PN 10018

The amino acid sequence around the reactive serine in alkaline phosphatase

Alkaline phosphatase (EC 3.1.3.1) has been shown to incorporate inorganic phosphate and Serine phosphate has been identified in partial acid hydrolysates of the ^{32}P-labelled enzyme[1]. It has been suggested this incorporation occurs at the active site of the enzyme[2,3]. A study of the amino acid sequence around the point of attachment of phosphate in alkaline phosphatase has been undertaken using radioactive techniques[4,5].

The enzyme was prepared from *Escherichia coli* following GAREN AND LEVINTHAL[6] with minor modifications, and the incorporation of [^{32}P]orthophosphate was made according to ENGSTRÖM[7].

Peptides from the labelled phosphatase were obtained by partial acid hydrolysis (5.7 N HCl in a boiling-water bath), subjected to ionophoresis and radioautographed. In Fig. 1 is shown the time course of the hydrolysis. Band 1 is inorganic phosphate and Band 3 matches perfectly with a serine phosphate marker on ionophoresis at pH 3.5.

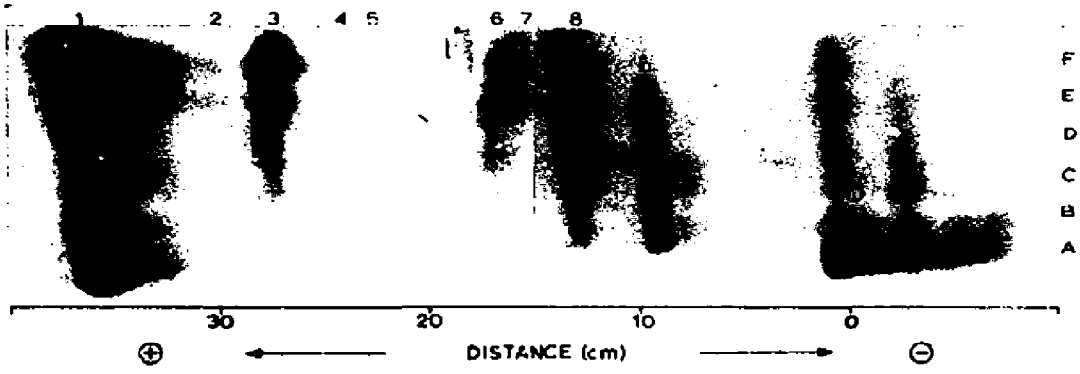

Fig. 1. Radioautograph of ionograms (Whatman No. 4 paper, pyridine–acetate buffer (pH 3.5), 35 V/cm, 2.5 h) of acid hydrolysates of [^{32}P]alkaline phosphatase with 5.7 N HCl in a boiling-water bath for different times: A: untreated, B: 8 min, C: 15 min, D: 30 min, E: 50 min, F: 90 min.

When Band 8 was rehydrolysed it gave bands in the positions of Band 7, unchanged peptide, serine phosphate and phosphate (in some experiments Bands 6 and 7 were not resolved). On the other hand Band 7 on rehydrolysis gives unchanged peptide, serine phosphate, phosphate and a trace of Band 8. This suggests that Bands 7 and 8 could be due to dipeptides one of which might be formed by inversion as in the case of Ser *P*–Gly and Gly–Ser*P* in chymotrypsin[8]. On ionophoresis in parallel with known dipeptides, Bands 7 and 8 match perfectly at pH 3.5 and 6.5 with two products obtained from a partial hydrolysate of [^{32}P]ovoalbumin and identified as Ala–Ser*P* and Ser*P*–Ala, respectively. The question is whether one is formed from the inversion of the other or if they are dipeptides derived from a sequence Ala–Ser*P*–Ala. If the latter is the case all the bands of the pattern should give on rehydrolysis Bands 7 and/or 8, since a second active serine is not present, as indicated by the comparative patterns of the partial hydrolysates of the peptides

obtained from proteolytic digestion. The fact that I have been able to isolate bands (as Band 6 for instance) which on rehydrolysis do not give Band 7 or 8, suggests that a third dipeptide must be present. The yields and partial hydrolysates made under different conditions show that Band 7 derives only from inversion of Band 8. That would mean that alanine is bound to the carboxyl group of serine phosphate and an amino acid different from alanine is bound to its amino group.

From the time course of the hydrolysis (Fig. 1) it can be seen that after prolonged hydrolysis there is no decrease of the intensity of Bands 2, 4 and 5. Rehydrolysis of Band 5 gave Band 4 and 2 together with serine phosphate and phosphate. Band 5 is faster than the dipeptide Gly Ser*P* (used as marker) suggesting that it contains an acidic amino acid. It might be the dipeptide Asp–Ser*P*, which on rehydrolysis gives a characteristic pattern due to conversion to the α and β forms[4]. A marker peptide which was known to contain Asp–Ser^{32}P was partially hydrolysed and run side by side on ionophoresis at pH 3.5 with a partial hydrolysate of [^{32}P]phosphatase. It was observed that Bands 2, 4 and 5 matched perfectly with the 3 forms of Asp–Ser*P*[4], indicating that aspartic acid is the amino acid attached to the amino group of Ser*P*. Rehydrolysis of Band 6 and the unnumbered bands present in the partial (for 30 min) acid hydrolysate (Fig. 1) gave Bands 7 and 8 (Ser*P*–Ala and its inverted form) and/or Bands 2, 4 and 5 (Asp–Ser*P* pattern) supporting the above conclusions.

The results obtained so far suggests that the sequence Asp–Ser–Ala is the site of the attachment of inorganic phosphate in Alkaline Phosphatase from *E. coli*. This is of interest in view of the known fact that an active serine in a sequence Asp–Ser–Gly– has been described in several proteases[4] and in a sequence Glu–Ser–Ala– in two esterases[7,8].

I am grateful to F. SANGER F.R.S. and D. C. SHAW for a kind gift of Ser*P* peptides.

División de Biología Molecular,
Instituto Nacional de Microbiologia,
Buenos Aires (Argentina)

C. MILSTEIN

[1] L. ENGSTRÖM, *Biochim. Biophys. Acta*, 52 (1961) 49.
[2] L. ENGSTROM, *Biochim. Biophys. Acta*, 54 (1961) 179.
[3] J. H. SCHWARTZ AND F. LIPMANN, *Proc. Natl. Acad. Sci. U.S.*, 47 (1961) 1996.
[4] M. A. NAUGHTON, F. SANGER, B. S. HARTLEY AND D. C. SHAW, *Biochem. J.*, 77 (1960) 149.
[5] C. MILSTEIN AND F. SANGER, *Biochem. J.*, 79 (1961) 456.
[6] A. GAREN AND C. LEVINTHAL, *Biochim. Biophys. Acta*, 38 (1960) 470.
[7] H. S. JANSZ, C. H. POSTHUMUS AND J. A. COHEN, *Biochim. Biophys. Acta*, 33 (1959) 396.
[8] H. S. JANSZ, D. BRONS AND M. G. P. WARRINGA, *Biochim. Biophys. Acta*, 34 (1959) 537.

Received September 21st, 1962