HYDROLYSIS OF THE GLYCOSIDIC BONDS IN β-*O*-XYLOSYL-L-SERINE AND β-*O*-D-XYLOSYL-L-THREONINE BY ENZYME PREPARATIONS FROM *HELIX POMATIA*

D. FISHER and P. W. KENT
Department of Biochemistry, University of Oxford, U. K.

Received 26 August 1968

In the course of investigating enzymes which bring about the cleavage of the carbohydrate-peptide linkage of mucopolysaccharide-protein complexes, we have studied the distribution and properties of β-xylosidases in a number of biological sources. Evidence indicates that in one form of the linkage, xylose (as a terminal sugar of a mucopolysaccharide chain) is attached glycosidically to the oxygen of seryl residues of a peptide chain. Using β-*O*-D-xylosyl-L-serine and β-*O*-D-xylosyl-L-threonine (chemically synthesized by M. Higham) as substrates, we find that the digestive juices of *Helix pomatia* are a source of powerful β-xylosidases which promote hydrolysis of both xyloside substrates. The enzyme has the greatest activity between pH 3 and pH 4.

Previous investigations have shown β-xylosidases to be present in emulsin [1], jack beans [2], mammalian tissues [2,3] and in the marine gastropod, *Charonia lampas* [4]. In each case, the enzymes were highly active towards chromogenic model substrates, *o*- and *p*-nitrophenyl-β-D-xylopyranosides [1,5]. Except that from *C. lampas*, none showed activity towards β-xylosides of serine, threonine or some of their related peptides. Purified preparations from *C. lampas*, removed xylose from xylosylserine and from stem bromolein glycoprotein, and were also strongly active towards chromogenic substrates.

Crop juice (or homogenates of digestive gland) taken from starved *Helix pomatia* and dialysed (cellophan) against 0.05 M-sodium acetate buffer (pH 5) for 24 hr at 0°, resulted in active enzyme preparations. Incubation of the enzyme (100 μl) at pH 3.2 (acetate buffer; 300 μl) in the presence of toluene at 37° with the 4mM-xylosylserine (0.8 mM final concentration; 100 μl) resulted in 30% liberation of the amino acid in 24 hr.

Portions of incubation medium were subjected to high voltage electrophoresis and the extent of hydrolysis was estimated colorimetrically from both the xylosylserine or xylosylthreonine remaining (Method A) and from the free serine or threonine produced (Method B) [6]. In comparable experiments with xylosylthreonine, 80% conversion to the amino acid was observed in 24 hr. The enzyme preparation was about 100 times more active towards the chromogenic substrates than towards the xylosylthreonine substrate.

The results of incubations at various pH values are given in table 1.

Table 1
Percentage hydrolysis of β-*O*-D-xylosides by *H. pomatia* enzyme

pH	Time of incubation					
	4.5 hr		9 hr		24 hr	
	Method A (%)	Method B (%)	A (%)	B (%)	A (%)	B (%)
(a) β-*O*-D-xylosyl-L-serine						
3.2	7	8	29	17	41	31
4.0	15	10	26	12	36	31
5.0	0	2	16	11	13	17
6.0	5	0	1	0	4	5
(b) β-*O*-D-xylosyl-L-threonine						
3.2	25	19	40	47	80	72
4.0	20	27	37	47	80	78
5.0	12	14	25	40	63	76
6.0	0	0	18	12	30	33

Investigations of the chemical behaviour of the seryl and threonyl substrates towards acid and alkaline hydrolysis, showed that the compounds possessed considerable stability and were unchanged at the pH conditions prevailing in the enzymic experiments [7]. The chemical properties of other xylosides have been investigated by de Bruyne & Wijnendaele [8].

The *H. pomatia* β-xylosidases offer considerable promise as readily available means of investigation of mucopolysaccharide-protein complexes.

References

[1] D. Fisher, M. Higham, P. W. Kent and P. Pritchard, Biochem. J. 98 (1966) 46P.
[2] D. Fisher, M. W. Whitehouse and P. W. Kent, Nature (London) 213 (1967) 204.
[3] D. Robinson and H. E. Abrahams, Biochim. Biophys. Acta 132 (1967) 212.
[4] M. Fykuda, T. Muramatsu, F. Egami, N. Takahashi and Y.Yasuda, Biochim. Biophys. Acta 159 (1968) 216.
[5] F. G. Lootiens and C. K. de Bruyne, Naturwiss. 51 (1964) 359.
[6] J. Heilmann, J. Barrolier and E.Watzke, Z. Physiol. Chem. 309 (1957) 219.
[7] M. Higham, P. W. Kent and D. Fisher, Biochem. J. 108 (1968) 47P.
[8] C. K. de Bruyne and F. van Wijnendaele, Carbohyd. Res. 6 (1968) 367.