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Note

An isolation of chymopapain*

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An agarose–mercurial column for the purification of papain has recently been described by Sluyterman and Wijdenes[1]. This can be used to isolate chymopapain, which is also found in papaya latex[2].

EXPERIMENTAL

The procedure adopted was that described[1] using columns, 2.5 × 50 cm, of the agarose–mercurial which were previously treated with 5,5′-dithiobis-2-nitrobenzoic acid (Calbiochem, Los Angeles, Calif., U.S.A.). Solutions[1] of 5 g of "Papain powder" (Mann Laboratories, New York, U.S.A.) were applied to the columns. After washing in the usual manner[1] enzyme was eluted as the mercurial compound as shown in Fig. 1. Recycling the enzymes so obtained, after dialysis and concen-

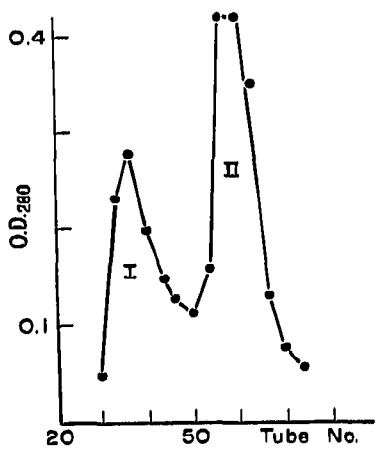

Fig. 1. Elution of papain (Fraction I) and chymopapain (Fraction II) as mercury derivatives from a 2.5 × 50 cm column as described[1]. Enzymatic activity measured with N-carbobenzoxy-L-glycine *p*-nitrophenyl ester in the presence of dithiothreitol.

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tration on a Diaflo apparatus (Amicon, Lexington, Mass., U.S.A.), yielded a single symmetrical elution diagram for each.

RESULTS

Conventional amino acid analysis and N-end group determination[3] showed that Fraction I was composed of papain. Fraction II, isolated in yields of 1 mg/g applied powder, was homogeneous on gel electrophoresis at pH 7, after activation with dithiothreitol and gel filtration on Sephadex G-25 (Pharmacia, Uppsala, Sweden). The enzyme so obtained contained two free sulphydryl groups per molecule, determined by the method of Ellman[4] (*cf.* ref. 5). The specific activity of the enzyme, assayed with N-carbobenzoxy-L-glycine *p*-nitrophenyl ester (Sigma, St. Louis, Mo., U.S.A.) as described for papain[6] was 2.4 $O.D._{410\ nm}$ units $\cdot min^{-1} \cdot \mu mole^{-1}$.

The amino acid composition of Fraction II is reported in Table I, except that analysis following gentle hydrolysis suggested the presence of 1 molecule/mole of an unidentified amino sugar. Comparison of Table I with results for chymopapain[5,7] shows that the material isolated was that enzyme.

TABLE I

AMINO ACID COMPOSITION OF FRACTION II FOLLOWING 22 h HYDROLYSIS IN 6 *N* HCl AT 110°

Tryptophan determinations were by the method of Spies and Chambers[8].

Amino acid	*Number of residues per mole**
Asp	27
Thr	17
Ser	19
Glu	29
Pro	17
Cys	10
Gly	42
Ala	21
Val	25
Met	1
Ile	12
Leu	17
Tyr	20
Phe	9
His	5
Lys	30
Arg	10
Trp	4

* Mol. wt. = 33,000 (ref. 2).

As a further check on the homogeneity of the product discussed here, N-end-group determination[3] revealed a single product, isoleucine. This is in contrast to the results reported by Yasunobu and colleagues (ref. 5 and refs. therein) for either of the forms of chymopapain which they isolated. However, it is identical with the end group of papain, and analogous with that (Leu) of ficin, a related sulphydryl enzyme[2].

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