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RECENT DEVELOPMENTS IN BIO-CALORIMETRY WITH MICRO-DSC

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SUMMARY

Different types of application (denaturation, transition requiring very slow scanning rates, liquid-liquid interactions) are presented : they correspond to the current needs of industrial control and research, and have now become possible owing to the progress of biocalorimetry.

INTRODUCTION

In recent years there has been considerable development in biochemistry and biology. Parallel to this evolution calorimetry has also made progress which now make possible the analysis of the behaviour of biochemical substances by calorimetry.

Biochemical and biological substances can lead to transformations such as configuration changes, denaturation, enzyme-substrate interactions, etc...

These processes generally involve low or very low energies. For this reason the micro-DSC, a very sensitive DSC has been developed.

DENATURATION AND AGGREGATION PROCESSES

Substances such as proteins, DNA, hormones, nucleic acids can undergo denaturation. It is very helpful to have information concerning this process and the corresponding thermodynamic data : temperature and heat of denaturation, change in heat capacity.

With the development of genetic engineering some substances such as Insulin, HGH (human growth hormone) are now produced in larger amounts : the practical need is then for an instrument able to test these molecules.

It is demonstrated that the denaturation and aggregation processes can be monitored in injectable solutions. In practice, for injectable solutions the aim of the research is to determine the medium in which the processes of denaturation and aggregation will be avoided.

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Ribonuclease A (Fig. 1)

When heated (0.3°C.min^{-1}), the Ribonuclease solution (2 %) presents an endotherm of denaturation with an enthalpy of ΔH = - 398.9 kJ.mole^{-1}.

The change in heat capacity during the transition is + 3.3 kJ.mole^{-1}.°C^{-1}.

The transition has a maximum at 56.1°C.

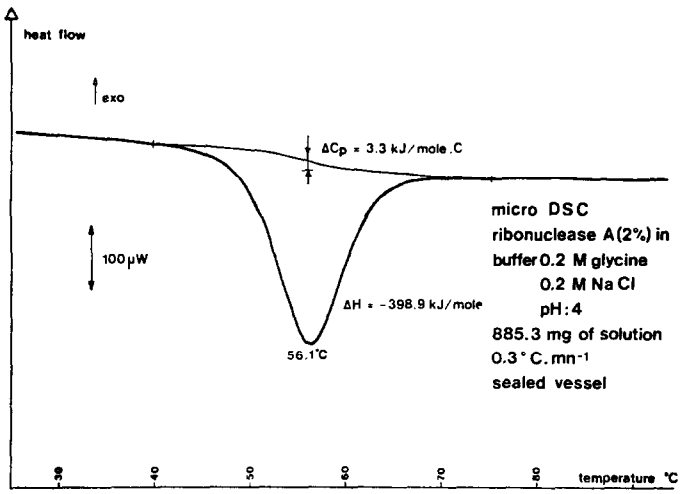

Fig. 1. Ribonuclease A

Stability of human growth hormone (hGH) (Fig. 2)

The hGH is investigated in solution (0.07 %) with Alamine (13.5 %) as a stabilizer. Even at such a low concentration the denaturation process can be monitored. Depending on the medium an exotherm of aggregation is generally observed.

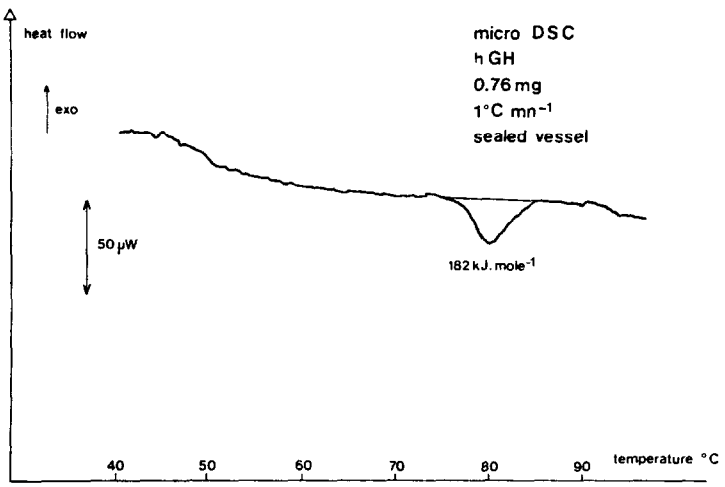

Fig. 2. Stability of human growth hormone (hGH)

Thermal stability of a protease (Fig. 3)

To improve the thermal stability of the protease different media have been used. The tests show that the Calcium solution produces a displacement of the denaturation process towards a higher temperature : it acts as a thermal stabilizer.

The EDTA complexation has demonstrated a contrary effect.

The micro-DSC is used here to test the thermal stability of the enzyme.

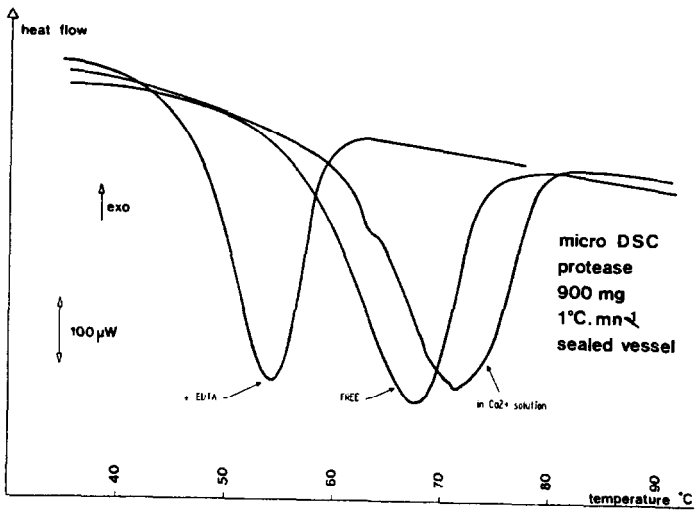

Fig. 3. Thermal stability of a protease

PHASE TRANSITIONS AT VERY SLOW SCANNING RATE (Fig. 4)

Bio-membranes like liquid crystals may present solid-mesophase and mesophase-liquid transitions which can be easily measured by conventional DSC.

But as far as the mesophase-mesophase transitions analysis is concerned, very slow scanning rate are generally required (a few $°C.h^{-1}$ or less). The main drawback is that under these conditions, conventional DSC is not sensitive enough. For these reasons the micro-DSC, a very sensitive DSC, is of great interest.

The capability of the micro-DSC to scan the temperature upwards and downwards is also essential, as it can provide information about the reversibility of the different transitions. This facility is especially important with the monotropic crystals which present mesophases only in the cooling mode.

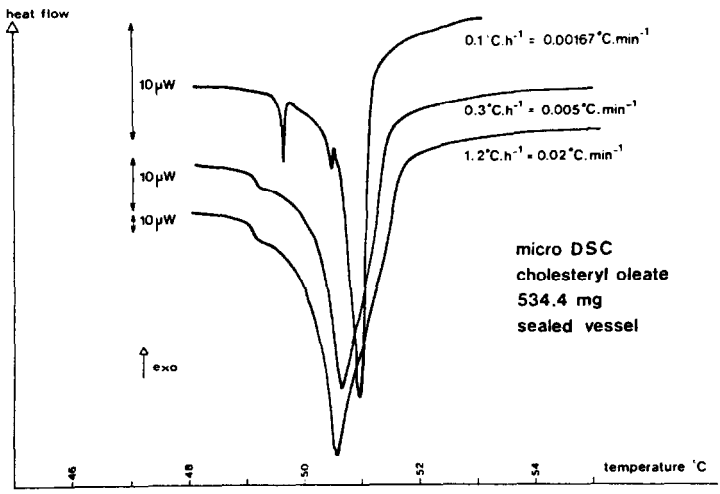

Fig. 4. Phase transitions at very slow scanning rate

FLOW MIXING : ENZYMATIC ACTIVITY (Fig. 5)

Using an adapted vessel it is possible to continuously inject two liquids in order to monitor liquid-liquid interaction.

This device makes the investigation of enzyme-substrate interaction possible. As an example, the action of a glucoamylase on a maltose solution is monitored. When the two solutions are injected, an exotherm due to hydrolysis is observed: this exotherm is proportional to the two flow-rates and of course to the activity of the glucoamylase.

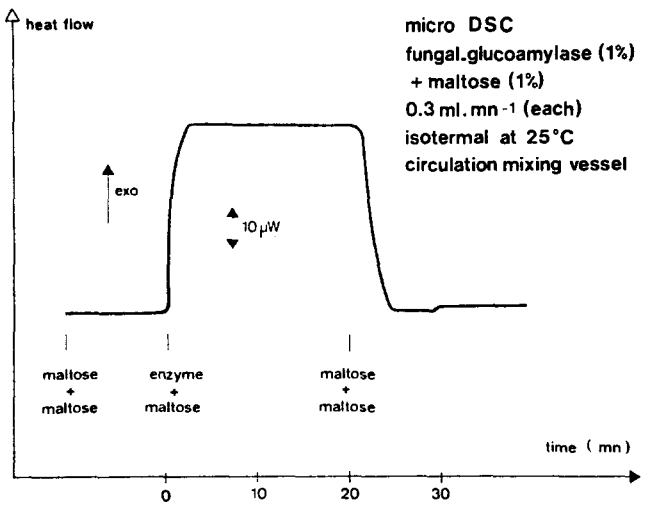

Fig. 5. Flow mixing : enzymatic activity

VESSEL - EXPERIMENTAL NOTICES (Fig. 6 - 7)

It must be pointed out that the use of removable vessels (in hastelloy) makes the filling very easy as it can be carried out outside the calorimeter. Another interesting point is the cleaning which becomes very simple : it is an important feature especially for proteins which tend to stick, after being denaturated.

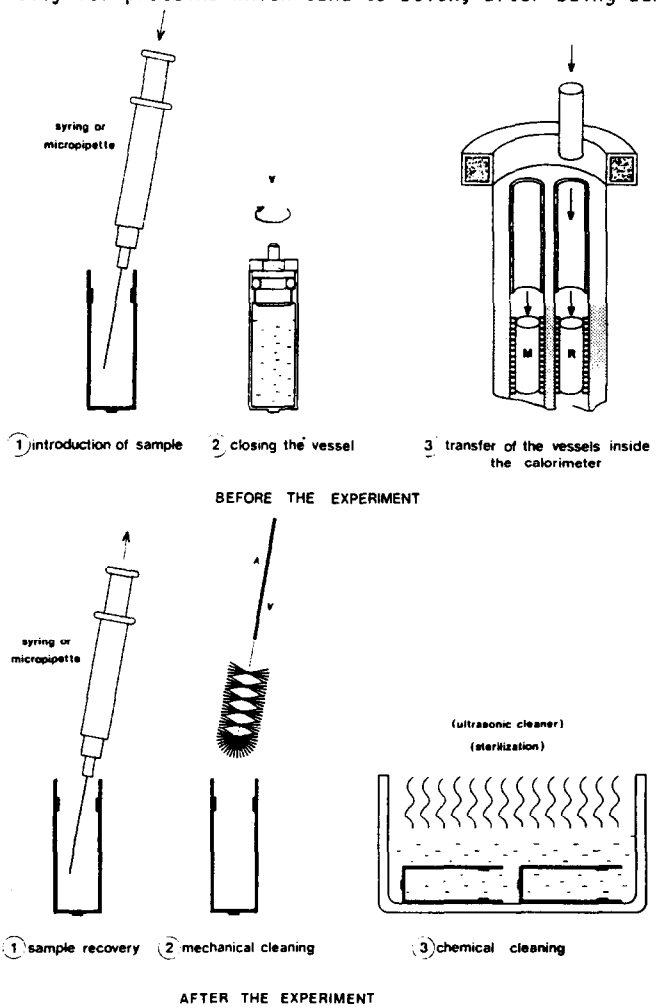

Fig. 6 - 7. Vessel - experimental notices

CONCLUSION

It is demonstrated that the micro-DSC, as a recent development, is a versatile instrument which can be used for the investigation of :

- thermal stability of biochemical compounds
- determination of tiny phase transitions requiring a very slow scanning rate
- enzyme activity with the flow mixing-vessel.