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review

Pancreatic colipase: chemistry and physiology

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The first indication for the existence of a cofactor for pancreatic lipase was reported in 1910 by Rosenheim (1). This paper is of some historical interest and contains the following statement: "It seems therefore that pancreatic lipase is a complex enzyme which can be separated into two inactive fractions by means of filtration, a fact which has so far not been observed in any other enzyme". The term co-enzyme of pancreatic lipase was proposed for the heat-stable substance that was contained in the clear filtrate of a glycerol extract of pig pancreas. Whether this "co-enzyme" is identical to the cofactor now discussed is unclear. It was not until 1963 that the existence of a lipase cofactor was again documented. That year Baskys, Klein, and Lever (2) reported the separation by ion exchange chromatography of a heat-stable cofactor for lipase. These authors did not follow up their findings, but it is important for an understanding of the further developments that the lipase assay used by Baskys et al. (2) contained 8 mM deoxycholate. Sarda et al. (3) were not able to confirm the observations of Baskys et al. (2) and the reason for this, most probably, has to be sought in differences in the lipase assay system. The substrate used by Sarda et al. (3) may not have contained bile salts in a concentration high enough to cause inhibition of the lipase, or the cofactor may not have been completely separated from the enzyme.

In 1969 Morgan, Barrowman, and Borgström (4) confirmed the results of Baskys et al. (2). They separated lipase from a heat-stable cofactor by gel filtration and could assign an approximate molecular size to the cofactor corresponding to ≃12,000 daltons. Of importance was the finding that the cofactor-free lipase was inactive when tested against substrate containing supramicellar concentrations of bile salts, but it retained its activity against a detergent-free tributyrin emulsion. It was subsequently realized that pure pancreatic lipase is inhibited by bile salts in concentrations over their critical micellar concentration (CMC) and that the function of the cofactor is to restore lipase activity in the presence of bile salt (5, 6). In the meantime, the cofactor had been partially purified and named colipase by Maylie et al. (7).

The following is an account of the present state of knowledge in this field which includes the chemistry, physical chemistry, and physiological chemistry of pancreatic colipase and its interactions.

CHEMISTRY OF PANCREATIC COLIPASE

Purification and characterization

Colipase has been purified from the pancreas of pig (8–11), ox (12), horse (13), human (14), rat[1] and dog.[1] The starting material for porcine colipase was either the fresh gland or a fortified preparation used for insulin production. The purification was based on a combination of ion-exchange and gel-filtration procedures (9, 10). A purification procedure using extraction and purification in the presence of the nonionic detergent Triton X-100 has been described (11, 13). This procedure seems to have definite advantages that result in a homogeneous preparation and Triton X-100 obviously protects colipase from proteolytic degradation.

Depending on the starting material, the porcine colipase shows two types of heterogeneity, in size and in charge. The size heterogeneity is most probably a result of the action of proteases present during purification; such colipase species contain 77 (7), 84, and 96 (10) amino acid residues. The porcine colipases containing 96 and 84 amino acid residues were named colipase I and II, respectively, by the authors (10) and will be referred to as colipase I-M and colipase II-M (M = Marseille), respectively.

Abbreviations: CMC, critical micellar concentration; CTAB, cetyltrimethylammonium bromide; IRS, interfacial recognition site; TDC, taurodeoxycholate; NMR, nuclear magnetic resonance.
[1] Erlanson-Albertsson, C. Unpublished results.

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The charge heterogeneity of colipase was observed in the DEAE chromatography step of the purification of porcine colipase (8). The colipase species, containing about 107 amino acid residues, differ only in the number of amidated aspartic and glutamic acids (17). The molecules were also named colipase I and II, colipase II being most negative at pH 7, and will be referred to as colipase I-L and colipase II-L, respectively (L = Lund). These two colipases have also been named colipase A and B (11). Charge heterogeneity has also been found in colipases purified from man (14) and rat,[1] while the colipase from horse (13) and dog[1] is present in one major form.

Pancreatic lipase prepared according to the standard method of Verger et al. (15) contains variable but mostly saturating amounts of colipase. This is due to the fact that porcine lipase and colipase have rather similar isoelectric points and bind with a dissociation constant of $\simeq 10^{-7}$ M in buffer. To obtain lipase low in colipase, therefore, dilute solutions have to be used in the purification procedure (16).

Structure of colipase

The porcine colipase first isolated was reported to have 77 amino acid residues and to contain a nonprotein component of some 15%; this was later found to be an artifact (7). In further work by Maylie et al. (10), two colipases were isolated with 94 (colipase I-M) and 84 (colipase II-M) residues. The *N*-terminal residue in both was glycine followed by three isoleucine residues.

Porcine colipases I-L and II-L, which were isolated subsequently, had nearly identical amino acid compositions and contained 101–107 residues, with the *N*-terminal valine (8). The compositions are shown in **Table 1.** The proteins contain 10 half-cystines, no methionine, no tryptophan, and a high content of hydroxylated and acidic amino acid residues. The *N*-terminal sequence is Val-pro-asp-pro-arg-gly-ile-ile-ile- for both proteins. Colipases I-L and II-L differ only in one amidated glu or asp (15). The amino acid compositions of colipase isolated from other species are listed in **Table 2** and are strikingly similar except for methionine which is present in horse (13), human (14), and rat[1] colipase but is absent in porcine (9), bovine (12), and dog[1] colipase.

The primary structure of the colipase II-M with 84 residues has been determined (18, 19). As noted above, the *N*-terminal amino acid is glycine, followed by three isoleucine residues, thus forming a hydrophobic region. The succeeding sequence contains several free carboxylic acid residues. Another interesting region is the three tyrosine residues which are situated close to one another, -tyr(53)-gly-val-tyr-tyr-lys-, in the middle of the molecule.

TABLE 1. Amino acid composition of colipase I and II (17)

Amino Acid	Number of Residues			
	Colipase I		Colipase II	
	Experimental[a]	Nearest Integer	Experimental	Nearest Integer
Alanine	5.01	5	4.72	5
Arginine	6.12	6	5.70	6
Asparagine *plus* aspartic acid	13.15	13	13.74	14
Cysteine[b]	10.26	10	9.29	9
Glutamine *plus* glutamic acid	10.14	10	9.11	9
Glycine	9.64	10	9.03	9
Histidine	2.04	2	2.34	2
Isoleucine[c]	5.78	6	5.86	6
Leucine	10.32	10	9.99	10
Lysine	4.71	5	4.71	5
Methionine				
Phenylalanine	2.57	3	2.04	2
Proline	3.32	3	3.32	3
Serine[d]	10.78	11	9.70	10
Threonine[d]	5.51	6	5.08	5
Tryptophan[e]				
Tyrosine	3.20	3	2.79	3
Valine	4.19	4	4.25	4
Total number of residues		107		102
Total weight of residues		11570		11020

[a] Except where noted, the figures are taken from the 24-hr hydrolysis value.
[b] Half-cystine was determined as cysteic acid after performic acid oxidation.
[c] 72-hr hydrolysis value.
[d] Values obtained by extrapolation to zero time of hydrolysis.
[e] Determined spectrophotometrically.

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TABLE 2. Amino acid compositions of pancreatic colipase from different species

	Pig (9)	Ox (12)	Dog[a]	Horse (13)	Rat[a]	Human (14)
Asp	13	12	13	12	12	11
Glu	10	9	10	11	11	11
Pro	3	5	1–2	5	3	4
Gly	10	10	11	8	10	10
Ala	5	7	6	10	4	5
His	2	2	3	2	2	2
Lys	5	5	5	6	4	4
Arg	6	6	5	5	6	2
Thr	6	6	6	5	7	6
Ser	11	8	10	9	7	9
Val	4	5	3	6	3	4
Met	0	0	0	2	2	1
Ile	6	6	7	6	8	6
Leu	10	3	11	7	8	6
Tyr	3	3	2	3–4	3	3
Phe	3	2	3	2–3	1–2	1
Cys	10	10	10	10	10	8
Total number of residues	107	104	106–107	110–112	101–102	93–94

[a] Erlanson-Albertsson, C. Unpublished results.

The locations of three out of five disulfide bridges ($\overset{17}{\text{cys}}$-$\overset{85}{\text{cys}}$, $\overset{23}{\text{cys}}$-$\overset{38}{\text{cys}}$, and $\overset{48}{\text{cys}}$-$\overset{67}{\text{cys}}$) were also established (19). The remaining two are so closely situated in the sequence that their locations could not be deduced at this stage (the cystine-containing peptides to be combined were $\overset{27}{\text{cys}}$-$\overset{28}{\text{cys}}$ and $\overset{59}{\text{cys}}$-$\overset{60}{\text{pro}}$-$\overset{61}{\text{cys}}$). The locations of the cystines suggest a structure of colipase having a tightly knitted "core" with two tails forming the *N*-terminal and *C*-terminal ends.

The *N*-terminal region of the porcine colipases I-L and II-L was determined by Erlanson, Fernlund, and Borgström (9) and the primary structure of the preformed colipase (except for its *C*-terminal part, which has not been determined) is shown in **Fig. 1.**

The *N*-terminal sequences of horse (13) and human (14) colipase are compared to the *N*-terminal sequence of porcine colipase in **Table 3.** Horse and porcine colipase start with the *N*-terminal pentapeptide val-pro-asp-pro-arg while the *N*-terminal in human colipase is gly. There then follows in the three colipases a hydrophobic region with the sequence ile-ile-ile (pig and human) or val-ile-ile (horse). Noteworthy in the *N*-terminal region in all three species is the existence of free carboxylic groups at positions 12 and 15. Also in this part of the molecule is found the methionine residue at position 18 of horse and human colipase.

In order to gain information about the tertiary structure of colipase, proton nuclear magnetic resonance investigations have been undertaken (20–23). NMR resonances could be assigned to specific amino acid residues in the primary structure of colipase. Thus Tyr 56 and Tyr 57 were found to be residues positioned on the surface of colipase while Tyr 53 is buried within colipase; this is important for the stability of the structure of the molecule. The pK_a values of His 30 and His 83 were calculated to be 7.8 and 6.9, respectively, with His 30 on the surface of the molecule. His 83 was found to be in close proximity to Tyr 56 and/or Tyr 57, forming a "sandwich" structure with the rings parallel or close to parallel. By this special arrangement of the amino acids, a hydrophobic–aromatic region is created by the sequences $\overset{49}{\text{Ala}} \rightarrow \overset{57}{\text{Tyr}}$ and $\overset{81}{\text{Asn}} \rightarrow \overset{89}{\text{Gly}}$.

PHYSICO-CHEMICAL PROPERTIES AND INTERACTIONS

Water solution

Colipase has a high water solubility. At high concentrations (>2 mM) linewidth broadening of the resonances in the proton NMR spectrum are consistent with the occurrence of self aggregation.[2] Colipase is only weakly surface active, as revealed by its extent of penetration of a monolayer film. The increase in surface pressure was comparable to that given by serum albumin (24).

Bile salt solution

Gel filtration and ultracentrifugation (26–28) experiments indicated that the properties of colipase

[2] Wieloch, T. Unpublished results.

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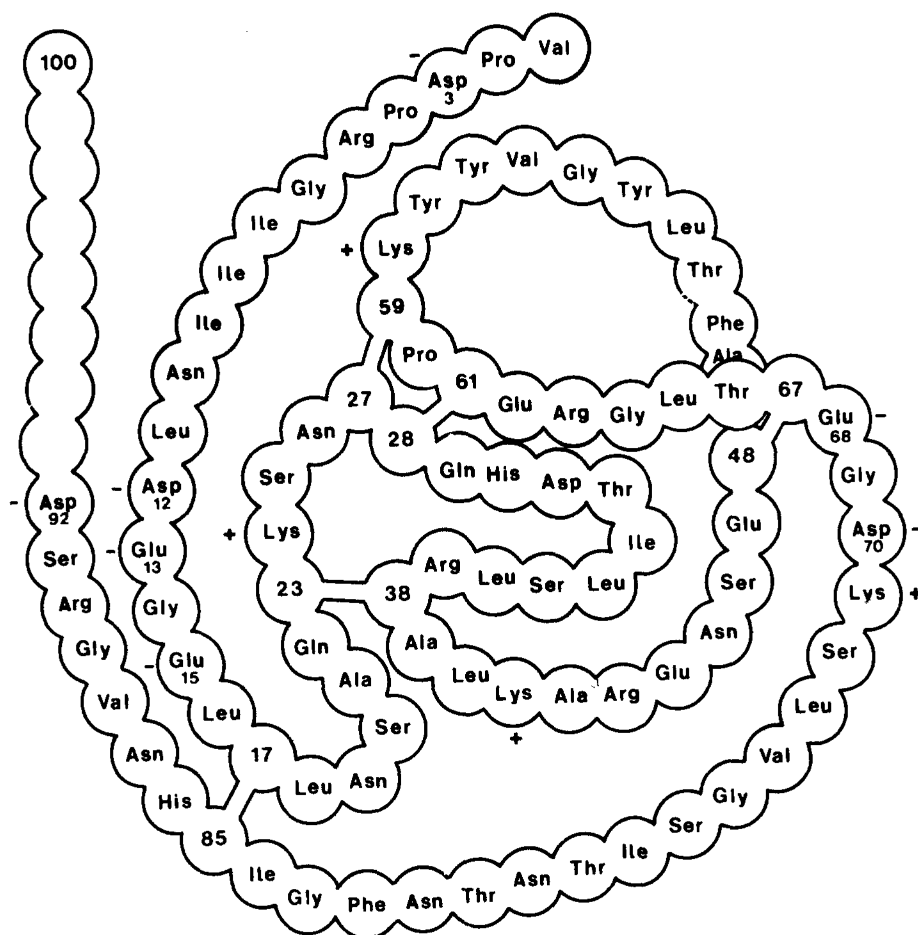

Fig. 1. Bidimensional structure of porcine colipase taken from ref. 31. The *C*-terminal amino acid has been numbered 100. It has been identified but the total number of residues in the intact molecules is not exactly determined. Within the limitations of the method there may be 101–107 residues (17).

change in bile salt solution at or above the CMC for the bile salt, suggesting an increase in molecular size. The apparent increase in molecular size was subsequently found to be due to the binding of bile salt to colipase. The results obtained by equilibrium dialysis and ultracentrifugation indicated that TDC binds to colipase in a way that varies with the degree of association of the bile salt to micelles and it was suggested that bile salts bind as micelles to colipase, i.e., one TDC micelle binds per molecule of colipase, and that colipase contains a single high-affinity site for micelle binding (26). The binding of dihydroxy bile salts to colipase shows the characteristics of cooperative binding—the aggregation number increases sharply in the region of the CMC of the bile salt and reaches numbers in the same range as those for the pure micelle. The binding of bile salts to colipase thus seems to be a manifestation of the same tendency of aggregation that results in micelle formation; a certain domain in colipase serves as a nucleus for micelle formation. The binding of TDC to colipase proceeds with an increase in entropy, indicative of hydrophobic interactions (28). This binding does not seem to induce any gross conformational change (28). Ultraviolet and circular dichroism spectroscopic studies, however, have revealed that the aromatic residues are affected by the binding (27). In proton NMR investigations it was shown that the resonances of Tyr 56, Tyr 57, and His 83 change chemical shift upon TDC binding. This seems to be a steric effect of the side chain of TDC with its bulky sulfate group, which is repelled as Tyr 56 and Tyr 57 are deprotonated. The TDC molecule(s) thus seems to bind to the aromatic–hydrophobic area on colipase as discussed earlier (22).

At high ratios of colipase to detergent, other kinds of complexes are formed which contain more than one colipase molecule (22, 26). In taurocholate solution, colipase does not change its gel filtration pattern due to the low association tendency of this trihydroxy bile salt (also manifested by its small primary micelles)

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TABLE 3. Comparison of the amino acid sequence of the *N*-terminal segments of human (14), horse (13), and porcine colipase (9)[a]

	6 10
Human	Gly-Ile -Ile-Ile-Asn-Leu-Glu-Asn-
	5 10
Horse	Val-Pro-Asp-Pro-Arg-Gly-Val-Ile-Ile-Asn-Leu-Glu-Ala-
	5 10
Pig	Val-Pro-Asp-Pro-Arg-Gly-Ile -Ile-Ile-Asn-Leu-Asp-Glu-
	15 20
Human	Gly-Glu-Leu- X -Met-Asn-Ser-Ala-Gln-Cys-Lys-
	15 20 25
Horse	Gly-Glu-Ile -Cys-Met-Asn-Ser-Ala-Glu-Cys-Lys-Ser-Glu
	15 20 25
Pig	Gly-Glu-Leu-Cys-Leu-Asn-Ser-Ala-Gln-Cys-Lys-Ser-Asn-
	30 35
Horse	Cys-Cys-Thr-Arg-Glu-Ser -Ser-Leu-Ser-Leu-Ala-Arg-Cys-
	30 35
Pig	Cys-Cys-Gln -His -Asp-Thr-Ile -Leu-Ser-Leu- ---- -Arg-Cys-
	40 50 55
Horse	Ala-Ala -Lys-Ala- X -Glu-Asn- X -Glu-Cys- X -Ala
	40 50 55
Pig	Ala-Leu-Lys-Ala-Arg-Glu-Asn-Ser-Glu-Cys-Ala-Phe

[a] The nonidentical residues are underlined.

(25). The binding of bile salts to colipase is not unique; it is also seen for the analogue sodium fusidate, for other anionic detergents such as sodium lauryl sulfate and dodecanoyl taurate, and for cationic detergents such as CTAB (29). Nonionic detergents such as Triton X-100 and a tridecylpolyethoxyethanol do not seem to bind to colipase, as judged by gel filtration experiments (29) (see **Table 4**).

The binding between lipase and colipase

The binding between lipase and colipase has been studied by several methods: calorimetry (28), gel filtration (16), two-phase aqueous partition, and affinity chromatography (30).

By calorimetry it was found (28) that porcine lipase and colipase in buffer solution bind to form a 1:1 complex with a dissociation constant of $5 \cdot 10^{-7}$ M. The binding proceeded with an increase in entropy and a negative heat capacity, suggesting that the binding was stabilized by hydrophobic interactions. In the presence of bile salt (4 mM TDC), lipase and colipase still form a 1:1 complex, although with a lower dissociation constant, $\simeq 10^{-5}$. Using an aqueous two-phase partition system, the interaction of lipase and colipase verified that they form a 1:1 complex with a dissociation constant of $4.8 \cdot 10^{-7}$ M in buffer (30).

The influence of different factors on the binding was also studied. It was found that ionic detergents like TDC and CTAB decreased the binding between lipase and colipase, while a nonionic detergent like Triton X-100 had no effect on the binding. The binding was also pH-dependent, being optimal at pH 5.8

TABLE 4. Gel filtration data (K_{av} values) for lipase and colipase on Sephadex G-100 columns preequilibrated by different detergent solutions in 0.15 M NaCl (29)[a]

Detergent	Conc.	Lipase	Colipase
	mM		
None		0.28–0.31	0.50–0.61
Taurodeoxycholate	6–12	0.24–0.25	0.41–0.45
Taurocholate	6		0.59
Fusidate	10		
Oleoyltaurate	1	0.28	
Dodecanoyltaurate	6		0.18
Dodecylsulfate (SDS)	4		0.17
Cetyltrimethylammonium bromide	6		0.19
N-Cholylcholamine	10	0.28	
N-Cholylglucosamine	10	0.27	
Nonipol DT15	0.4%	0.30	0.60
Triton X-100	1	0.30	0.60

[a] A decrease in K_{av} indicates association between protein and detergent.

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and decreasing to a minimum at pH 8.6. Neutral salts such as sodium chloride (0–1 M) did not influence the binding between lipase and colipase. From these studies it was concluded that the binding between lipase and colipase was both hydrophobic and ionic, hydrophobic in that it was decreased in the presence of detergents and was unaffected by varying the concentration of salt, and ionic because it was pH-dependent and unaffected by a nonionic detergent.

Chemical modification studies of colipase and lipase have indicated that one or two free carboxylic groups situated in the tails of colipase, at positions 12, 13, 15, or 68 and 70, are important for the lipase binding (31). At least one positively charged amino group on lipase, a lysine residue, seemed important for the colipase binding. Lysine-modified lipase could still hydrolyze an emulsion of tributyrin but could not be reactivated by colipase in the presence of bile salt (32). In addition, it did not bind to colipase, as determined by affinity chromatography using immobilized colipase on Sepharose (30). Taken together these experiments indicate that the interaction between lipase and colipase is hydrophobic and ionic; the hydrophobic binding gives the energy for complex formation and the ionic interaction is important for the proper orientation between the two proteins. This is analogous to other protein–protein interactions described (33).

Most of the experimental results with the colipase–lipase system have been obtained with the porcine enzyme and coenzyme. Colipase, however, has been found in the vertebrate series down to the shark, and a general cross reaction of colipase and lipase from different species has been indicated (30, 34). Lee (35), however, reported that canine colipase stimulated lipolysis with dog, pig, or ox lipase but not with human or rat lipase. It is of interest that the colipase of man (14) and rat[1] (and horse) contain methionine while the others (dog, pig, and ox) do not. Microbial lipases may be inhibited by bile salt, but they display no specific interactions with colipase of animal origin (35, 36).

Interactions of lipase and colipase with lipase substrate

Pancreatic lipase adsorbs at the interface between water and various dispersed hydrophobic substances such as hydrocarbons, triglycerides, and siliconized glass beads (37–39), and the lipase is rapidly inactivated at these interfaces. The interactions are mainly hydrophobic and can be visualized as an exposure of hydrophobic regions of the lipase molecule that leads to irreversible conformational changes. The inactivation process for lipase has been studied in quantitative terms at the interface of siliconized glass beads (40) and a substrate monolayer film (39). The inactivation is first order, complete, and apparently irreversible, suggesting that the inactivation reflects denaturation at the interface. The adsorption to a substrate monolayer was found to be relatively slow and an adsorption flux and an inactivation flux were recognized (39). The former is indicated by a lag phase before the lipolytic activity becomes maximal. The nature of the lag phase is not definitely known but can be visualized as a penetration to the substrate to which the lipase must bind before the enzyme–substrate binding at the catalytic site can occur. It has been suggested (41) that this orientation of lipase to the oil (substrate)/water phase is due to hydrophobic interaction.

The adsorption of lipase to hydrophobic interfaces is prevented by the presence of bile salt in the solution (42–44), and the inhibition of lipase activity by bile salts is thus caused by a physical separation of the enzyme and the substrate. The desorption of lipase from the substrate interface by bile salts has been ascribed to the formation of a lipase–bile salt complex (44, 45) with low affinity for the substrate interface. Desorption may also be a general detergent effect and a competition for the interface between the enzyme and the detergent, analogous to that demonstrated by Cochbein (46) for the sodium lauryl sulfate–hydrocarbon–serum albumin system. The desorption of lipase from the interface is seen for all detergents tested and is not necessarily related to the CMC of the detergent or to whether the detergent binds to lipase or not (29). That the binding of lipase to the substrate does not take place solely by hydrophobic interactions is shown by the demonstration that it binds in bile salt solution at bulk pH values below the pI of the enzyme (42). This binding most probably is a hydrogen bonding between the carbonyl oxygen of the substrate ester bonds and the enzyme; it does not occur with an emulsified hydrocarbon or with a trioctyl ether interface (42), nor does lipase bind to siliconized glass beads under these conditions (47). Lipase activity is inhibited by the simultaneous presence of hydrophobic proteins such as serum albumin and β-lactoglobulin and this inhibition occurs as a competition for the substrate interface (48). The behavior of lipase at the lipase–substrate interface has been discussed in detail because it is important for an understanding of the function of colipase.

Colipase interacts with the lipase–substrate interface but the binding is quantitatively different. The binding of colipase to the lipase substrate (triglyceride) is dependent on pH and salt concentration of the medium (42, 47, 49). At moderate salt concentrations (0.15 M NaCl) colipase binds to lipase triglyceride substrate also in supramicellar bile salt concentrations, but the binding is decreased with increasing bile salt con-

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centration; it is suggested that bile salt micelles competitively inhibit the reaction (50). The binding of colipase to the lipase–substrate interface most probably occurs by hydrogen bonding (49, 51). Chemical modification of colipase indicates that a free NH_2 group is important for the binding to the substrate in the presence of bile salt (31, 43). In a carbon-13 NMR study of methylated colipase, the individual pK_a values of methylated amino groups of colipase were determined (52). By this procedure the apparent pK_a value of the α-amino terminal was calculated to be 7.8. Because both the binding of colipase to the lipase substrate and the activity of lipase–colipase decrease at high pH with a "pK_a" value of $\simeq$8.0 (42, 53), it was concluded that the ionization state of the α-amino terminal is crucial for the colipase–triglyceride binding. There is, however, no absolute specificity for the *N*-terminal amino acid whether it is valine in the intact colipase molecule or glycine in colipase I-M and II-M.

The complete system

In the previous sections we have dealt with the structure of colipase and its reactions with lipase, bile salt, and the lipase substrate. In the physiological situation, i.e., in the contents of the small intestine, it can be assumed that the role of colipase is to anchor lipase to the interface of the substrate in the presence of bile salts. In this situation there is formed a quarternary system whose components are lipase, colipase, bile salt, and lipase substrate. Before going into a discussion of the complete system, studies with the lipase–colipase–substrate combination will be discussed.

Lipase activity is increased about 40–50% in the presence of colipase (53). This increase in activity is not explained by an increase in the number of lipase molecules at the substrate interface but rather by a protective effect of colipase on the irreversible surface inactivation of lipase (39). This effect most probably is nonspecific as it is seen also with serum albumin.

The monolayer technique used in the above experiments cannot be used with bile salt in the system, because the monolayer becomes unstable with bile salt in the subphase. An increase in surface pressure of the monolayer film, assumed to simulate a progressive saturation of the interface with bile salt, leads to inactivation of lipase, parallel to a decrease in the amount of lipase in the surface layer at a critical surface pressure of 22 dynes/cm. In the pressure interval of 25–29 dynes/cm, colipase can penetrate the surface film, anchor lipase, and reestablish its activity (36). Translated into the situation with an emulsified substrate, this would indicate that the surface pressure allowing penetration of lipase is below 23 dynes/cm.

An increase in bile salt in the aqueous phase also has been found to result in a lag phase before the lipase reaches a linear reaction velocity (29). The lag phase is increased with an increase in bile salt concentration and, in the CMC region of bile salt, the lipase activity is completely inhibited. The presence of bile salts at the emulsified substrate interface thus prevents lipase penetration, an effect that seems analogous to the effect of an increase in surface pressure in the monolayer experiments. In the emulsified substrate experiments, the lag phase in the presence of bile salt is diminished by an increase in colipase and can finally be completely abolished (29). The effect of colipase in the presence of bile salt may therefore be to overcome the surface pressure of a mixed detergent–substrate interface and to anchor lipase to the substrate in a way that allows the proper formation of an enzyme–substrate complex at the active site. Under these conditions, i.e., in the presence of bile salts, the binding of colipase to the substrate most probably is dependent on polar interactions, and hydrophobic interactions may be of little importance (51).

Kinetic and partition experiments indicate that the binding constant for lipase–colipase binding is increased 2–3 orders of magnitude in the presence of substrate (16, 42, 54). This could be due to the fact that the binding is actually stronger at the interface or that it only appears so due to the high local concentration of colipase and lipase at the substrate surface. The question of the formation of a ternary complex of lipase, colipase, and a bile salt micelle has attracted interest (55–57) and evidence for the formation of such a complex has been obtained from gel filtration experiments.

On the basis of experimental evidence, different tentative models illustrating the role of colipase for lipase function have been constructed (58–61). The main difference between these schemes is the functional importance of bile salts for the binding of lipase to colipase. Chapus et al. (58) postulated that adsorption to micelles (or monolayers) leads to a conformational change of colipase that creates or unmasks a recognition site for lipase. The finding that colipase binds to lipase in the absence of bile salts and that an increase of bile salt concentration into the micellar range will decrease the binding of colipase to lipase are evidence against this hypothesis (30).

Another point of different opinion concerns the mode of the binding of colipase to the substrate in bile salt solution. Brockerhoff (61) hypothesized that the colipase–lipase complex has a stronger hydrophobic affinity for the lipase substrate than lipase alone and is able to disrupt the electrostatic shield formed at the interface by bile salt. This hypothesis is based

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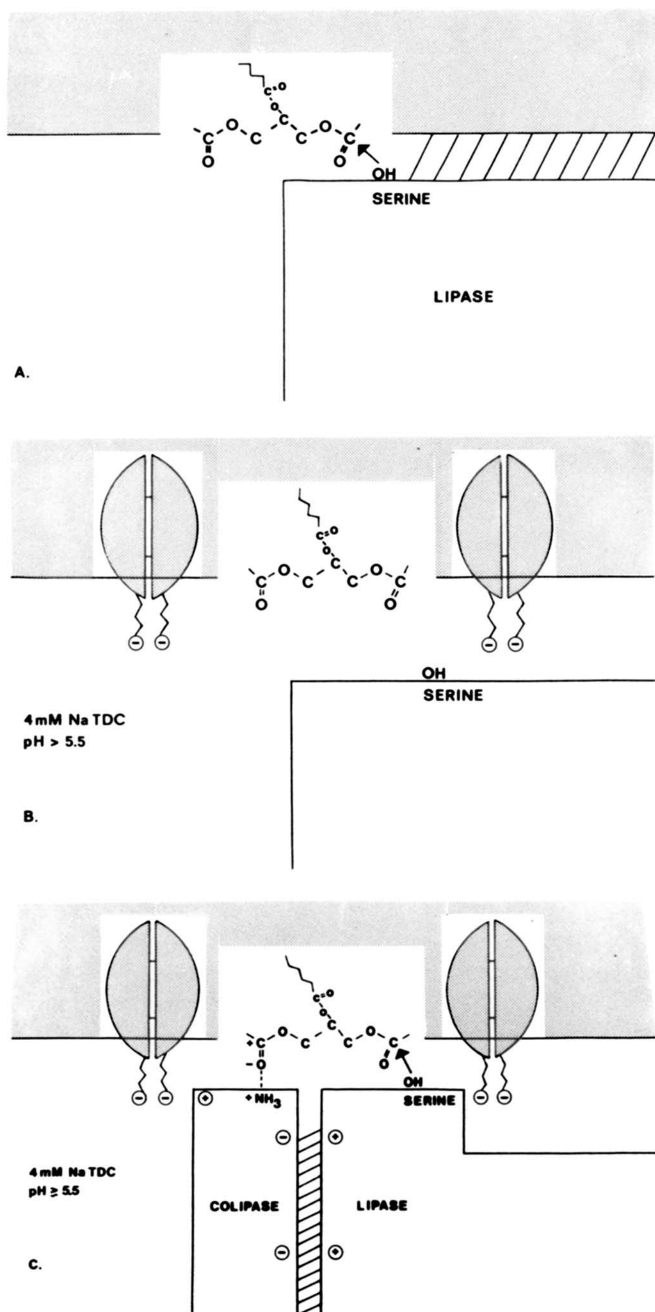

Fig. 2. Tentative models for the mode of action of pancreatic lipase and colipase under different conditions. It is assumed that the triglyceride oil droplets have a distribution of hydrophobic areas = hydrocarbon chains and hydrophilic areas = ester bond regions in circumscribed patches. *Panel A.* In the absence of bile salts lipase binds to the hydrophobic patches and the active site can bind to the ester bond and the enzyme is active. *Panel B.* In the presence of bile salt the hydrophobic patches are covered by bile salts and the lipase is prevented from binding to the now overall hydrophilic surface. No activity of lipase can be measured. *Panel C.* In the presence of bile salt and colipase, colipase attaches to the ester bond region by hydrogen bonding or electrostatic interactions and the lipase anchors to colipase by electrostatic and hydrophobic bonding. The lipase molecule is now in a position to the ester bond that allows the formation of an active site reaction. In panel *C*, lipase and colipase are depicted as binding to the same glyceride molecule. This is done for the sake of simplicity and is not proposed as a model for binding. In the same diagram, the possibility of a conformation change of lipase is indicated, resulting from the binding to colipase. Opposite charges in colipase and the side chain of bile salt may be of importance for the binding of colipase to the substrate interface. The average molecular area occupied by lipase at saturation at a hydrophobic surface has been calculated to be 4150 (58) and 6000 Å^2 (38). One lipase molecule therefore may cover a large number of triglyceride molecules (50 or more) at the substrate interface.

on the assumption that the colipase–substrate interactions are mainly hydrophobic. Evidence obtained later, however, favors the interpretation that, in bile salt solution, hydrophobic interactions at the interface are weak and that hydrogen bonding between substrate and lipase dominates (60).

As previously discussed, colipase is only weakly surface active because the surface pressure of a monomolecular film is only increased by 0.5 dyn/cm when colipase is added to the subphase (24). Colipase therefore cannot be considered to penetrate into the surface to any appreciable extent and must bind superficially at the substrate interface in the presence of bile salts. The different behavior of colipase and lipase at the substrate interface in bile salt solution may be explained by differences in the ionization state of specific groups involved in the binding. The finding that porcine lipase can bind to the substrate in bile salt solution under certain pH conditions indicates that electrostatic interactions can occur also in this case. This binding occurs only at pH values that are too low to be favorable for the activity at the active site of the enzyme and lower than the pH most often observed in the contents of the small intestine. Colipase, on the other hand, can interact electrostatically or by hydrogen bonding to the substrate at higher pH values and so can anchor lipase under conditions more favorable for the enzyme activity. Different functional groups of lipase and colipase that can favorably compete with bile salt for the interface may therefore be engaged in the binding to the substrate. Experiments using bile salts differing in net charge indicate that the concentration range of these molecules in which colipase reactivates lipase is larger for the normally occurring negatively charged bile salts than for positively charged or nonionic bile salt derivatives (29). The gross surface charge of the substrate seems to be important for the diffusion of colipase to the interface.

Certain analogies can be drawn between lipase and colipase substrate interactions and the interaction of pancreatic phospholipase A_2 with its substrate. This latter reaction has been analyzed in detail and an interfacial recognition site (IRS) on the enzyme molecule has been recognized (62). An important role of the *N*-terminal sequence of phospholipase A_2 in

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the specific interaction with the enzyme and the substrate was demonstrated, and evidence was obtained that a salt bridge between the protonated *N*-terminal amino group and a negatively charged carboxylate group stabilizes the IRS and allows the enzyme to penetrate the micellar surface. Evidence was obtained for interactions in which the *N*-terminal polypeptide chain of phospholipase A_2 penetrates to a certain extent into the lipid phase (63). Both lipase and colipase obviously interact with the emulsified lipase substrate by hydrophobic and electrostatic interactions, the latter being especially important in the presence of bile salts. As discussed previously, the binding of lipase to its substrate in bile salt solution suggests that a group with a pK of $\simeq$5.8 (42) is important. This binding may also involve hydrogen bonding to the substrate ester bond. An IRS has been postulated in analogy for lipase in which a serine residue is implicated in the interfacial binding and a carboxyl group for the interfacial activation (64).

Colipase binding to the substrate interface in the presence of bile salt is also pH dependent and involves a free amino group. This may be the *N*-terminal amino acid (pK estimated to 7.8), which is in close proximity to a hydrophobic structure with a sequence of $\overset{7}{\text{Ile}}$-Ile-Ile.

In analogy with phospholipase A_2, the *N*-terminal of colipase would thus bind to the substrate interface by a combination of hydrogen bonding (the *N*-terminal to the substrate C═O) and hydrophobic bonding (the ile sequence).

Tentative models for the relation of colipase and lipase to the substrate interface under different conditions are given in **Fig. 2.**

THE FUNCTION OF COLIPASE: PHYSIOLOGICAL CONSIDERATION

With the knowledge of the properties of colipase and its interactions in in vitro systems, it is now appropriate to discuss the question of what is the actual role of colipase in the physiological situation, i.e., in the contents of duodenum and upper jejunum? If we first consider the pancreatic juice in which lipase and colipase are secreted and restrict the conditions to those in the pig (as most in vitro work has been done with porcine lipase and colipase), the concentrations of these two proteins in pig pancreatic juice are on the order of 1×10^{-6} M (30). With a dissociation constant of 4.4×10^{-6} M calculated for colipase–lipase binding (in the absence of bile salt) at pH 8.6 (30), it can be estimated that about 70–80% of the lipase and colipase will be present in the free form. When the pancreatic juice empties into the duodenum it is diluted with the chyme coming from the stomach and also by bile. The intestinal contents represent a very complex mixture whose composition varies with the food taken and also with the composition of the bile, gallbladder bile in the beginning of the digestion period or liver bile in the later period. This dilution in combination with a competitive inhibition of binding by bile salt would indicate that the lipase–colipase association in solution in the intestinal contents is further decreased and that colipase and lipase are more than 90% free. In man the concentration of lipase and colipase in the intestinal contents is about ten times higher than in the pig (65) but it appears that the association constant between lipase and colipase is almost one order of magnitude lower, resulting in binding conditions very close to those discussed for the pig.[1]

With the bile comes the bile salt, and the question is whether bile salt associates with lipase and colipase to form micellar complexes as it does in in vitro experiments. The bile salts are found in the bile as mixed micelles with lecithin and, most probably (again extrapolating from in vitro experiments), the concentration of free bile salt is too low to associate to form pure bile salt micelles or to aggregate on the colipase or lipase molecules (66). The dietary triglyceride fat of the chyme is emulsified to a variable extent and is covered at the interface by protein and phospholipid. The bile salt has been shown to compete with protein for the substrate interface and most probably will momentarily clear the interface of such proteins as albumin and β-lactoglobulin of milk (48). Additionally the peptidase of stomach and pancreatic secretions may also be important in this regard, but no experimental evidence is available (67). The in vitro experiments indicate that the apparent association constant between lipase and colipase is increased 2–3 orders of magnitude in the presence of substrate and that, at the concentration of lipase and colipase in the luminal contents, at least 75–90% of lipase and colipase will exist as a complex at the interface. The introduction of substrate therefore will decrease the concentration of enzyme and coenzyme in the aqueous phase and any colipase–lipase complex in this phase will further dissociate.

As pointed out previously, the unresolved question is "whether the binding (between lipase and colipase) is actually stronger at the interface or the same as in solution but which appears stronger because of high localized concentrations at the substrate surface" (30). No actual measurements have been undertaken of the concentrations of lipase and colipase in the bulk and aqueous phases of intestinal content to verify if the in vitro results can be transferred to the in vivo

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situation. The in vitro experiments indicate that sequential reaction occurs under the conditions found in the intestinal contents: colipase first binds to the clean substrate interphase and then the binding of lipase to the colipase–substrate complex occurs. The possible functional advantages of the colipase–lipase system (two molecules) over a system with these properties united into one molecule has been discussed (30) but will of course only be a speculation on the wisdom of nature.

A role for the biliary phospholipids in the binding of lipase and colipase to the triglyceride substrate interface has been suggested (57). The results of another study (16) indicate that fatty acids, rather than phospholipids, in mixed bile salt micelles are important for such a high-affinity binding of lipase to colipase (17). The significance of such a binding under physiological conditions is not fully understood, but the dietary triglyceride fat entering the duodenum already contains free fatty acids as a result of the activity of the pharyngeal lipase (68). An important function of this enzyme therefore may be to initiate triglyceride hydrolysis early in the digestion sequence and prepare the dietary fat for the attachment of lipase–colipase in the duodenal contents.

The formation of a ternary association lipase–colipase–bile lipoprotein in bile salt solution has been ascribed a physiological role in the protection of lipase from proteolysis in the duodenal contents (69).

The very complex physico-chemical interactions that take place when the dietary fat empties into the duodenal contents and is mixed with pancreatic juice and bile are further complicated by the rapid activation of the pancreatic prophospholipase A_2. The elucidation of this complex sequence of interactions is a challenge for further research in this field.

We want to stress the fact that, due to the presence of bile salts, the milieu of small intestinal contents is largely incompatible with hydrophobic interactions. This can be said to be advantageous for lipid digestion. The interface of the dietary triglycerides is cleared from interfering amphiphilic substances (protein, phospholipids?). The lipase is also cleared from this and other unspecified interfaces and is thereby prevented from irreversible inactivation. Colipase provides a mechanism to specifically anchor lipase to the substrate interface in proximity to the ester bond largely directed by polar interactions.

Definite evidence for the importance of colipase in the digestion of triglyceride fat in animal or man has not been demonstrated. An isolated colipase deficiency has not been reported and in the cases of congenital lipase deficiency studied in man, colipase activity is in the normal range (70).

Addendum in proof

Recent results in our laboratory strongly suggest that colipase I-L with *N*-terminal valine is a *pro-colipase*, which on limited proteolysis with trypsin is completely converted to colipase I-M with 96 amino acid residues and *N*-terminal glycine and a pentapeptide val-pro-asp-pro-arg. The cleavage of the *N*-terminal pentapeptide by trypsin results in only insignificant increase in the activity in a tributyrin-based colipase assay. In contrast to colipase with *N*-terminal valine, the colipase with the *N*-terminal sequence gly-ile-ile-ile has the property to bind to a phospholipid-covered triglyceride emulsion, thereby making it available for pancreatic lipase.

In the earlier work the occurrence of a pro- and activated form of colipase has in general not been recognized. These two forms of colipase most probably have different physico-chemical properties, a fact that has to be considered in the interpretation of previous experiments and in the planning of new ones.

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