Further work is in progress, and a detailed report will be published later.

H. R. SCHREINER
A. P. RINFRET

Research Laboratory,
Linde Co.,
Division of Union Carbide Corporation,
Tonawanda,
New York.

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Activation of Hurain by Bivalent Iron

HURAIN¹, the active principle of the sap of jabillo trees (*Hura crepitans*) which is responsible for its proteolytic action, apparently represents a proteinase with special chemical properties differing from those so far known for this class of enzymes. During purification and assay of the enzyme, it was found that the enzymatic activity of hurain is greatly affected by metal ions.

Experimental results, to be described in this preliminary communication, have been performed with crude enzyme obtained as a dry, almost white powder from the tree sap by cutting the bark and centrifuging, dialysing and freeze-drying the pooled samples. Proteolytic activity was determined by present techniques²⁻⁵. The enzyme preparation shows certain activity on haemoglobin and egg albumin (Fig. 1), gelatine and Witte's peptone¹, without further additions; maximal activity is reached, however, only when bivalent iron ions are added (Table 1).

The crude hurain preparation was found to act also on a synthetic substrate. Hurain slowly hydrolyses

Fig. 1. Relative activity of crude hurain on haemoglobin —●— and egg albumin —○— without further additions. Method, ref. 2

Table 1. REVERSIBLE ACTIVATION AND INHIBITION OF HURAIN ACTION BY Fe^{2+} , Zn^{2+} AND EDTA. Method, REF. 3

Enzyme (mgm.)	Fe^{2+} (μmoles)	Zn^{2+} (μmoles)	EDTA (μmoles)	Relative activity, $\log \frac{I_0}{I}$, 280 m μ
1.0	—	—	10.0	0.267
	1.0	—	—	0.678
	1.0	—	10.0	0.237
0.25	—	—	—	0.180
	—	10.0	—	0.044
	—	10.0	10.0	0.108
0.50	10.0	—	—	0.720
	10.0	10.0	—	0.573
	10.0	20.0	—	0.351

benzoyl-L-arginine-methylester, and the velocity of hydrolysis is increased by the addition of Fe^{2+} (Fig. 2). It appears that hurain does not act on the corresponding amide. The effect of bivalent iron appears to be specific, since Ca^{2+} , Mg^{2+} , Mn^{2+} and Fe^{3+} had no influence on hurain activity; Zn^{2+} , Cd^{2+} and Ni^{2+} inhibit the enzyme action. The mechanism of activation by bivalent iron is not produced by reduction of S-S to SH-groups of the protein. Hurain is not affected by cysteine or other reducing agents¹, and *p*-chloromercuribenzoate.

Fig. 2. Activity of crude hurain on benzoyl-L-arginine-methylester without —×— and with —●— addition of Fe^{2+} . Method, ref. 4

Apparently, active hurain represents a reversibly dissociating iron complex, in which Fe^{2+} can be displaced by inhibiting ions as Zn^{2+} or by complexing agents such as ethylenediamine tetraacetic acid (EDTA) (Table 1). The bivalent iron is firmly bound by the protein moiety, at least more firmly than the inhibiting ions. An excess of Zn^{2+} is required to displace the iron from its site of binding. Other evidence for the strong binding of Fe^{2+} is shown by the fact that after 48 hr. of dialysis against EDTA the preparation has 2.2 mgm. per cent iron, that is, about 10 per cent of its original iron content (23.5 mgm. per cent), calculated for dry weight. The amount of iron present in the preparation after dialysis of the crude extract is sufficient to activate partly the hurain.

DINAH SHARON DE SEIDL
KARL GAEDE

Department of Biochemistry,
Instituto Venezolano de Investigaciones Científicas,
Caracas/Venezuela.

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