Reaction of an Extracellular Proteolytic Enzyme from a Strain of *Arthrobacter* with Diisopropylphosphofluoridate (DFP)

SVANTE WÄHLBY,
LORENTZ ENGSTRÖM, ULF BJARE
and BENGT VON HOFSTEN

Institutes of Medical Chemistry and Biochemistry, University of Uppsala, Uppsala, Sweden

Several proteolytic enzymes are inactivated by DFP with a concomitant phosphorylation of a serine residue at the active site of the enzymes.[1] In most of these enzymes, *e.g.* chymotrypsin, the amino acid sequence around the reactive residue is Asp-Ser-Gly. However, subtilisin and a mold protease have been reported to have a different sequence.[1]

The purification of a proteinase from a strain of *Arthrobacter* has recently been reported [2] and the enzyme was shown to be inhibited by DFP (see Ref. 3). In this preliminary communication, some experiments are described which demonstrate that a particular serine residue of the *Arthrobacter* proteinase reacts with DFP. The amino acid sequence around the reactive serine is different from that in chymotrypsin.

20.4 mg of crystalline *Arthrobacter* proteinase were dialyzed overnight against 0.05 M Tris-HCl buffer (pH 7.5) and then incubated with 3×10^{-3} M $DF^{32}P$ (2100 counts/min/mμ mole) for 2 h at 20°. No enzyme activity remained after this treatment, as measured by a milk-clotting assay.[4] In order to remove unreacted $DF^{32}P$, the incubation mixture (1.5 ml) was filtered through a 1.2×22 cm column with Sephadex G-50 (Pharmacia, Uppsala, Sweden), in equilibrium with 0.05 M Tris-HCl buffer (pH 7.5). The eluted enzyme was radioactive and was well separated from the free $DF^{32}P$. The enzyme was precipitated by adding 1/3 volume of 25 % (w/v) trichloroacetic acid, which was 0.01 M with respect to silicotungstic acid. The precipitate was washed twice with acetone and dried *in vacuo*.

7 mg of the dry, labelled enzyme were hydrolyzed with 1 ml of 2 N HCl for 20 h at 100°. $Ser^{32}P$ was isolated from the hydrolysate and identified with the same methods as used previously.[5] Another sample of the inactivated enzyme was analyzed for phosphorus [6] and nitrogen. Since the molecular weight (23 041) and nitrogen content (15 %) of the enzyme are known,[2] it could be calculated that 0.87 mole of phosphorus was bound per mole of enzyme, indicating one active site per enzyme molecule.

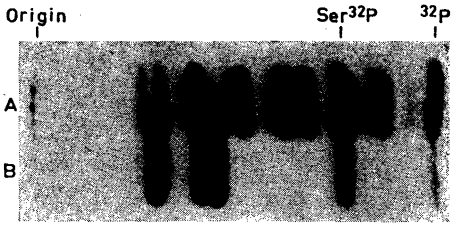

Fig. 1. Radioautograph of electropherogram of partial acid hydrolysates of $DF^{32}P$-incubated chymotrypsin (A) and *Arthrobacter* enzyme (B). Amounts corresponding to (A) 0.13 mg (25 000 cpm) and (B) 0.10 mg (23 000 cpm) applied to Whatman No. 3 paper on a continuous line at 5-cm intervals. Buffer: 0.05 M pyridine-acetic acid (pH 3.5). Voltage: 65 V/cm; electrophoresis time (anodic migration): 85 min. X-Ray film (Ilford, Goldseal) exposed for 60 h.

In order to compare the amino acid sequence around the reactive serine of the *Arthrobacter* enzyme with that of chymotrypsin, 7 mg of each enzyme was treated with $DF^{32}P$ as described and was hydrolyzed with 1 ml of 5.7 N HCl for 30 min at 100°. After removal of the HCl *in vacuo*, the hydrolysates were subjected to high-voltage electrophoresis at pH 3.5 to separate the labelled peptides, which were located by radioautography. From Fig. 1 it is seen that the phosphopeptide patterns obtained from the two hydrolysates are different, indicating that the amino acid sequence around the reactive serine is different in the two enzymes. Except for some labelled orthophosphate and phosphorylserine, only three labelled bands were obtained from the *Arthrobacter* enzyme. The structure of these peptides is now being studied.

This work was supported by grants from the *Swedish Medical Research Council* (Project No. 40X-665-01), the *Swedish Technical Research Council*, and the *Medical Faculty*, University of Uppsala.

1. Bender, M. L. and Kézdy, F. J. *Ann. Rev. Biochem.* **34** (1965) 49.
2. v. Hofsten, B., van Kley, H. and Eaker, D. *Biochim. Biophys. Acta* **110** (1965) 585.
3. v. Hofsten, B. and Reinhammar, B. *Biochim. Biophys. Acta* **110** (1965) 599.
4. v. Hofsten, B. and Tjeder, C. *Biochim. Biophys. Acta* **110** (1965) 576.
5. Engström, L. *Biochim. Biophys. Acta* **52** (1961) 49.

Received August 24, 1966.

Thermodynamic Properties of Stannous Sulfide Around 900°C

ARNE W. ESPELUND

Metallurgisk Institutt, Norges tekniske høgskole, Trondheim, Norway

The equilibrium relations found between the condensed phases of the system Sn—SnS are in good agreement as reported in Ref. 1, shown in Fig. 1. However, determination of the stability of SnS and vapour pressures in the system has presented difficulties because of the high escaping tendency and the complexity of the gas phase. There are great discrepancies between some results reported in the literature,[2-4] and it was found worth-while to investigate the system.

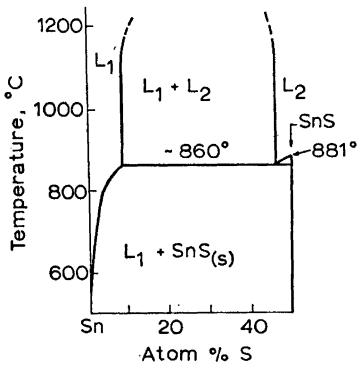

Fig. 1. The phase diagram Sn—SnS.

The experiments were performed at constant temperature on a sample composed of the two phases metallic tin and tin sulfide in contact with each other held in a crucible in a vertical tube furnace. Purified hydrogen was passed through the sample. The gas had a double function: it would react with the sulfide and transport the resulting hydrogen sulfide as well as gaseous tin sulfide away from the hot zone. The tin sulfide condensed in a cooler region. The remaining gas passed through absorption tubes containing NaOH ("Ascarite") and finally through an integrating flow meter.

The presence of two condensed phases in the crucible throughout the experiment ensured constancy of activities.

The hydrogen gas was bubbled through the upper sulfide layer of the charge. Results for experiments at flow rates varying from about 50 to 120 ml H_2 (NTP)/min showed no systematic variation, indicating completion of reaction.

During the heating and cooling of the reactor the gas was passed in the direction opposite to normal to ensure that the results would represent the period with constant temperature only.

After completion of each run the condensate of SnS needles was carefully collected and weighed, the weight increase of the absorption tubes noted and the amount of hydrogen gas registered.

In Fig. 2 are shown the results for the reaction

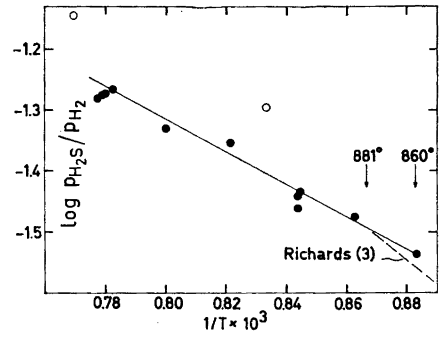

Fig. 2. Ratios of H_2S/H_2-pressures above the binary mixture "Sn"—"SnS".
Open circles: calculated from Kellogg's values.
Filled circles: present work.