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Dimeric Structure and Allosteric Properties of Spleen Acid Deoxyribonuclease

Acid DNase from different sources degrades native DNA according to both "single hit" and "double hit" kinetics (Bernardi & Sadron, 1961,1964*a*,*b*). The latter mechanism is associated with the random splitting of one or another of the two strands and is similar to that already known for pancreatic DNase. The "single hit" degradation, the existence of which has been confirmed by MacHattie, Bernardi & Thomas (1963), and by the independent work of Young & Sinsheimer (1965), appears to take place through the splitting of both DNA strands at the same level and at the same time. It has been suggested that this peculiar mechanism is due to the simultaneous action of two catalytic sites located on the same enzyme molecule (Bernardi & Sadron, 1964*a*,*b*; Young & Sinsheimer, 1965), which may be a dimeric protein with two identical subunits (Bernardi & Sadron, 1964*a*).

A first result in agreement with this hypothesis was the finding (Bernardi, Appella and Zito, 1965) of an even number of residues per mole of protein (mol. wt 38,000) for all amino acids present at a low level in hog spleen acid DNase. The results briefly reported here provide now unequivocal evidence for the correctness of the dimer hypothesis.

Hog spleen acid DNase was prepared according to a modification (to be published) of the method of Bernardi & Griffé (1964). The enzyme was reduced, carboxymethylated and digested with crystalline trypsin treated with L-1-tosylamido-2-phenethyl-chloromethyl-ketone to inactivate contaminating chymotrypsin. The digest was mapped according to Katz, Dreyer & Anfinsen (1959). Seventeen to nineteen peptides were shown, as opposed to 32 to 34 arginine + lysine residues found by Bernardi *et al.* (1965) in the supposedly dimeric enzyme (mol. wt 38,000). Arginine, tryptophan and histidine peptides were found to be present in half, or less than half, the number of the respective amino acids in the dimeric protein.

Physical evidence for the dimeric structure of hog spleen acid DNase was provided by the complete dissociation into monomer units which occurs in 8 M-urea–0·1 M-2-mercaptoethanol; and by the partial dissociation which takes place in 4 M-urea–0·05 M-2-mercaptoethanol (Fig. 1). No dissociation into subunits is caused by high dilution (Bernardi, *et al.*, 1965).

A study of the hydrolysis of bis(*p*-nitrophenyl)phosphate, which is a synthetic substrate slowly hydrolysed by the enzyme (Bernardi & Griffé, 1964), provided additional, although indirect, evidence for the oligomeric structure of acid DNase. In fact, plots of the initial velocity of hydrolysis *versus* substrate concentration have a sigmoidal shape, a phenomenon which was not found when using DNA as the substrate (Fig. 2). This result indicates that the system bis(*p*-nitrophenyl)phosphate–acid DNase exhibits a co-operative type of substrate–enzyme interaction (Monod, Wyman & Changeux, 1965); this has not been reported so far for any monomeric enzyme.

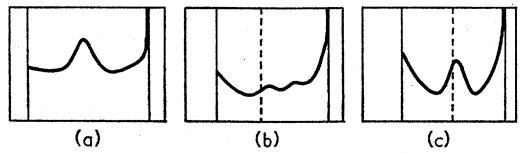

FIG. 1. Sedimentation patterns of hog spleen acid DNase centrifuged at 59,780 rev./min in a Spinco model E apparatus.

(a) In 0·15 M-acetate buffer pH 5·0 + 0·01 M-EDTA (after 122 min). (b) In acetate–EDTA + 4 M-urea–0·05 M-2-mercaptoethanol (after 76 min). (c) In acetate–EDTA + 8 M-urea–0·1 M-2-mercaptoethanol (after 93 min).

Experiment (a) was carried out in a conventional cell; experiments (b) and (c) in synthetic boundary cells; the position of the starting boundary is indicated by the broken line. The sedimentation coefficients of the enzyme in acetate–EDTA and of the fast component in 4 M-urea were close to 2·8 S (c=1%); those of the enzyme in 8 M-urea and of the slow component in 4 M-urea were close to 0·8 S.

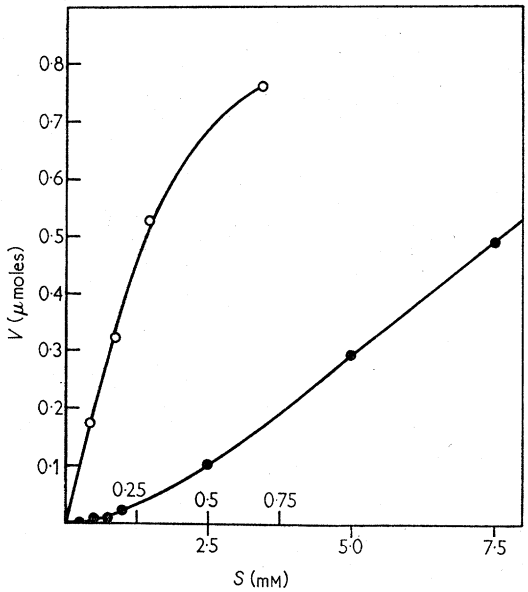

FIG. 2. Velocity, V, of hydrolysis at 37°C of DNA (open circles) and bis(p-nitrophenyl)phosphate (closed circles) by hog spleen acid DNase at different substrate (S) concentrations.

V is given in μmoles of acid-soluble nucleotide phosphorus liberated in 15 min (DNA) or of p-nitrophenol liberated in 120 min (synthetic substrate). The enzyme concentration used in the DNase assay was about 100 times lower than that in the phosphodiesterase assay. Substrate concentrations higher than those shown in the Figure were not used because of substrate inhibition in the case of DNA, or low solubility in the case of bis(p-nitrophenyl)phosphate.

It appears, therefore, that the "single hit" mechanism of action shown by acid DNase can be explained by the simultaneous attack of opposite bonds located at the same level on the DNA duplex by the active sites of the dimeric enzyme. An important implication of this conclusion and of the antiparallel arrangement of the DNA strands is that the two enzyme subunits are antiparallel. The observation that acid DNase is competitively inhibited by antiparallel, but not by coparallel, double-stranded polyribonucleotides (Bernardi, 1964; and work to be published) lends additional support to this prediction, which is in agreement with the model postulated by Monod *et al.* (1965).

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