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Composition of a crude lipase from *Candida Cylindracea* as studied by differential scanning calorimetry and thermogravimetry

A. Gentili, M. Repetto, G. D'Ascenzo, R. Curini*, S. Materazzi

Department of Chemistry, University La Sapienza of Rome, P.le A. Moro, 5-00185 Rome, Italy

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Abstract

Lipases are carboxyl-esterases (EC 3.1.1.3.) which, in vivo, catalyze the hydrolysis of triglycerides and isoluble substrates by a heterogeneous process. In vitro, they may also hydrolyze soluble esters, but the presence of a water–lipid interface significantly increases their lipolytic activity.

The commercially available crude lipase contains ca. 30% of lactose as 'extender'. In fact, the addition of sugars or polyols stabilizes the biological macromolecules, thereby preventing loss of enzyme activity and increasing resistance to denaturing conditions.

By means of differential scanning calorimetry (DSC) and thermogravimetry (TG), it has been possible to evaluate the composition of a commercial lipase and also to demonstrate the addition of a monohydrate α-lactose, and the time of addition.

1. Introduction

Lipases are carboxyl-esterases (EC 3.1.1.3.) which, on the basis of their origin and properties, can catalyze the hydrolysis of a large number of esters, in particular of long-chain acylglycerols. The catalysis is heterogeneous in that it occurs at the water–lipid interface [1–3].

Lipase from *Candida cylindracea*, commonly known as *Candida rugosa*, has a low substrate specificity and, thus, can act on a wide variety of substrates; in addition, it is able to catalyze both, esterification and transesterification reactions in non-aqueous solutions. [4–7].

The commercially available crude product contains ca. 30% of lactose as 'extender'. In fact, many other proteins are commercially available in freeze-dried form with the addition of mono- and disaccharides or in 50 : 50 water–glycerol solutions. Introduction of polyols and sugars stabilizes the biological macromolecules, thereby preventing loss of enzyme activity and increasing resistance to denaturing conditions [8,9].

In this work, we have studied the composition of a commercial lipase preparation by differential scanning calorimetry (DSC) and thermogravimetry (TG), to demonstrate with this method the addition of a particular lactose and also the time of addition.

2. Experimental

2.1. Materials

Lipase (EC 3.1.1.3.), type VII, from *Candida cylindracea* and D-lactose monohydrate were purchased

*Corresponding author. Tel.: +39 6 4958251; fax: +39 6 490631.

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from Sigma, St. Louis, MO; di-potassium hydrogen orthophosphate (98%) and potassium dihydrogen (99.5%) for preparation of buffer were purchased from Farmitalia Carlo Erba.

All others chemicals of the purest grade were available.

2.2. Thermal analysis

The Perkin–Elmer series 7 differential scanning calorimeter (DSC-7) was used to characterize the thermal behaviour of the enzyme preparation (CL: commercial product Sigma) and of lactose (L). Samples of 4 mg were placed in the standard, crimped aluminum sample pans (Perkin–Elmer). DSC analysis was performed using a temperature scan rate of 10°C/min and nitrogen (99.9) as carrier gas.

The Perkin–Elmer series 7 thermogravimetric analyzer (TGA-7) was used on 4-mg samples of L, and CL. The carrier gas was nitrogen (99.9) at a flow rate of 50–100 ml/min and with a temperature scan rate of 10°C/min.

3. Results and discussion

The Sigma enzyme preparation used in the present work contains, according to the manufacturers' specification, ca. 30% lactose as 'extender'. From our DSC and TG investigations of the commercial lipase, it is clear that the lactose it contains is monohydrate α-lactose and that disaccharide was added to the lyophilized product.

The lactose (4-O-β-D-galactopyranosyl-D-glucopyranose) has two anomeric forms, α and β, which are distinguished in terms of chemico–physical properties [10]. The more common α form is monohydrate, whereas the β form has no water of crystallization. However, they can be identified by DSC through their melting point and the presence, or absence, of a peak for crystallization water release [11,12].

The DSC curve for crude commercial lipase (Fig. 1(A)) obtained in the 25–250°C range shows a first endothermal process between room temperature and 125°C, followed by a second more acute one with a maximum at 146°C. At 175°C, a slight exothermal process occurs, followed by two consecutive endothermal processes with maxima at 220° and 230°C (start of protein decomposition), respectively. The first endothermal process, with a maximum at 86°C, is the result of two concomitant events: proteic thermal unfolding and water of hydration loss [13]. The ensuing processes are typical of monohydrate α-lactose transformations, as is confirmed by further studies on pure lactose carried out by DSC and TG.

As can be seen from the pure α-lactose DSC curve (Fig. 1(B)), the peak at 148°C corresponds to the crystallization water. The exothermal process at 175°C refers to the recrystallization of small amorphous amounts present in the sample. Finally, the endothermal peak at 220°C refers to the melting and simultaneous decomposition of sugar.

The TG and derivative TG (DTG) curves of commercial lipase (Fig. 2(A)) in the 23–250°C range, evidence an initial loss of weight of 6.4% between room temperature and 170°C. In reality, there are two loss-of-weight processes, the first between 23° and 110°C with a 3.7% loss (hydration water of proteic matrix) and a second one between 110° and 170°C with a 2.7% loss (crystallization water of lactose).

The TG and DTG curves of pure α-lactose (Fig. 2(B)) evidence a loss of weight at ca. 155°C (water of crystallization).

Comparison of DSC and TG thermograms for pure lactose and enzyme preparation confirm that, in the latter, the anomer used as extender is in fact monohydrate α-lactose.

Another datum obtained was a confirmation that the lactose is added to the proteic sample in solution not before, but after freeze-drying, when the lyophilization is completed, to the solid sample. That could be shown, since lactose in aqueous solution gives mutarotation and, thus, the anomer is partly converted into the β one [11].

We, therefore, dissolved the commercial enzyme preparation and the monohydrate α-lactose separately in the water at pH 7 and subjected them to lyophilization. Then, DSC and TG investigations were repeated. The results are shown in Fig. 3(A and B), respectively, DSC thermograms of the protein and sugar, where the water of hydration peak is no longer observed, whereas a clear exo peak at 90°C, followed by an endo one at 125°C ($\alpha \rightleftarrows \beta$ transformation) are now present [12]. Also, TG and DTG curves confirm the disappearance of the water of crystallization.

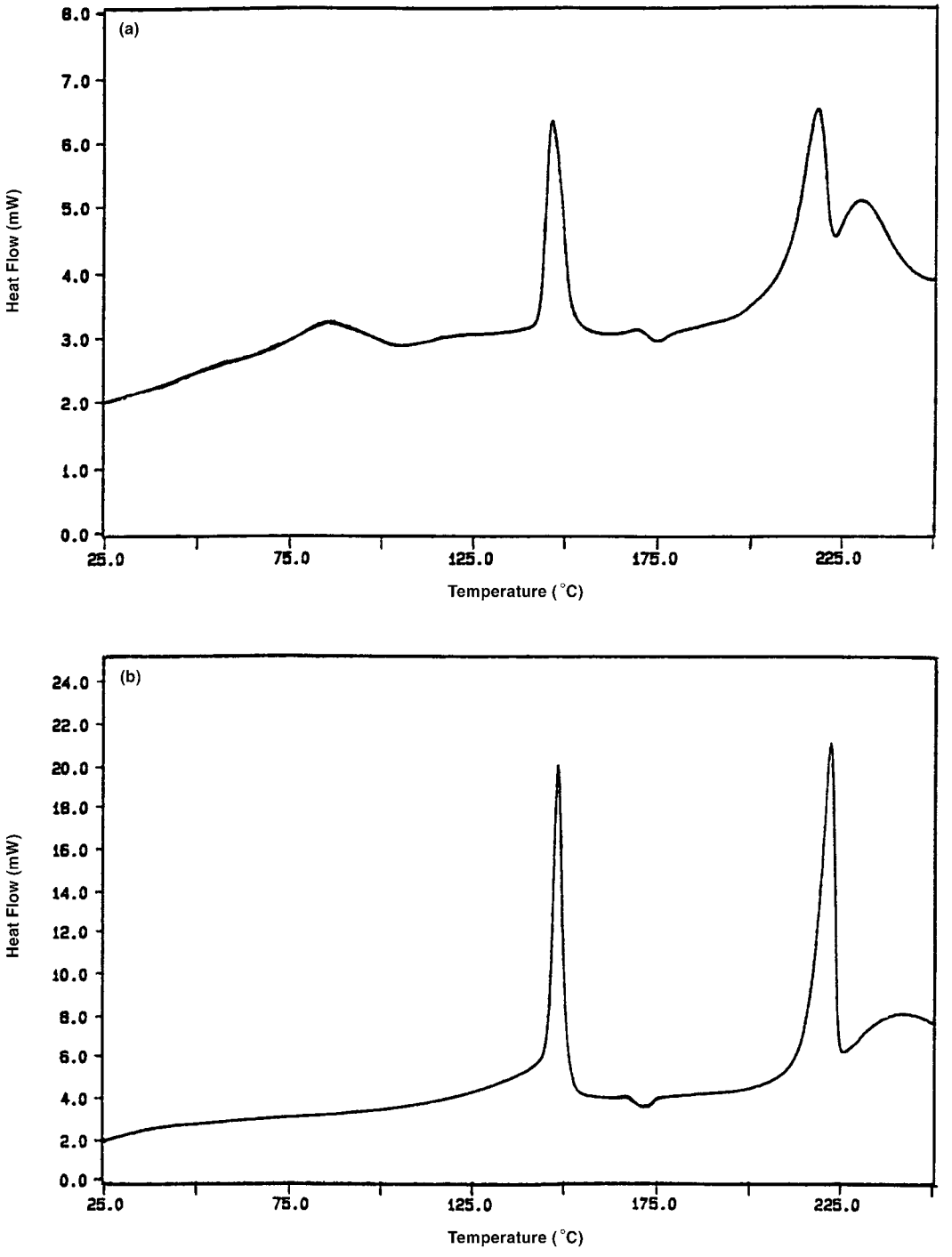

Fig. 1. (A) DSC curve of commercial lipase from *Candida cylindracea* with lactose. Heating rate, 10°C/min; nitrogen at flow rate of 50–100 ml/min. (B) DSC curve of α-lactose monohydrate standard. Heating rate, 10°C/min, nitrogen at flow rate of 50–100 ml/min.

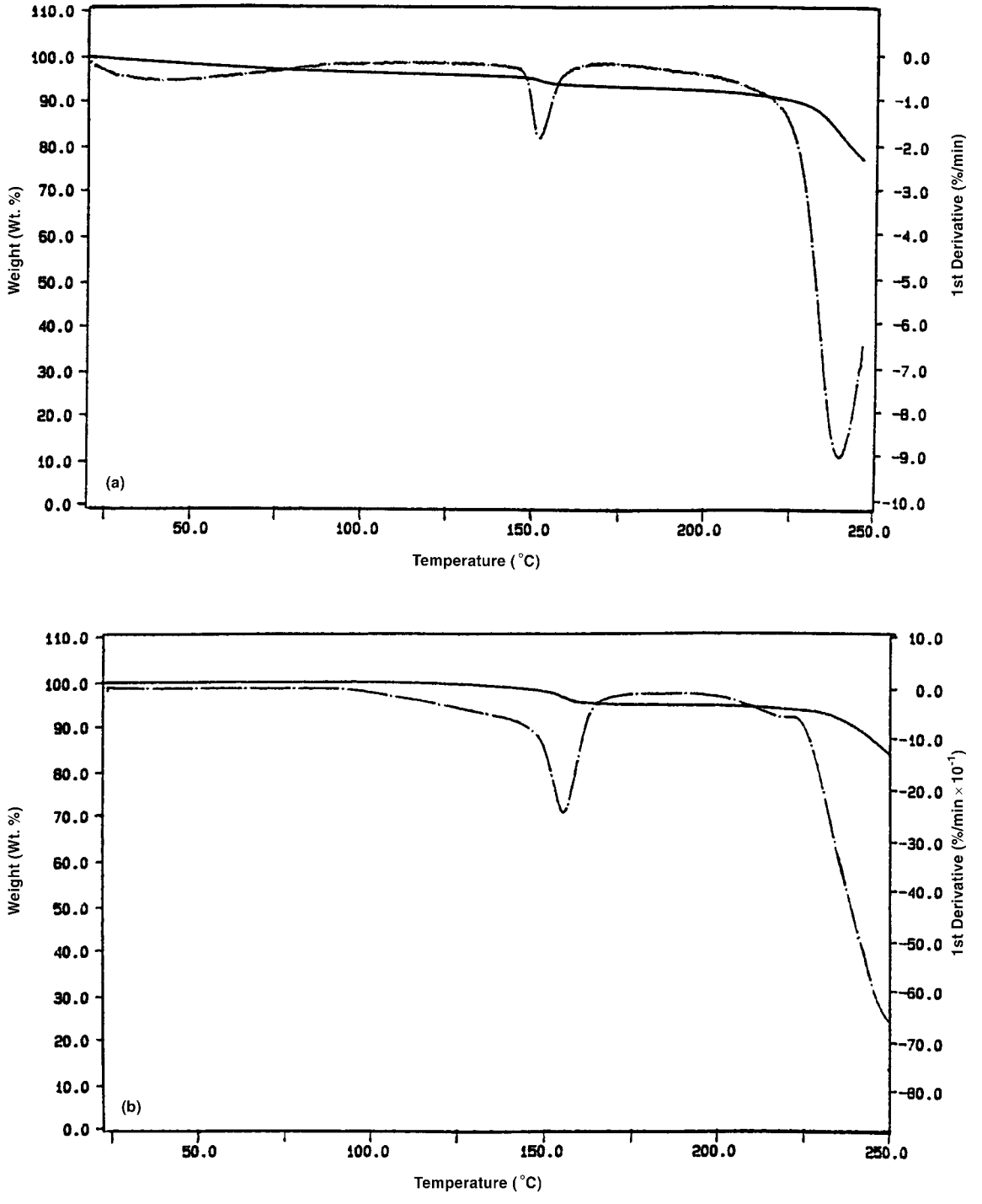

Fig. 2. (A) ——— TG and ● – DTG curves of commercial lipase from *Candida cylindracea* with lactose. Heating rate, 10°C/min, nitrogen at flow rate of 50–100 ml/min. (B) ——— TG and ● – DTG curves of α-lactose monohydrate standard. Heating rate 10°C/min, nitrogen at flow rate of 50–100 ml/min.

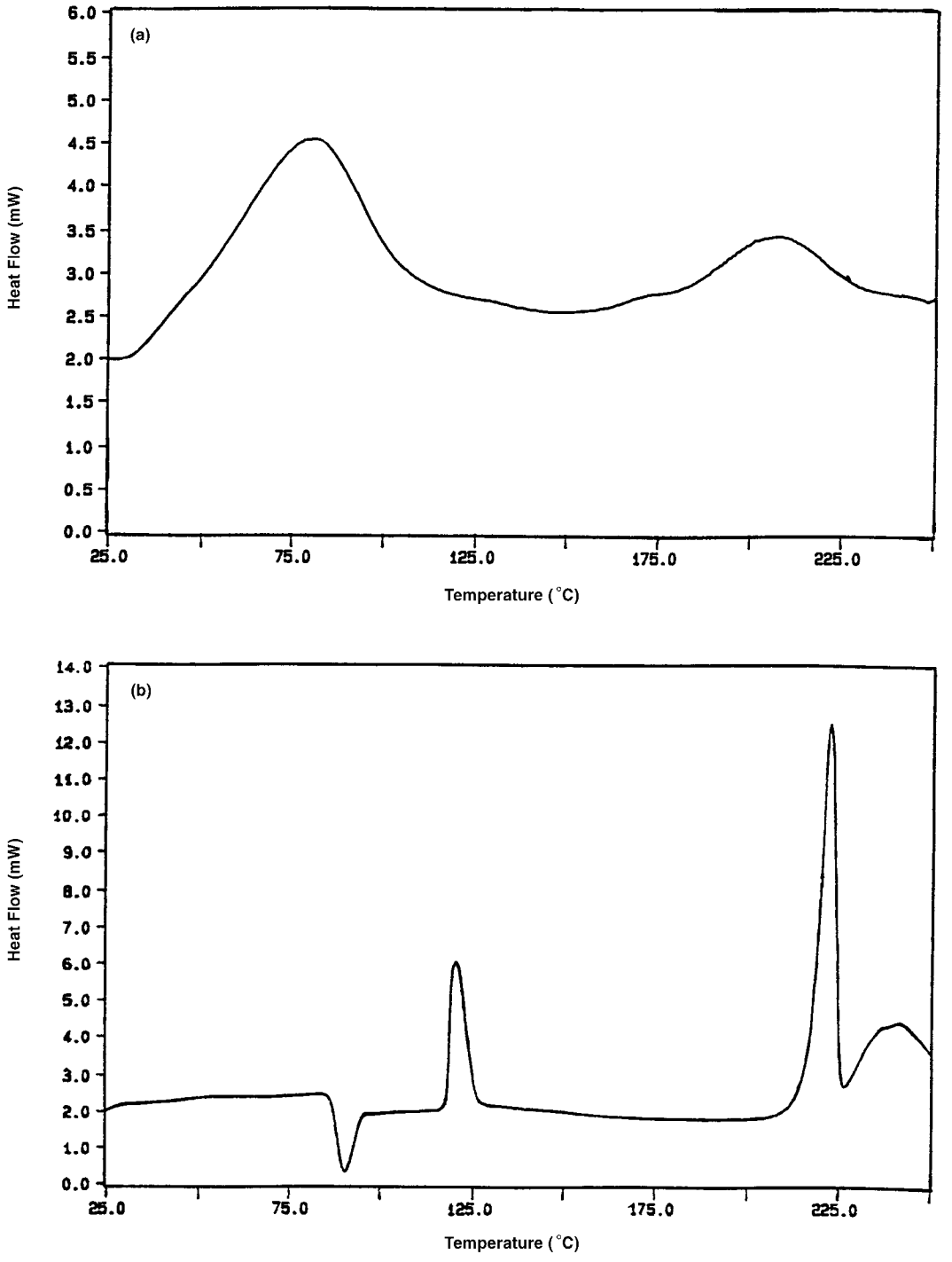

Fig. 3. (A) DSC curve of commercial lipase from *Candida cylindracea* with lactose after lyophilization. Heating rate 10°C/min, nitrogen at flow rate of 50–100 ml/min. (B) DSC curve of α-lactose monohydrate standard after lyophilization. Heating rate 10°C/min, nitrogen at flow rate of 50–100 ml/min.

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