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Interactions between Pancreatic Lipase, Co-Lipase, and Taurodeoxycholate in the Absence of Triglyceride Substrate†

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ABSTRACT: We have studied the interactions between lipase, co-lipase, and taurodeoxycholate by calorimetry and circular dichroism determinations. Porcine pancreatic lipase binds to co-lipase to form a 1:1 complex with an association constant of 2×10^6 M^{-1}. The binding is exothermic and proceeds with an increase of entropy and the ΔC_p of the reaction is highly negative, -1.31 kJ K^{-1} (mol of lipase)$^{-1}$ at 25 °C. This is interpreted to be due to hydrophobic interactions between lipase and co-lipase. The binding of taurodeoxycholate to lipase and co-lipase is an endothermic process, which proceeds without any gross conformational changes as judged by circular dichroism measurements, although spectral changes are observed in the aromatic region. No binding between taurodeoxycholate and co-lipase is detected until concentrations near the critical micellar concentration of the former are reached. It is suggested that taurodeoxycholate and co-lipase form mixed micelles.

Pancreatic lipase (EC 3.1.1.3), which preferentially hydrolyzes water-insoluble esters of fatty acids in the diet, is inhibited by conjugated bile salts, which are normally found in the duodenal contents. The lipase activity is restored in the presence of a polypeptide cofactor, co-lipase. Co-lipase also enhances lipase activity in the absence of bile salts (Borgström and Erlanson, 1973). The system lipase-co-lipase-bile salt-substrate surface involves several important binding reactions, the nature of which are not well understood. Recently, equilibrium dialysis experiments were employed to elucidate the characteristics of the binding between some conjugated bile salts and lipase and co-lipase, respectively (Borgström and Donnér, 1975). As far as co-lipase-bile salt interactions are concerned, the results of these experiments have been confirmed by interaction studies in the ultracentrifuge (Charles et al., 1975).

In order to provide a basis for a better understanding of the effect of the different binding reactions mentioned above on the enzymatic activity, the lipase-co-lipase-bile salt system was studied by calorimetry and circular dichroism measurements.

Experimental Procedure

Porcine pancreatic lipase L_B was prepared by a modification (Donnér, 1976) of the method of Verger et al. (1969); the lipase obtained was free of co-lipase activity. Porcine pancreatic co-lipase was purified as described by Erlanson and Borgström (1972) and TDC[1] was synthesized by us (Norman, 1955). Sodium dodecyl sulfate of approximately 95% purity was recrystallized three times from 95% ethanol (Ray et al., 1966).

Protein concentration was determined spectrophotometrically using $E_{280nm}^{1\%} = 13.3$ for lipase and $E_{280nm}^{1\%} = 4.2$ for co-lipase. The molecular weights for lipase and co-lipase were taken to be 52 000 and 11 260, respectively. The solutions were stored at +4 °C. All experiments were carried out at pH 7.0.

In the calorimetric experiments, solutions of lipase, co-lipase, and TDC were made such that the final buffer composition was 2 mM Tris-HCl, 150 mM NaCl, 1 mM $CaCl_2$, and 0.02%

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[1]Abbreviations used: TDC, sodium taurodeoxycholate; cmc, critical micellar concentration; CD, circular dichroism; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; UV, ultraviolet; NMR, nuclear magnetic resonance.

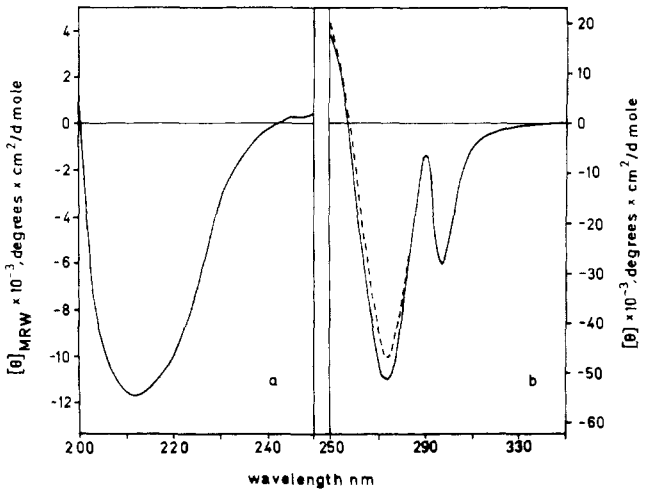

FIGURE 1: The circular dichroism spectrum of lipase (a) in the far-ultraviolet region at a concentration of 19.2 μmol/l. and (b) in the near-ultraviolet region at a concentration of 2.9 μmol/l. The broken line shows the spectrum obtained in the presence of 5 mM TDC.

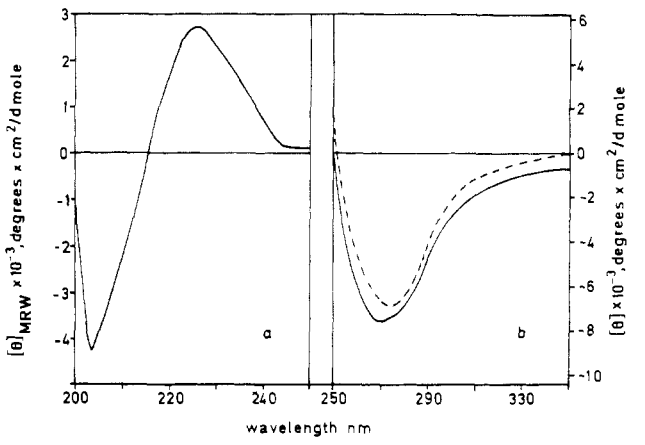

FIGURE 2: The circular dichroism spectrum of co-lipase (a) in the far-ultraviolet region at a concentration of 88.8 μmol/l. and (b) in the near-ultraviolet region at a concentration of 435 μmol/l. The broken line shows the spectrum obtained in the presence of 5 mM TDC.

NaN_3. When the reaction between lipase and co-lipase in the presence of TDC was studied, the concentration of TDC in the buffer was 3 mM. In a few control experiments, Tris-HCl was exchanged for Tris-maleate of the same concentration. Solutions of lipase and co-lipase were dialyzed twice against each other for 24 h at 25 °C to prevent any spurious pH effects upon mixing.

The calorimeter used in the experiments was the prototype to the LKB Batch Micro Calorimeter 10700-2, which is a twin heat conduction calorimeter designed by Wadsö (1968). It was thermostated at 25.0 °C except when ΔC_p was to be determined for the purpose of which it was thermostated at 21.0 or 30.7 °C. The differential voltage signal was amplified by a Keithley 150 B microvolt ammeter and the amplified signal was recorded on a Sargent SR recorder fitted with a ball and disc integrator.

The calorimetric vessels were charged by means of glass syringes and the sample quantities determined by differential weighing. In the lipase–co-lipase interaction experiments, 2 ml of a 0.20% lipase solution and 4 ml of co-lipase solutions of varying concentrations were taken into the two reaction vessel compartments. When these experiments were repeated in the presence of 3 mM TDC, a 0.18% lipase solution was used instead. A 0.21% lipase solution was used when the binding of TDC to lipase was investigated while 4 ml of TDC solutions of varying concentrations were taken. For the co-lipase–TDC experiments 4 ml of TDC solutions of different concentration was added to fill one of the calorimetric vessel compartments, the other compartment being filled with 1 ml of a 0.48% co-lipase solution. The two compartments in the reference cell were correspondently charged with buffer solution. Separate dilution experiments were made on the reactants.

The samples prepared for cicular dichroism measurements were dissolved in 2 mM Tris, 150 mM NaCl, and 1 mM $CaCl_2$. In the near ultraviolet (aromatic) region (250–350 nm), the concentration of lipase was 0.015%, that of co-lipase 0.49%, and in the far ultraviolet region (200–250 nm) 0.1% solutions were used throughout.

CD was measured in a JASCO J-20 or J-40 spectropolarimeter standardized with *d*-10-camphorsulfonic acid. The samples were measured at 25 °C in 0.1 to 75 mm cells, chosen to optimize the measuring conditions. The molar ellipticity, $[\theta]$ or $[\theta]_{MRW}$ is expressed as deg cm^2 dmol^{-1} calculated from the equation

$$[\theta] = \frac{\theta M}{lC \times 10} \quad (1)$$

where θ is the observed ellipticity, M the mean residue weight (used in spectra below 250 nm) or the molecular weight (used beyond 250 nm), l the path length in cm, and C the protein concentration in g per ml. The mean residue weight was calculated from the amino acid compositions and was found to be 112 for lipase (Donnér, 1976) and 108 for co-lipase (Erlanson et al., 1973).

Results and Discussion

Figure 1 shows the CD spectra of lipase in the far-UV and aromatic UV regions. The former clearly indicates that lipase does not contain any significant amounts of α-helix structures, as the characteristic negative maxima at 208 and 222 nm are missing. On the other hand, it can be deduced that the content of β structure is about 75%, when the method of Greenfield and Fasman (1969) is used. The effect on the CD spectrum of addition of TDC (0–7 mM) is negligible. The aromatic CD spectrum shows two characteristic negative maxima at about 274 and 298 nm, which originate from tyrosine and tryptophan transitions, respectively (Horwitz et al., 1970; Sjöholm, 1975; Strickland et al., 1969). In this case, addition of TDC (5 mM) perturbs the tyrosyl side chains, while the tryptophanyl groups apparently are unaffected. This finding indicates that the tryptophanyl groups of lipase are not present at the protein surface (the solvatized parts of the protein).

The far-UV spectrum of co-lipase (Figure 2) exhibits low ellipticities indicative of the absence of ordered structures and no changes could be detected when TDC was added. Co-lipase does not contain any tryptophan residues (Charles et al., 1974) and the aromatic CD spectrum is then dominated by the phenylalanine transitions at 268 nm (Horwitz et al., 1969) and the tyrosine transitions at 275 nm (Horwitz et al., 1970; Sjöholm, 1975). The spectrum is significantly altered, when TDC (5 mM) is added. By ultraviolet difference spectroscopy, Charles et al. (1975) showed that TDC was perturbing the tyrosyl side chains. The CD spectrum indicates that phenylalanine residues are also involved in the interaction.

The binding of lipase and co-lipase, which did not result in any detectable circular dichroism spectral changes either in

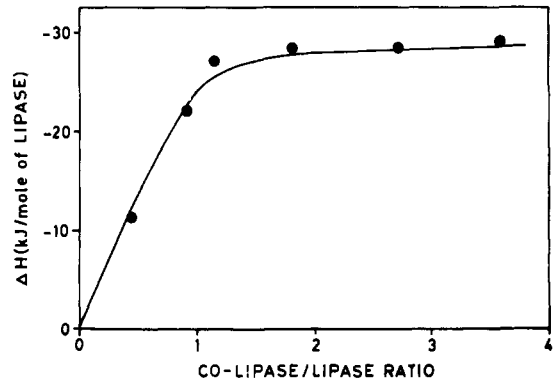

FIGURE 3: Experimental heats of the binding of co-lipase to lipase. The total concentration of lipase was kept constant at 13.0 μmol/l. after mixing the reactants in the calorimeter.

the far-UV or in the aromatic UV region, was found to be exothermic (Figure 3). From the results of the five experiments represented by the three points at the highest co-lipase to lipase ratio in Figure 3, a ΔH of -28.7 ± 0.6 kJ/mol of lipase is derived (uncertainty limits given as twice the standard deviation of the mean). The nearly straight arms of the binding isotherm extrapolate to a point near a lipase-co-lipase ratio of 1:1.

The reaction enthalpy of the lipase-co-lipase reaction was found to be the same with Tris-HCl and Tris-maleate. Considering the different heats of proton ionization, the interaction obviously does not involve any proton transfer to the solvent.

Figure 3 also shows a calculated binding isotherm for 1:1 binding between lipase and co-lipase using 2×10^6 M^{-1} as the value of the association constant, K. The experimental points were found between the calculated binding isotherms with $K = 10^6$ M^{-1} and $K = 4 \times 10^6$ M^{-1}. Being the best fit, the K for the binding between lipase and co-lipase was taken to be 2×10^6 M^{-1} and for that case $\Delta H_f = -29.0 \pm 0.6$ kJ/mol of lipase (ΔH_f represents the enthalpy of reaction for the hypothetical situation where all the lipase is bound to co-lipase and it would be synonymous to $\Delta H°$ under standard state conditions). These values correspond to a $\Delta G°$ of -36.0 ± 1.7 kJ/mol of lipase and a $\Delta S°$ of 23.5 ± 7.7 J K^{-1} (mol of lipase)$^{-1}$ when calculated from

$$\Delta G = -RT \ln K \quad (2)$$

and

$$\Delta G = \Delta H - T \Delta S \quad (3)$$

respectively. The findings that the binding of lipase to co-lipase is accompanied by an increase in entropy suggests that the interaction is hydrophobic or that conformational changes take place. However, as was mentioned above, no conformational changes could be detected by the circular dichroism measurements. In order to get further support for the suggestion that hydrophobic interactions may be important in the binding the reaction enthalpy was studied at different temperatures. The results of these experiments are shown in Figure 4 where they have been fitted to the equation

$$\Delta H = a + bt + ct^2 \quad (4)$$

a, b, and c are constants with the numerical values of -38.5, 2.09, and -0.068, respectively, and t is the temperature expressed in °C. ΔC_p is obtained as the derivative of eq 4 and at 25.0 °C $\Delta C_p = -1.31$ kJ K^{-1} (mol of lipase)$^{-1}$. This is a highly negative value (Suurkusk and Wadsö, 1972), which

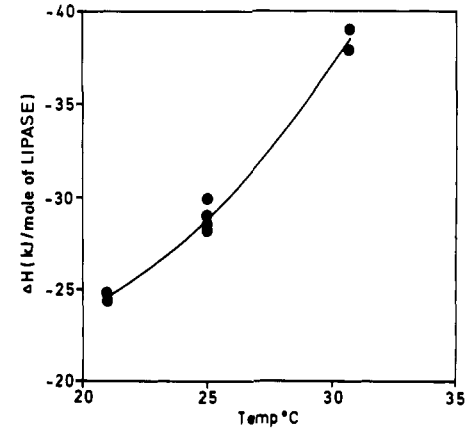

FIGURE 4: ΔH for the binding of co-lipase to lipase as a function of temperature. The results have been fitted to the equation $\Delta H = a + bt + ct^2$, where a, b, and c are constants and t the temperature in °C. $\Delta C_p = b + 2ct$ and at 25.0 °C ΔC_p has the value of -1.31 kJ K^{-1} (mol of lipase)$^{-1}$.

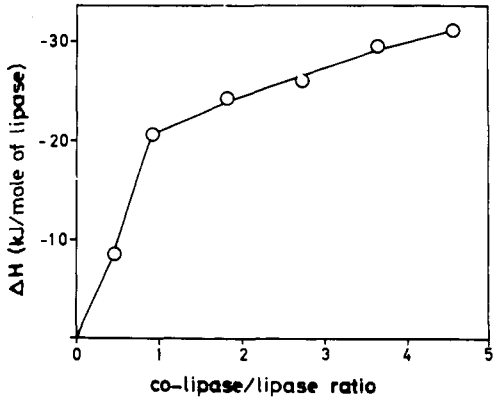

FIGURE 5: Experimental heats of the binding of co-lipase to lipase in the presence of 3 mM TDC (see under Experimental Procedure for details). The total concentration of lipase was kept constant at 11.8 μmol/l. after mixing the reactants in the calorimeter.

supports the hypothesis that hydrophobic interactions are important, although there will always be a risk, of course, in comparing the ΔC_p for the interaction between macromolecules with the ΔC_p for the transfer of hydrocarbon chains from water to liquid hydrocarbon phase.

When reaction enthalpies for the binding of lipase and co-lipase in the presence 3 mM TDC (Figure 5) were determined, the K was found to be decreased to 10^5 M^{-1} (K was estimated similarly as described above). This finding, which is interpreted in such a way that there is a competition between co-lipase and TDC for the binding to lipase, agrees with the inhibition studies presented by Borgström and Erlanson (1973). These results are compatible with a model where lipase and co-lipase interact with hydrophobic regions located at the protein surfaces which are easily accessible to TDC, which binds hydrophobically (see below) to one or both of the hydrophobic regions. In an experiment like the one which is presented in Figure 5, TDC is bound to lipase and co-lipase before mixing the latter two in the calorimeter reaction vessel. If every molecule of TDC which is bound per molecule of lipase and co-lipase is replaced in the binding reaction between lipase and co-lipase, the binding isotherm in Figure 5 would be expected to level off at a reaction enthalpy value that would be equal to the difference of the reaction enthalpy in Figure 3 and the reaction enthalpies.

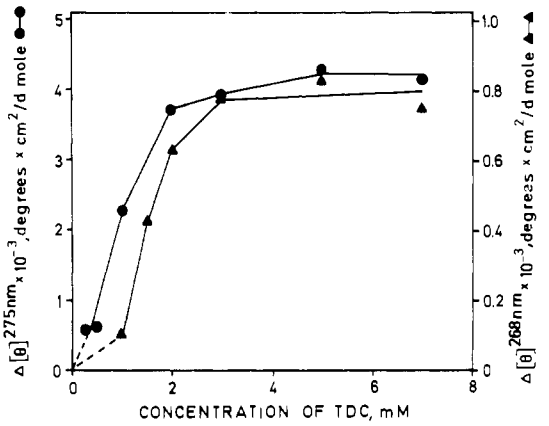

FIGURE 6: The effect of taurodeoxycholate on the spectrum of lipase (●—●) at 275 nm at a concentration of 2.9 μmol/l. and on the spectrum of co-lipase (▲—▲) at 268 nm at a concentration of 435 μmol/l.

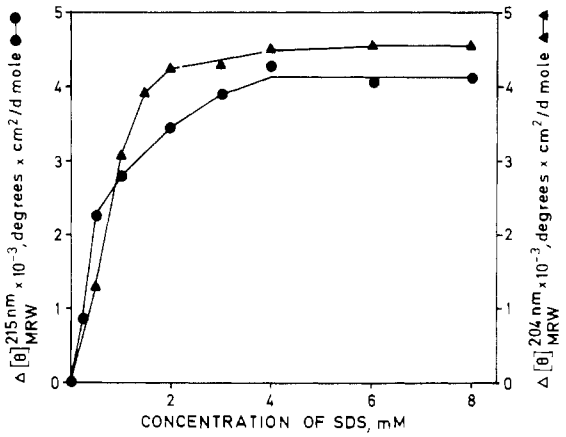

FIGURE 7: The effect of sodium dodecyl sulfate on the spectrum of lipase (●—●) at 215 nm and on the spectrum of co-lipase (▲—▲) at 204 nm. The protein concentration was 19.2 and 88.8 μmol/l., respectively.

in Figure 8 (see below). However, this is not the case and, hence, not all of the TDC is replaced in the binding between lipase and co-lipase.

Figure 6 shows the effect of TDC on the aromatic region of the circular dichroism spectrum of lipase and co-lipase. Earlier co-lipase has been shown by equilibrium dialysis to bind 12–15 mol of TDC above the cmc of TDC (Borgström and Donnér, 1975). In the same study it was reported that lipase only bound 2–4 mol of TDC per mol. A reappraisal of the latter data indicate that the binding of TDC to lipase is of the same magnitude as that to co-lipase on a mole per mole basis (Borgström and Donnér, submitted for publication). The extrapolation of the curve for co-lipase also shows that CD changes will not take place at concentrations below cmc. The lipase curve is less conclusive in this respect. As was mentioned earlier no changes in the far-UV spectra were detected with increasing TDC concentrations. Changes in the far-UV spectrum are seen when conformational changes affecting the polypeptide backbone structure occur. Thus the inhibitory effect of TDC on lipase activity is not due to a typical detergent induced unfolding of the protein structure. This also means that the random structure of co-lipase is retained after the interaction with TDC.

For comparison, the effect of sodium dodecyl sulfate on the circular dichroism spectra of lipase and co-lipase was also investigated (Figure 7). In addition to the effects sodium dodecyl sulfate induces in the aromatic region of these spectra, it has a dramatic effect, in contrast to TDC, in the far-ultraviolet region. As far as lipase and co-lipase are concerned, the designation of sodium dodecyl sulfate as a "hard" detergent in contrast to, e.g., TDC (Helenius and Simons, 1975) is justified. Earlier approximately 200 mol of sodium dodecyl sulfate have been found to bind per mol of lipase (Borgström and Donnér, submitted for publication) and 40–50 mol per mol of co-lipase (Borgström and Donnér, 1975). These results also explain the different nature of binding to lipase which results in a reversible inhibition of enzyme activity when TDC is bound and in an irreversible inactivation when sodium dodecyl sulfate is bound (Borgström and Erlanson, 1973).

The circular dichroism measurements and the results of the calorimetric experiments (Figure 8) on the binding of TDC to co-lipase confirm the results of the equilibrium dialysis experiments in that there is no binding of TDC to co-lipase until concentrations near the cmc of TDC are reached (0.8–1.0 mM under the conditions of these experiments). However, it was not recognized in the circular dichroism and equilibrium dialysis experiments that the binding of TDC to lipase or co-lipase exhibits a two-step behavior as in the calorimetric experiments presented in Figure 8. The significance of this difference is not clear at the moment.

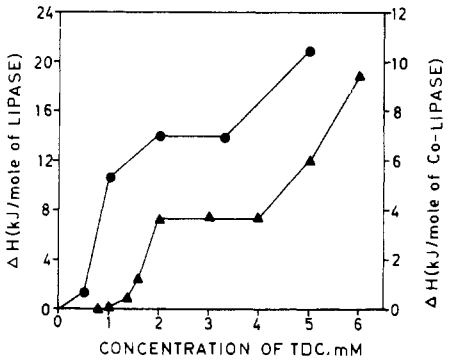

FIGURE 8: Experimental heats of the binding of TDC to lipase (●—●) and co-lipase (▲—▲) as a function of the total concentration of TDC in the final solution after mixing the reactants in the calorimeter. The total concentration of lipase was kept constant at 13.3 μmol/l. in the final solution and that of co-lipase at 86.0 μmol/l.

Figure 8 also shows that the bindings of TDC to lipase or co-lipase are endothermic processes which implies that these reactions are entropy driven. It is thus not probable that any polar forces between the proteins and TDC monomers or micelles are of any importance for the binding. This agrees with the findings of Robinson and Tanford (1975) regarding the cooperative binding of deoxycholate, Triton X-100, and sodium dodecyl sulfate to cytochrome b_5.

It has been shown by Charles et al. (1975) that the strong dependence on ionic strength of TDC micellar weight is also exhibited by the co-lipase–TDC complex. Furthermore, it was shown by Borgström and Donnér (1975) that the average number of moles of TDC bound per mole of co-lipase is the same as the aggregation number of TDC micelles. These observations are all compatible with a model where co-lipase forms mixed micelles with TDC. Such an interpretatio was also done by Robinson and Tanford (1975) regarding the binding of cytochrome b_5 to the detergents mentioned above.

Finally, it should be mentioned that Wieloch et al. (1975) have shown in a NMR investigation of the binding of TDC to co-lipase that hydrophobic amino acids (alanine, valine, leucine, and isoleucine) are involved in the binding reaction.

Acknowledgments

We thank Professor Ingemar Wadsö for placing his resources at our disposal.

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