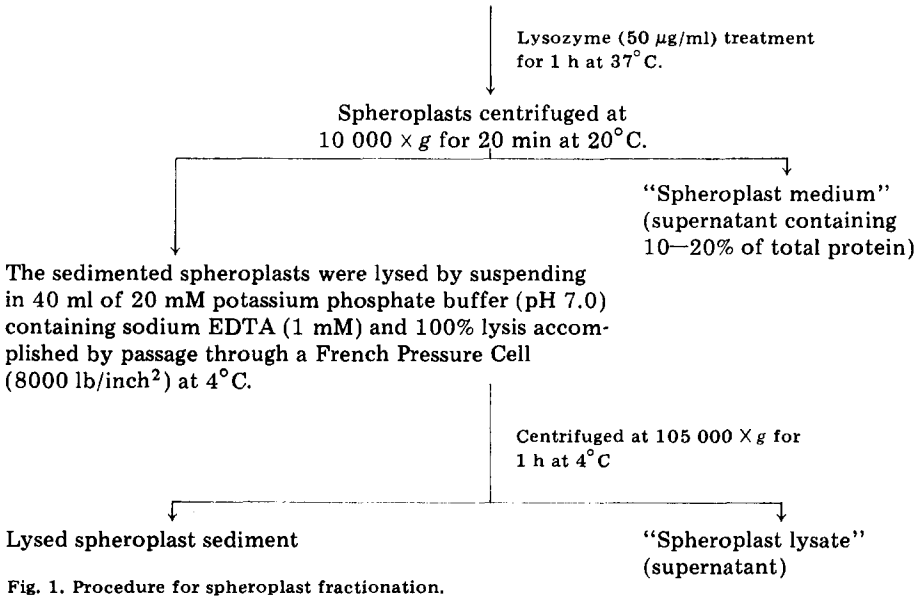

Fig. 1. Procedure for spheroplast fractionation.

then eluted with a linear (10—100 mM) gradient of sodium phosphate buffer (pH 7.0). Protein elution was monitored spectrophotometrically by measuring absorbance of eluant at 280 nm. Fractions were assayed for enzymatic activity immediately following elution and fractions containing peak activities were pooled and frozen (−20°C) for use in subsequent enzyme characterization experiments. The enzyme did not detectably lose activity when stored under these conditions for 3 months.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis. The most purified (128-fold) bile salt hydrolase preparation obtained (spec. act. 160 $\mu mol \cdot min^{-1} \cdot mg\ protein^{-1}$) from pooled fractions following Bio-Gel A 1.5-M and DEAE column chromatography was applied to 7% sodium dodecyl sulfate polyacrylamide gels overlayed with 4% acrylamide gel using the method of Davis [17]. Proteins were incubated for 3 h at 37°C in a 0.2—0.3 ml solution of 1% SDS containing 1% 2-mercaptoethanol before applying onto gels. Samples were electrophoresed at 25°C with a current of 5 mA per gel for 6 h. Proteins were stained overnight with 0.05% Coomassie Brilliant Blue according to the method of Weber et al. [18].

Materials. Glycine and taurine conjugates of cholic acid, deoxycholic acid and lithocholic acid were obtained as the sodium salts from Calbiochem (San Diego, Calif.). Lysozyme (9000 I.U./mg) used in spheroplast formation was obtained from Worthington Biochemical Corp. (Freehold, N.J.). Bio-Gel A 1.5-M was obtained from Biorad Laboratories (Richmond, Calif.). Acrylamide was obtained from Eastman Kodak Co. (Rochester, N.Y.) and was purified by recrystallization from chloroform wash. Sodium dodecyl sulfate (SDS) was obtained from Sigma Chemical Co. (St. Louis, Mo.). Diethylaminoethyl-cellulose (DE-52) was purchased from Whatman (W. & R. Balston Ltd, England).

Results

Enzyme purification. The results of the purification procedure are summarized in Table I. Pooled fractions (57—59) from Bio-Gel A 1.5-M (Fig. 2) showed a 64-fold purification over whole spheroplasts. The enzyme was further purified (2-fold) using DEAE-cellulose column chromatography (Table I). Bile salt hydrolase eluted from DEAE-cellulose columns showed a high degree of congruency between enzyme activity and protein profile (Stellwag, E.J. and Hylemon, P.B., unpublished). The bile salt hydrolase isolated and purified from the spheroplast lysate fraction had a specific activity of 160 μmol/min per mg of protein under standard reaction conditions. Because of the limited quantity of 128-fold purified enzyme, the 64-fold purified preparation was utilized in all subsequent experiments, except for SDS polyacrylamide gel electrophoresis.

Enzyme purity and molecular weight estimations. The molecular weight of the 64-fold purified hydrolase was estimated by gel filtration using Bio-Gel A 1.5-M column (3 by 90 cm) chromatography (Fig. 2). Elution volumes of three reference proteins, V_e/V_0 (V_0, void volume; V_e, elution volume) values were plotted against molecular weight on a log scale. From the standard curve a molecular weight of approx. 250 000 was obtained for the 64-fold purified bile salt hydrolase isolated from the "spheroplast lysate" fraction of *B. fragilis.*

Enzyme purity was evaluated by SDS polyacrylamide gel electrophoresis. The 128-fold purified enzyme preparation yielded only one major protein band which corresponded to a molecular weight of approx. 32 500.

Optimum pH for enzyme catalysis. The initial rates of enzyme catalysis were measured over a time course of 15 min under standard assay conditions and a

TABLE I

PURIFICATION OF BILE SALT HYDROLASE FROM *B. FRAGILIS* SUBSP. FRAGILIS ATCC 25285

Procedure	Vol. (ml)	Total activity (I.U.) *	Total protein (mg)	Specific activity (I.U./mg protein)	Purification (fold)	Recovery (%)
Whole spheroplasts **	50	1230	984	1.25	1.0	100
Spheroplast lysate (105 000 × *g* supernatant)	30	540	135	4.0	3.2	44
Biogel A 1.5-M chromatography	18	360	4.5	80.0	64.0	30
DEAE-cellulose ** chromatography	10	133	0.83	160.2	128.0	10

* I.U., international units.

** Whole spheroplasts were prepared from 16 g wet weight whole cells of *B. fragilis* subsp. *fragilis* ATCC 25285 as described previously [13].

*** DEAE, diethylaminoethyl.

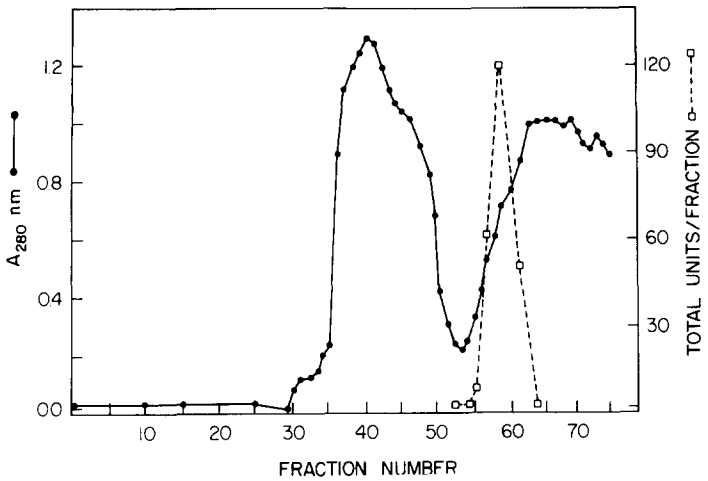

Fig. 2. Elution profile of bile salt hydrolase from Bio-Gel A 1.5-M column (3 by 90 cm). Fractions (6 ml) were collected and assayed for absorbance at 280 nm (•) and bile salt hydrolase activity (□).

pH range of 3.5—7.0. Hydrolysis of glycocholic acid resulted in pH alterations of less than 0.1 pH unit during the incubation. 64-fold purified bile salt hydrolase isolated from the spheroplast lysate fraction of *B. fragilis* had an optimal pH of 4.2 (Fig. 3B) compared to a value of 4.5 for whole cells of *B. fragilis* (Fig. 3A).

Substrate specificity. Several glycine and taurine conjugates of bile acids were examined for activity as substrates of bile salt hydrolase. The enzyme hydrolyzed both glycine and taurine conjugates and demonstrated greatest activity on dihydroxyl taurine conjugates (Table II) as compared to dihydroxyl glycine conjugates or trihydroxyl conjugates of glycine or taurine. The least activity was demonstrated using glycochenodeoxycholic acid and taurocholic acid

TABLE II

SUBSTRATE SPECIFICITY OF BILE SALT HYDROLASE FROM *B. FRAGILIS* SUBSP. *FRAGILIS* ATCC 25285

Each steroid was assayed at a concentration of 1.0 mM under standard assay conditions. The reaction was initiated by the addition of 25 μg of 64-fold purified enzyme and activity is reported as μmol substrate hydrolyzed/min per mg protein.

Steroid	Bile salt hydrolase activity
Glycocholic acid	80
Glycochenodeoxycholic acid	37
Glycodeoxycholic acid	57
Glycolithocholic acid *	<1
Taurocholic acid	53
Taurochenodeoxycholic acid	40
Taruodeoxycholic acid	48
Taurolithocholic acid *	<1

* These steroids were sparingly soluble under standard assay conditions.

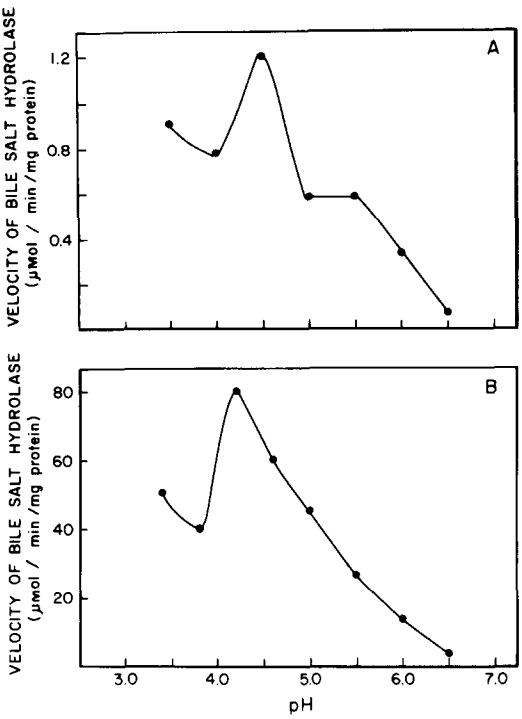

Fig. 3. Effect of pH on catalysis of bile salt hydrolase. The pH optimum of bile salt hydrolase using intact whole cell suspensions (A) and 64-fold purified bile salt hydrolase from the intracellular lysate fraction (B).

substrates under our assay conditions. Bile salt hydrolase from *B. fragilis* showed undetectable activity with glycine or taurine conjugates of lithocholic acid as substrates (Table II).

Kinetic properties. Substrate saturation kinetics were performed and the results are shown in Figs. 4, 5, 6 and 7. These data show that substrate saturation kinetics were biphasic. For example, an intermediate plateau was observed with taurocholic acid, glycochenodeoxycholic acid, glycodeoxycholic acid, taurochenodeoxycholic acid and taurodeoxycholic acid (Figs. 5, 6 and 7). A complete loss of enzymatic activity at high substrate concentrations was observed with glycochenodeoxycholic acid, taurochenodeoxycholic acid, and taurodeoxycholic acid (Figs. 6A, 7A and 7B). In contrast, substrate saturation kinetics performed using glycocholic acid, glycodeoxycholic acid and taurocholic acid showed saturable kinetic behavior without concomitant decrease in enzymatic activity at high substrate concentrations (Figs. 4, 5 and 6B). It is of interest to note that a decrease in enzymatic activity at a particular substrate concentration was paralleled by the appearance in the reaction vessel of a flocculent precipitate at the time of reaction initiation. Additional experiments were performed to determine if the loss of enzymatic activity at high substrate concentrations was reversible. For example, enzymatic reaction mixtures were incubated 15 min with bile salt substrate concentrations known to cause inactivation of bile salt hydrolase. Various dilutions were made in acetate buffer (pH 4.2) and the diluted reaction mixtures were further incubated for 30 min.

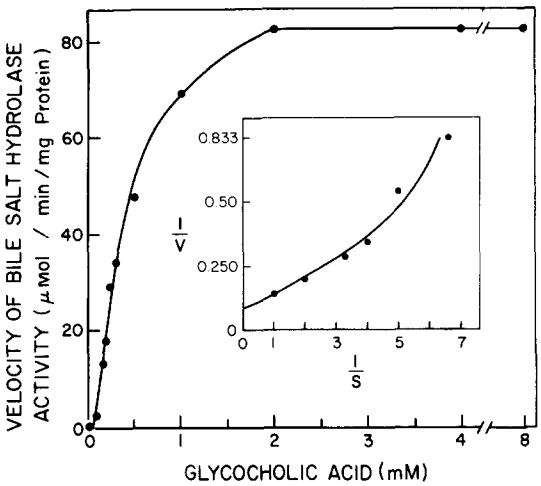

Fig. 4. Effect of glycocholic acid concentration on bile salt hydrolase activity. Bile salt hydrolase (25 μg of 64-fold purified enzyme) was added to initiate the reaction. Initial rates of hydrolysis were measured over a time course of 15 min.

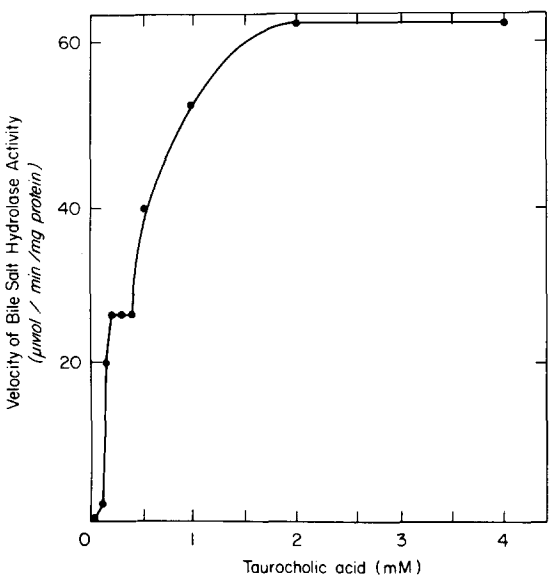

Fig. 5. Effect of taurocholic acid concentration on bile salt hydrolase activity. Bile salt hydrolase (25 μg of 64-fold purified enzyme— was added to initiate the reaction. Initial rates of hydrolsysis were measured over a time course of 15 min.

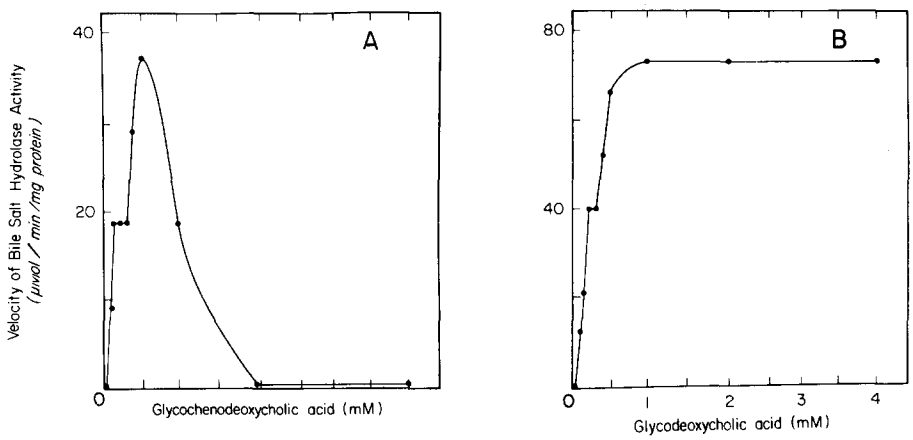

Fig. 6. Effect of glycochenodeoxycholic acid (A) and glycodeoxycholic acid (B) on bile salt hydrolase activity. Bile salt hydrolase (25 μg of the 64-fold purified enzyme) was added to initiate the reaction. Initial rates of hydrolysis were measured over a time course of 15 min.

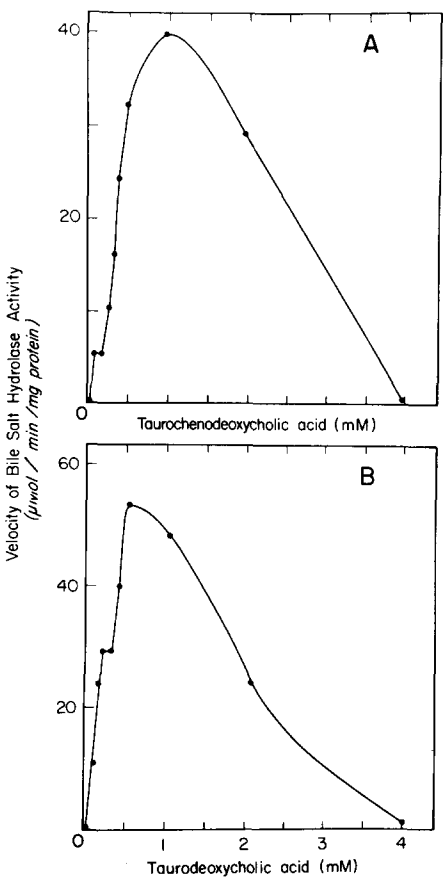

Fig. 7. Effect of taurochenodeoxycholic acid (A) and taurodeoxycholic acid (B) on bile salt hydrolase activity. Bile salt hydrolase (25 μg of 64-fold purified enzyme) was added to initiate the reaction. Initial rates of hydrolysis were measured over a time course of 15 min.

TABLE III

APPROXIMATED VALUES FOR V AND K_m

The V values were determined from the substrate saturation curves shown in Figs. 4, 5, 6 and 7 and the apparent K_m values were calculated by estimating 1/2 V.

Substrate	V (μM/min per mg protein)	K_m (mM)
Glycocholic acid	80	0.35
Glycodeoxycholic acid	72	0.20
Glycochenodeoxycholic acid	38	0.26
Taurocholic acid	66	0.45
Taurodeoxycholic acid	53	0.17
Taurochenodeoxycholic acid	40	0.29

Samples were taken every 5 min during this incubation and assayed for product accumulation. Enzyme activity was detectable following dilution of these reaction mixtures; however, the amount of activity recovered was variable.

The data shown in Figs. 4, 5, 6 and 7 demonstrate intermediate plateaus in plots of initial reaction rates versus substrate concentrations for all substrates except glycocholic acid (Fig. 4). Interestingly, the intermediate plateaus were all located in the region of 0.2—0.3 mM substrate concentration irrespective of the substrate being hydrolyzed. The apparent K_m and V values for glycine and taurine conjugates of cholic, deoxycholic and chenodeoxycholic acids are summarized in Table III.

Distribution of bile salt hydrolase and 7-α-hydroxysteroid dehydrogenase in

TABLE IV

DISTRIBUTION OF 7-α-HYDROXYSTEROID DEHYDROGENASE AND BILE SALT HYDROLASE IN DIFFERENT DEOXYRIBONUCLEIC ACID HOMOLOGY GROUPS OF *B. FRAGILIS*

Strain No.	Deoxyribonucleic acid homology group *	Bile salt hydrolase **	7-α-Hydroxysteroid dehydrogenase ***
ATCC 25285	Subsp. *fragilis* (1)	+	+
VPI 2393	Subsp. *fragilis* (2)	+	+
VPI 4243	Subsp. *distasonis* (1)	—	—
VPI 3452-A	Subsp. *distasonis* (2)	+	+
VPI 4245	Subsp. *vulgatus*	—	—
VPI 0061-1	Subsp. *thetaiotaomicron* (1)	—	—
VPI 5482	Subsp. *thetaiotaomicron* (2)	+	+
VPI 2302	Subsp. *thetaiotaomicron* (3)	+	+
VPI 0038-1	Subsp. *ovatus* (1)	+	+
VPI 3524	Subsp. *ovatus* (2)	+	+

* Reported by Johnson [19].

** Cells of *B. fragilis* were cultured in chemically defined medium containing 0.5 mM glycocholic acid and bile salt hydrolase assayed chromatographically as described in Materials and Methods.

*** 7-α-Hydroxysteroid dehydrogenase was determined by identification of 7-ketodeoxy[^{14}C]cholic acid generated by growing cultures of *B. fragilis* in chemically defined medium containing [^{14}C]-cholic acid as described previously [14].

strains of B. fragilis. The distribution of bile salt hydrolase as compared to the distribution of 7-α-hydroxysteroid dehydrogenase in different DNA-DNA homology groups [19] of *B. fragilis* is shown in Table IV. It should be noted that the presence or absence of bile salt hydrolase activity correlates absolutely with the presence or absence of 7-α-hydroxysteroid dehydrogenase in all 10 strains of *B. fragilis* assayed.

Discussion

The presence of a relative high molecular weight (250 000) bile salt hydrolase with a pH optimum of 4.2 exhibiting non-Michaelis-Menten saturation kinetics was unexpected in view of the previous reports. Aries and Hill [7] described an oxygen (O_2)-sensitive, 50 000—100 000-dalton bile salt hydrolase in the same strain of *B. fragilis* using a thin-layer chromatography enzyme assay. Such a low molecular weight value may have resulted as a consequence of the isolation procedure (i.e. sonic disruption using glass beads), which could easily have disrupted the native oligomeric structure of bile salt hydrolase. For example, a single protein band, corresponding to a molecular weight of approx. 32 500, was observed on SDS polyacrylamide gel electrophoresis of the 128-fold purified enzyme. These data indicate that the native intact enzyme is composed of eight identical subunits.

Previously reported pH optima for bile salt hydrolase were 5.6—5.8 in *C. perfringens* [10] and 5—6 for a strain of *B. fragilis* identical to that utilized in our studies [7]. Such results are in contrast to a pH optimum of 4.2 for bile salt hydrolase purified from the spheroplast lysate fraction of *B. fragilis.* The discrepancies encountered with regard to pH optima may be a result of differences in the enzyme form with respect to the clostridial enzyme, whereas the method of isolation, enzyme assay and purity of bile salt hydrolase may be a factor in the bile salt hydrolase isolated from *B. fragilis.*

The possibility that the enzyme isolated and characterized from *C. perfringens* by Nair et al. [10] is different from the enzyme isolated from *B. fragilis* is strengthened by the reported typical Michaelis-Menten saturation kinetic behavior of the clostridial enzyme. In contrast, we observed biphasic substrate saturation kinetics with the 64-fold purified bacteroides enzyme with an intermediate plateau (0.2—0.3 mM) and substrate inhibition with certain substrates at high concentrations. Kagan et al. [20] reported that the plateau kinetic phenomenon is highly dependent on enzymatic form. These investigators reported that in a theoretical analysis of allosteric enzyme systems for which equilibrium between oligomeric forms occurs, the rate of equilibration is slower than the enzymatic reaction. Moreover, the allosteric transition in the oligomer is of high velocity, showing that in such systems the kinetics of allosteric interactions may be expressed by V versus $[S_0]$ plots of a number of inflection points (one or two intermediate plateaus). Such an explanation may be one interpretation of the biphasic kinetic behavior of bile salt hydrolase isolated from *B. fragilis.* This explanation is also consistent with the fact the enzyme consists of several monomeric subunits (probably 8) that appear to exist in loose association as is evidenced by the apparent instability of the complex to sonic oscillation. Although this explanation may be valid for bile salt hydrolase from *B.*

fragilis, further experimentation will be necessary to determine whether the shape of the V versus $[S_0]$ plots is a phenomenon of slow equilibrium installation between oligomeric forms having different kinetic parameters. Based on the apparent K_m values, it appears that the enzyme has the strongest binding affinities for the glycine and taurine conjugates of deoxycholic acid and the weakest binding affinities for glycine and taurine conjugates of cholic acid. The relative binding affinity of the enzyme for various substrates does not seem to influence the shape of the saturation curves. With the exception of glycocholic acid, an intermediate plateau is present at 0.2—0.3 mM substrate concentration for all substrates regardless of the K_m values.

The distribution of bile salt hydrolase in different DNA homology groups of *B. fragilis* deserves note, particularly because its presence or absence correlates absolutely, in all strains tested, to the presence or absence of 7-α-hydroxysteroid dehydrogenase. We have previously reported [13] that bile salt hydrolase and 7-α-hydroxysteroid dehydrogenase activities increase markedly, with approximately the same time course, during the stationary growth phase. These data indicate that bile salt hydrolase and 7-α-hydroxysteroid dehydrogenase may be regulated in a similar fashion in *B. fragilis*. Further, the physiological significance of 7-α-hydroxysteroid dehydrogenase and bile salt hydrolase to *B. fragilis* needs to be further clarified with respect to the marked ability of this organism to survive and grow in the gastrointestinal tract of animals.

Acknowledgements

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References

1 Danielsson, H. (1963) Advances in Lipid Research (Pavletti, R. and Kritchevsky, E., eds.), Vol. I, pp. 335—385, Academic Press, New York
2 Dietschy, J.M. (1968) J. Lipid Res. 9, 297—309
3 Playoust, M.R. and Isselbacher, K.J. (1964) J. Clin. Invest. 43, 467—476
4 Berstrom, S. (1961) Fed. Proc. 20, 121—126
5 Norman, A. and Bergman, S. (1960) Acta Chem. Scand. 14, 1781—1789
6 Hill, M.J., Crowther, J.S., Drasar, B.S., Hawksworth, G., Aries, V.C. and Williams, R.E.O. (1971) Lancet 1, 95—100
7 Aries, V.C. and Hill. M.J. (1970) Biochim. Biophys. Acta 202, 526—534
8 Ekwall, P., Rosendahl, T. and Lofman, N. (1957) Acta Chem. Scand. 11, 590—598
9 Hill, M.J. and Drasar, B.S. (1968) Gut 9, 22—27
10 Nair, P.P., Gordon, M. and Reback, J. (1967) J. Biol. Chem. 242, 7—11
11 Aries, V.C., Crowther, J.S., Drasar, B.S. and Hill, M.J. (1969) Gut 10, 575—576
12 Holdeman, L.V. and Moore, W.E.C. (1972) Anaerobe Laboratory Manual, 2nd edn., pp. 27—36, V.P.I. Anaerobe Laboratory, Blacksburg, Va.
13 Hylemon, P.B. and Stellwag, E.J. (1976) Biochem. Biophys. Res. Commun. 69, 1088—1094
14 Hylemon, P.B. and Sherrod, J.A. (1975) J. Bacteriol. 122, 418—424
15 Troll, W. and Cannan, R.K. (1953) J. Biol. Chem. 200, 803—811
16 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
17 Davis, B.J. (1964) Ann. N.Y. Acad. Sci. 121, 404—427
18 Weber, K., Pringle, J.R. and Osborn, M. (1972) Methods in Enzymology (Hirs, C.H.W. and Timasheff, S.N., eds.), Vol. XXVI, pp. 3—27, Academic Press, New York
19 Johnson, J.L. (1973) Int. J. Syst. Bacteriol. 23, 308—315
20 Kagan, Z.S., Doroshko, A.I., Kovaleva, S.V. and Yakavleva, L.I. (1975) Biochim. Biophys. Acta 403, 208—220