# EFFECT OF COLIPASE ON ADSORPTION AND ACTIVITY OF RAT PANCREATIC LIPASE ON EMULSIFIED TRIBUTYRIN IN THE PRESENCE OF BILE SALT

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Received 11 November 1974

## 1. Introduction

Conjugated bile salts at levels higher than their critical micellar concentration strongly inhibit the activity of pancreatic lipase (glycerolester hydrolase, EC 3.1.1.3). This inhibition is more effective in the alkaline pH range and results in an 'acid shift' of optimal pH [1]. The addition of the protein cofactor colipase overcomes this inhibition [1,2]. Whether or not colipase clearly abolishes the bile salt mediated acid shift in the pH activity curve of lipase is a debated question [1,3]. The present work was undertaken to examine rat pancreatic lipase activity in relation to its ability to be adsorbed on emulsified tributyrin. This study was conducted at a supramicellar concentration of sodium taurodeoxycholate, between pH 6.0 and 8.0, and at different colipase concentrations. A pH-adsorption curve was differentiated from the pH-activity curve of lipase. We concluded that the so-called acid shift of the optimal pH for lipase action described earlier [1,4] is due to the low adsorption rate of lipase on its substrate at alkaline pH rather than to a change of the pH dependence of the $V_{max}$ and $K_m$ of the enzyme.

## 2. Materials and methods

Rat pancreatic lipase was purified as previously described [4]. Bovine colipase was purified by a procedure adapted from Erlanson and Borgström [6] as reported recently [7]. Tributyrin was purchased from Fluka (Buchs, Switzerland). Sodium taurodeoxycholate came from Sigma (St. Louis, Missouri, USA). *N*-Morpholino-3-propanesulfonic acid (MOPS), *N*-Tris (hydroxymethyl) methyl-3-amino-propanesulfonic acid (TAPS) and maleic acid were obtained from Serva (Heidelberg, Germany). These 3 acids were used at a concentration of 2 mM to adjust the pH of 2% tributyrin emulsions between 6.0 and 8.0 with NaOH. These emulsions were prepared daily with an Ultra-Turrax type TB 18 homogenizer from Janke and Kunkel KG (Staufen, Germany) for 5 min at 10 000 rev/min. The substrate emulsion was continuously agitated with a magnetic stirrer before and during lipase assays conducted potentiometrically at 25°C with a combititrator 3 D from Metrohm (Herisau, Switzerland).

The effect of colipase on lipase-tributyrin interaction was examined on a tributyrin emulsion containing as much as 5 mM sodium taurodeoxycholate and 100 mM NaCl. Various amounts of colipase (0.25 to 50 μg from a stock solution at 500 μg/ml) were added to 10 ml of the emulsion in order to study enzyme activity as well as enzyme adsorption on emulsified particles. The reactions were started with 5 μl of an aqueous lipase solution (0.6 mg/ml) and the activity was recorded with 100 mM NaOH as titrating reagent.

The fraction of the enzyme bound to its substrate was determined by removing a 1 ml aliquot from the reaction medium at zero time. This aliquot was immediately centrifuged at 0°C for 10 min at 800 *g*. 500 μl of the clear aqueous supernatant was taken to estimate the percentage of lipase still non adsorbed by a measurement of lipase activity under optimal assay conditions [7,8]. The complete system contained 9.2 ml of 2 mM Tris–HCl buffer (pH 8.0), 0.2 ml of tributyrin, 0.1 ml of colipase solution (50 μg) and 0.5 ml of enzymatic sample. The final medium contained 0.25 mM sodium taurodeoxycholate and 5 mM NaCl. Constant titration was performed under magnetic stirring with 10 mM NaOH.



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## 3. Results and discussion

All the kinetic graphs were linear at pH 6.0–6.5 but tended to become convex in the alkaline pH range as if an increasingly long lag period delayed the course of the reaction at higher pH (fig.1).

The addition of colipase improved the rate of lipolysis throughout the range of pH tested and shortened the lag period at neutral and alkaline pH. The amount of colipase required for maximum initial velocity increased from acidic to alkaline pH. At pH 6.0, 0.5 μg of colipase (i.e. a molar ratio of colipase:lipase equal to 1) sufficed to ensure maximum lipase activity, while at pH 8.0, hundred times more colipase allowed only 70% of maximum activity (fig.1 and 2).

Convex kinetic curves indicated that steady states were not reached and suggested that the adsorption of lipase on its substrate might be the rate limiting step of lipolysis in the pH 7.0 to 8.0 range.

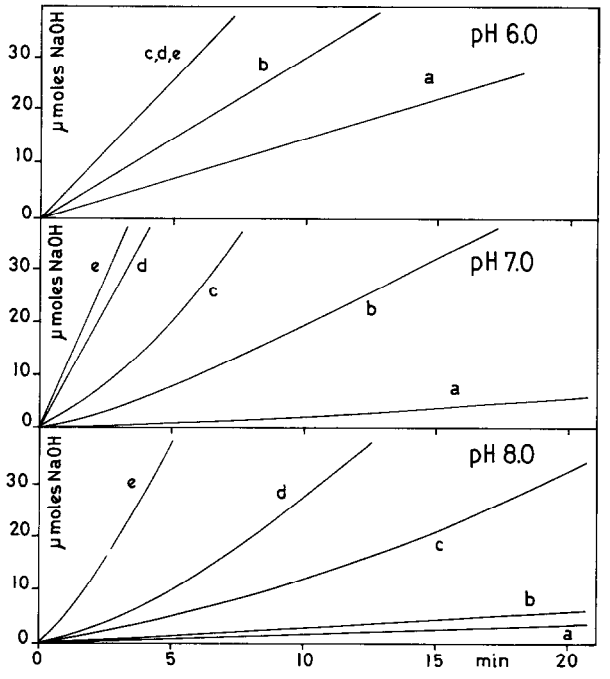


Fig. 1. pH-stat recordings obtained at 3 different pH for a single amount of lipase. Bovine colipase was absent (a) or added in increasing amounts: 0.25 μg (b), 0.50 μg (c), 5.0 μg (d), and 50 μg (e) per assay. In all assays the substrate was a buffered emulsion of tributyrin prepared in 5 mM sodium taurodeoxycholate and 100 mM NaCl.

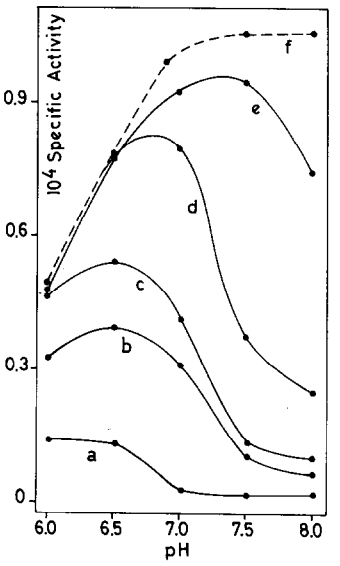


Fig. 2. pH-activity curves of rat lipase based on initial velocities in the presence of increasing amounts of bovine colipase: 0 μg (a), 0.25 μg (b), 0.50 μg (c), 5.0 μg (d) and 50 μg (e,f) per assay. In all assays the substrate was a buffered emulsion of tributyrin prepared in 5 mM sodium taurodeoxycholate and 100 mM NaCl except for curve f (●–●–●) where the bile salt was omitted. All reactions were started by adding lipase to the emulsion adjusted to the proper pH. The specific activities were expressed in units/mg pure rat lipase. One unit corresponds to one μmol of butyric acid liberated/min at 25°C.

Fig.2 and 3 compare the activity and the adsorption of lipase on tributyrin as determined by centrifugation (see under Materials and methods). Adsorption was maximal at pH 6.0, and remained high at pH 6.5 in the presence of minute amounts of colipase so that the colipase effect at those pH mainly consisted in increasing the specific activity of lipase. At neutral and alkaline pH, adsorption and activity at zero time were very low. Addition of colipase increased both parameters in parallel and it is thus evident that colipase exerted its effect mainly by improving lipase adsorption.

It was important to exclude the possibility that neutral or alkaline pH were in fact decreasing the affinity of lipase towards tributyrin rather than the adsorption rate. We therefore reexamined the kinetics under new assay conditions. We first ensured rapid lipase adsorption by mixing the various ingredients (lipase included) at pH 6.0. Immediately after addition of lipase to the stirred emulsion, the pH was shifted to the desired

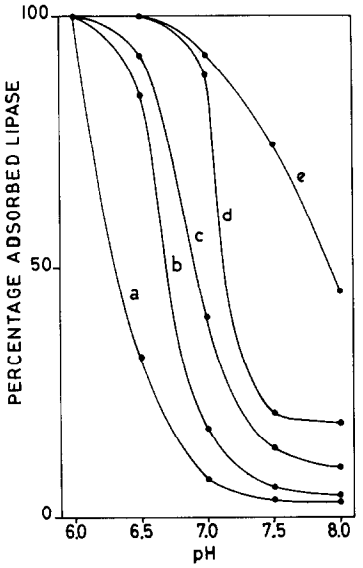


Fig. 3. pH-adsorption curves of rat lipase on emulsified tributyrin particles. A one ml aliquot was taken from the incubation mixtures prepared as in fig.2 immediately after lipase addition. After centrifugation, lipase adsorption was estimated by a measurement of its residual activity in the clear supernatant (see Materials and methods).

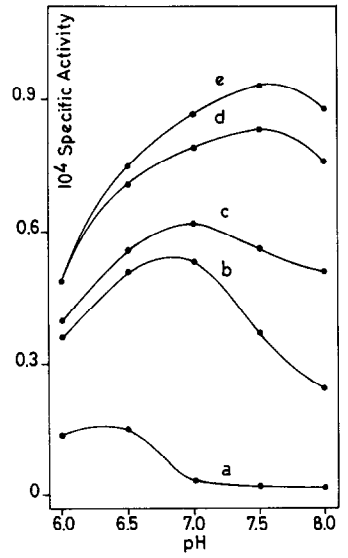


Fig. 4. Same experiments as in fig.2 except for a preincubation at pH 6.0. The final pH was adjusted immediately after addition of lipase to the substrate at pH 6.0.

value. This time, all kinetics were linear and the highest initial velocities were recorded in the alkaline pH range as is the case in the absence of sodium taurodeoxycholate (fig.4 as compared to fig.2). These results are consistent with a single mechanism of catalysis independent of the precence or absence of bile salt [9]. They point out that the rate limiting step of lipolysis at pH 7.0–8.0 is the adsorption of lipase on the tributyrin emulsion or possibly a still earlier event controlling lipase adsorption in the presence of taurodeoxycholate and colipase.

In conclusion, colipase clearly exhibited two distinct stimulatory effects on lipolysis in the presence of sodium taurodeoxycholate. Firstly, colipase increased the rate of lipase adsorption on its substrate. Secondly, colipase ensured maximum velocities though at alkaline pH the lag period due to poor adsorption had first to be overcome. The real limits of colipase action may therefore be the increasingly difficult adsorption of lipase on its substrate. When natural proportions of lipase and colipase were tested in the form of a crude rat pancreas homogenate or of human pancreatic juice, with tributyrin or triolein as substrate, we observed pH-activity curves very similar to those obtained under the conditions illustrated by curves c and d in fig.2 (data not shown). Under most if not all circumstances, including probably those occurring in vivo in the duodenum, the rate limiting step of lipolysis at alkaline pH appears to be the slow organization of a ternary complex involving lipase, colipase and bile salt [3] and (or) its adsorption on the emulsified triglyceride particles.

## Acknowledgements

This work was supported by Fonds de la Recherche Scientifique Médicale (Belgium) contract number 20 403. We wish to express our thanks to Mrs Ballinckx and Mr Gelston for preparation of the manuscript.

## References

[1] Borgström, B. and Erlanson, C. (1971) Biochim. Biophys. Acta 242, 509–513.
[2] Maylié, M. F., Charles, M. and Desnuelle, P. (1971) Biochim. Biophys. Acta 229, 286–289.

[3] Maylié, M. F., Charles, M., Astier, M. and Desnuelle, P. (1973) Biochem. Biophys. Res. Commun. 52, 291–297.
[4] Borgström, B. and Erlanson, C. (1973) European J. Biochem. 37, 60–68.
[5] Vandermeers, A. and Christophe, J. (1968) Biochim. Biophys. Acta 154, 110–129.
[6] Erlanson, C. and Borgström, B. (1972) Biochim. Biophys. Acta 271, 400–412.
[7] Vandermeers, A., Vandermeers-Piret, M. C., Rathé, J. and Christophe, J. (1974) Biochim. Biophys. Acta, in the press.
[8] Erlanson, C. and Borgström, B. (1970) Scand. J. Gastroent. 5, 293–295.
[9] Brockerhoff, H. (1973) Chemistry and Physics of Lipids 10, 215–222.