Stability of Pancreatic Deoxyribonuclease in the Presence of Proteases

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Bovine pancreatic deoxyribonuclease was found to be remarkably stable in the presence of relatively high concentrations of bovine plasmin, while comparable or much lower fibrinolytic levels of chymotrypsin, trypsin, or plant proteolytic enzymes gave rapid and extensive destruction of the nuclease activity. The results are considered to illustrate a sharp divergence in substrate preference between plasmin and the other proteases.

In the evaluation of a mixture of bovine pancreatic deoxyribonuclease and bovine plasmin[1] (hereafter referred to as DNase-P) for use as a debriding preparation, the possibility that the plasmin could inactivate the deoxyribonuclease was a matter of great concern since a number of proteins may serve as substrates for plasmin (1). As no pertinent information was available in the literature, it was necessary to design a study bearing on the question; the study included comparisons with other proteolytic enzymes, particularly chymotrypsin, which was considered as an alternative proteolytic constituent of the mixture. The results are worthy of note in illustrating unexpected degrees of substrate preferences by the two proteolytic enzymes.

MATERIALS AND METHODS

Enzymes.—Vials of DNase-P were obtained from production lots. Bovine plasmin was prepared as described by Loomis, *et al.* (2), and the deoxyribonuclease was the pharmaceutical grade material supplied by Worthington Biochemical Corporation. Pharmaceutical grade α-chymotrypsin and crystalline trypsin were also obtained from the latter source.[2] Bromelin concentrate was obtained from Takamine Laboratory, ficin concentrate from Merck and Co., and papain N.F. VIII from Difco Laboratories.

Enzyme Assays.—Previously described methods were used for the determination of fibrinolytic (3), caseinolytic (4), and deoxyribonuclease (5) activities. In the case of the latter activity, results are expressed for the present purposes as the numerical decrease of relative viscosity units in the 10-minute period from the initial relative viscosity of 4.0. All solutions and dilutions of the plant enzymes contained 0.025*M* cysteine except for the final dilutions for deoxyribonuclease assay. Exposure to cysteine alone was without effect on the deoxyribonuclease.

Stability Studies.—As indicated for clinical use, vials of DNase-P were reconstituted with 10 ml. of 0.9% NaCl. Corresponding mixtures were made with the same concentration of deoxyribonuclease, and the same or different concentrations of other proteolytic enzymes as established in each case by fibrinolytic assays. Solutions were prepared at 0° and then incubated in a 37° water bath. The pH of all solutions was adjusted to 7.0, which was that of the DNase-P. At intervals during incubation identical dilutions of the various mixtures were assayed for residual deoxyribonuclease and fibrinolytic activities.

RESULTS AND DISCUSSION

As shown in Table I the level of plasmin contained in DNase-P had no effect on the deoxyribonuclease in 4 hours; the same fall in viscosity was obtained if aliquots were assayed immediately after solution of the mixture at 0°. Significant destruction of the deoxyribonuclease activity was found only when the same level of deoxyribonuclease was incubated with 8× the DNase-P level of plasmin for 4 hours. In contrast, 0.1 as much fibrinolytic activity in the form of chymotrypsin completely destroyed the deoxyribonuclease in 1 hour; even the 0.01 level of chymotrypsin gave greater loss of deoxyribonuclease in 1 hour than the 8× level of plasmin did in 4 hours.

TABLE I.—PROTEASE EFFECT ON DEOXYRIBONUCLEASE

Enzyme[a]	Initial Fibrinolytic Level	Incubation, Hr.	Deoxyribonuclease Activity Δ Vr[b]
Plasmin (DNase-P)	1.0	1	2.8
"	"	2	2.7
"	"	4	2.8
Plasmin	3.0	2	2.7
"	"	4	2.6
Plasmin	8.0	2	2.3
"	"	4	2.0
Chymotrypsin	1.0	1	0
"	0.1	1	0
"	0.01	1	1.2
Trypsin	1.0	1	0
Ficin	1.0	3	0
Bromelin	1.0	3	0
Papain	1.0	3	1.6

[a] In terms of 280 mμ absorbance, concentrations at the 1.0 level were as follows: plasmin, 6.6; chymotrypsin, 0.087; trypsin, 0.067; ficin, 4.3; bromelin, 2.1; papain, 2.7. [b] Fall in relative viscosity units in 10 minutes from the initial value of 4.0.

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[1] Marketed as Elase by Parke, Davis and Co.

[2] According to the manufacturer, pharmaceutical grade deoxyribonuclease has 70 to 90% of the activity of crystalline material. The pharmaceutical grade α-chymotrypsin has 90 to 100% of the activity of crystalline material, and trypsin contamination is "considerably" lower than one per cent.

Because plasmin is a more impure preparation than the chymotrypsin, the possibility was considered that inert proteins associated with the plasmin might protect the deoxyribonuclease from proteolytic attack. However, an equivalent amount of chymotrypsin added to the DNase-P mixture again completely destroyed the deoxyribonuclease in 1 hour. According to Feinstein and Hagen (6), ovalbumin had a remarkable effect in stabilizing deoxyribonuclease activity in crude pancreatic extracts; therefore, one per cent ovalbumin was added to a chymotrypsin-deoxyribonuclease mixture corresponding to the DNase-P formulation. The deoxyribonuclease was completely destroyed in 1 hour, indicating that the situation must be much different with the purified enzymes since this is even a higher level of inert protein than found in the DNase-P solution (about 0.6%). In this connection it might be noted that it is claimed in a recent patent (7) that solutions of crystalline deoxyribonuclease lost all activity in 3 hours at 31°. The deoxyribonuclease used in the present study showed no loss of potency in 4 hours at 37° in the presence or absence of plasmin, so that either the preparations or conditions used are not comparable in this respect. It is also now obvious that the possibility of chymotrypsin contamination must be ruled out in studies of the stability of any pancreatic deoxyribonuclease preparation.

By comparing the activities of bovine plasmin and chymotrypsin under optimum conditions of the fibrinolytic and caseinolytic assays, it was concluded that chymotrypsin "preferred" casein over fibrin as a substrate by a factor of about 3.5. In view of the above results it is apparent that the disparity may be orders of magnitude greater with respect to deoxyribonuclease (or perhaps some peptide bond related to its active center) as a substrate.

Under the conditions used for these stability studies plasmin was fairly unstable, presumably as a result of autodigestion in the absence of substrate. At the DNase-P level about 50% of the fibrinolytic activity remained after 1 hour and about 30% after 2 hours; in the case of chymotrypsin there was no significant decrease in this time. From the product processing point of view, therefore, it was possible to compensate for plasmin autodigestion by addition of excess plasmin without endangering the deoxyribonuclease activity; on the other hand, the amount of excess deoxyribonuclease required to compensate for its unexpectedly greater destruction by chymotrypsin eliminated consideration of such a mixture. The possibility of destruction of tissue deoxyribonuclease at the point of application by chymotrypsin and other proteolytic enzymes used in debriding preparations may have clinical implications, and it is hoped that information bearing on this question may be obtained in the future.

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Variation of pKa'-Values of Tetracyclines in Dimethylformamide-Water Solvents

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The variation of pKa'-values for several tetracycline antibiotics with per cent dimethylformamide (DMF) in per cent DMF-per cent H_2O solvents (v/v) is given. Quantitative expressions for the pKa' dependence on per cent DMF for the various functional groups have been obtained. This information is utilized in the assignment of pKa'-values to functional groups.

THE DISSOCIATION CONSTANTS or pKa'-values of many of the tetracycline antibiotics have been determined in various nonaqueous-water solvents because of the difficulties of solubilization and maintenance of homogeneous solutions over the entire titratable range for one solvent alone (1–4). A frequently used solvent mixture has been dimethylformamide-water.

In addition, a traditional routine procedure has been to titrate a substance in a nonaqueous-water solvent with an aqueous titrant giving an apparent pKa' at half-neutralization difficult to compare with the results from other routine titrations, since amounts of titer may vary and the relation of pKa' with per cent nonaqueous solvent has not been established.

This communication presents information on the variation of pKa'-values for several tetracycline antibiotics, presents quantitative expressions for their dependence on per cent dimethylformamide (DMF) in %DMF–%H_2O solvents, and attempts to demonstrate their potential usefulness in deducing pKa' assignments to functional groups.

EXPERIMENTAL

The titrations of tetracycline free base and benzenesulfonyltetracyclinonitrile were conducted on 200 mg. samples in 20–50 ml. of solvents of varying %DMF–%H_2O composition at 25° with

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