BBA 63383

The non-essentiality of the N-terminal amino group of papain

Studies concerning the pH dependence of the hydrolysis of a number of specific substrates by papain have shown that on the alkaline side of the pH optimum (pH 6), K_m is governed by a protein group with a pK of 8.5–9.0 (refs. 1, 2). In chymotrypsin a group of similar pK with a similar function has been identified as the α-amino group of N-terminal isoleucine[3]. Therefore it was considered worthwhile to find out whether or not the N-terminal isoleucine is involved in substrate binding in papain, too. It will be shown in this communication that this is not the case.

Guanidination[4] or acetylation[5] of most of the amino groups does not affect the activity of papain, but it was not proved in these experiments that the α-NH_2 was modified.

Therefore, in the present investigation, papain was treated with 1-fluoro-2,4-dinitrobenzene (FDNB) and the product was tested for modification of the N-terminal isoleucine group and for activity.

Crystals (modification C) of 30 mg of mercuri-papain, were suspended in 10 ml of 60% (v/v) of methanol, 15 mM in bicarbonate and 5 mM in FDNB. After storage for one night at 20° the crystals were washed 3 times with 60% (v/v) methanol and brought to the initial volume for assay. Since the modified crystals proved to be insoluble in water, all activities were determined in the crystalline state towards 0.05 M acetylglycine ethyl ester in 20% of Na_2SO_4 (ref. 6). The activity of DNP-papain was found to be the same as that of control crystals.

The remaining portion of DNP-papain was washed with 60% methanol, methanol and ether. The dry preparation (20 mg) was hydrolyzed (16 h in 6 M HCl at 105°). The hydrolysate was taken up in ethanol and analyzed on thin-layer plates (Kieselgel F 254, Merck), run with propanol–ammonia (7:3, v/v) together with reference DNP-compounds. The spots of DNP-isoleucine were scraped off and eluted with ethanol. The absorbance of the eluates was read at 348 nm.

The DNP-isoleucine content was found to be 96 ± 5% of the calculated value. Furthermore 4.5 moles of ε-DNP-lysine and about 3 moles of O-DNP-tyrosine per

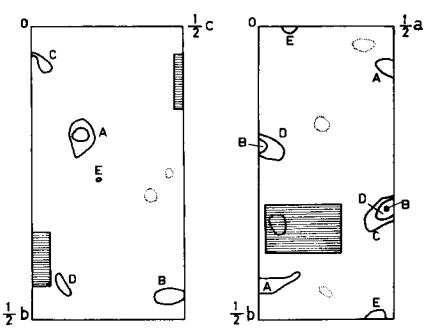

Fig. 1. Difference Fourier maps, the *Okl* and *hkO* projection two-dimensional space group *pgg*, of DNP-papain *versus* papain. Contour lines drawn at a level significantly above the normal background noise. Holes in the projections are surrounded by a dotted line. The shaded area indicates the active center.

mole of protein were estimated in the ether-extracted hydrolysate from the absorbance at 350 and 390 nm in 1 M HCl (*cf.* Table 11–1 of ref. 7).

X-ray patterns of crystals treated in the same way were made and compared with those of unmodified crystals. Two difference Fourier maps, the *Okl* and *hkO* projections, are shown in Fig. 1. Although the electron density peaks do not rise much above the background noise, six of them can be clearly seen. Peaks B, C, D overlap in the *hkO* projection. Peak A, elongated in the *hkO* projection, can most likely be ascribed to two partially overlapping peaks; this overlap also occurs in the other projection, as Peak A here has a rather high value. By correlation of the *y*-coordinates in the two projections, three-dimensional coordinates of the peaks can be calculated, thus determining which amino acid side chains have reacted.

Peak A can be ascribed to reaction with the α-NH_2 group and Lys-137, Peak B with Lys-54, Peak C with Tyr-176, Peak D with Tyr-161, and Peak E with Tyr-183. No reaction with Cys-25 or His-106 in the active site could be seen. Of course, sites of minor reactivity do not show up.

The present data exclude the participation of the α-NH_2 group in the action of papain, which apparently differs from chymotrypsin in this respect. Furthermore a number of other groups are not essential for catalysis either.

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Received December 18th, 1968

Biochim. Biophys. Acta, 171 (1969) 392–393

BBA 63377

Allosteric effects and phlorizin inhibition of intestinal trehalase

Intestinal sucrase (sucrose glucohydrolase)[1,2], and isomaltase (isomaltose glucohydrolase)[3], are activated by Na^+, and at least in the case of sucrase, the kinetic parameters of this Na^+ activation are essentially the same as those of Na^+ activation of sugar transport, although sucrase itself does not participate in sugar transport[4,5]. Sucrase shows an evident cooperative interaction both among substrate sites and among Na^+ sites[6].

Biochim. Biophys. Acta, 178 (1969) 393–396