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EVIDENCE FOR DIFFERENT SPECIFICITIES OF DNASE I AND DNASE II*

by

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Up till now the conclusion that the desoxyribonuclease from pancreas (DNase I) is different from the DNase II from thymus was based on differences in the optimum pH and thermolability of the two enzymes, their behaviour toward magnesium and citrate[1,2,3,4], and toward a naturally occurring inhibitor[4,5,6,7]. A partial purification of the DNase II from thymus[8] established that the two enzymes have also different characteristics of solubility and stability, and offered a new opportunity to compare the two enzymes in respect to the products formed. While only very few of these products have been identified so far, they were strikingly different for the two enzymes. Therefore the conclusion that the DNase I and the DNase II are non-identical is now supported by apparent differences in their specificity.

Desoxyribonucleic acid (DNA) was prepared according to DOUNCE and co-workers[9]. Once crystallized DNase I*** was prepared according to KUNITZ[10], and partially purified DNase II was prepared according to the previously described method[8]. The digests were made using 100 or 200 mg DNA in 5 or 10 ml, at 37°. In studies of DNase II, 2 to 4 mg of the lyophilized enzyme were used in 0.1 *M* acetate buffer pH 5.0 without Mg; incubation time 4–8 hours. In case of DNase I either 0.2 *M* borate buffer pH 7 or 0.1 *M* acetate buffer pH 6 were used; in either case Mg was added to attain 0.025 *M*, 5–10 mg of enzyme was used and the incubation time was 8–16 hours. Invariably (with both enzymes) toward the end of the digestion period when the viscosity of the solution decreased a white precipitate became apparent. This precipitate was centrifuged down and discarded.

Fig. 1 represents a chromatogram obtained according to the method of MARKHAM AND SMITH[11] using 5 mg quantities of the digests. Among the products of DNase II digestion, adenylic acid and guanylic acid were identified by their spectra. Among the digestion products of DNase I thymidylic and cytidylic acids were identified. Similar experiments were then repeated on a preparative scale using 100 mg quantities of the digests. The bands thus obtained were then either rechromatographed according to MARKHAM AND SMITH[11], CARTER[12], or HOTCHKISS[13] (the latter to exclude nucleosides), or ionophorized according to MARKHAM AND SMITH[11]. In addition to the previously identified nucleotides thymidylic acid was found in the digest of the DNase II.

In the next series of experiments, 200 mg quantities of digests were fractionated on columns of Dowex 1, 2% cross linkage, according to a slightly modified method of SINSHEIMER[14]. We are greatly indebted to Dr. R. L. SINSHEIMER for letting us read his manuscript prior to publication. In order to obtain salt-free fractions, SINSHEIMER's sodium acetate buffer pH 5.5 was substituted by ammonium formate buffer pH 4.5, which sublimated *in vacuo*; formic acid of indicated molarity was titrated with ammonia to pH 4.5 (glass electrode). Under these conditions a fair resolution of mononucleotides and dinucleotides was obtained in the first run. Only initial parts of elution patterns are reproduced in Fig. 2. The patterns of the digests by the two DNases were strikingly different. In agreement with the previous data[15,16,17], only a small portion of the DNase I digest was represented

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by mononucleotides, a fairly large portion appeared as di- and trinucleotides[14] and all of the digest was eluted with 2 *M* buffer[14] (not reproduced). In the case of DNase II digest the mononucleotide fraction was somewhat larger (purine mononucleotides predominated), the di- and trinucleotides fraction was considerably smaller, and 3 *M* buffer was required to complete the elution.

From the digest of DNase I peak 3 (cytidylo-cytidylic acid), peak 4 (a dinucleotide of cytidylic and thymidylic acids), and peak 5 (a dinucleotide of cytidylic and adenylic acids), were rechromatographed, lyophilized, and digested each with 100 γ of DNase II for 60 minutes. They were then rechromatographed under the identical conditions. The unchanged dinucleotides were quantitatively recovered. In view of this experiment it appears that the preparation of DNase II was free from a contamination by the non-specific phosphodiesterase.

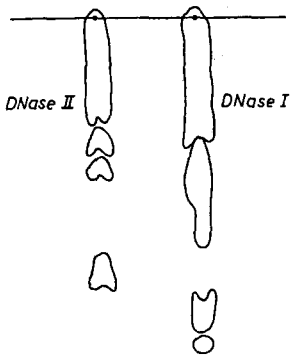

Fig. 1. Descending paper chromatogram according to MARKHAM AND SMITH[11]. 5 mg of digests, *iso*propyl alcohol 7/water 3 in ammonia atmosphere, 60 hours. Low spots in DNase I digest were identified as pyrimidine nucleotides; low spots in DNase II digest as purine nucleotides.

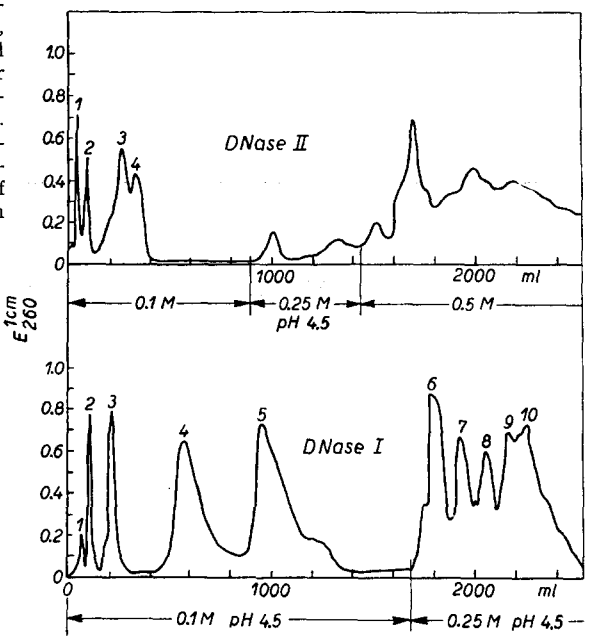

Fig. 2. Initial parts of elution patterns from the Dowex 1-X2 columns according to the method of SINSHEIMER[14] on 200 mg samples of digests. DNase I digest: peak 1 – cytidylic acid, peak 2 – thymidylic acid, peak 3 – cytidylo-cytidylic acid, peak 4 – dinucleotide of cytidylic and thymidylic acids, peak 5 – dinucleotide of cytidylic and adenylic acids; DNase II digest: peak 1 – cytidylic acid, peak 2 – thymidylic acid, peak 3 – adenylic acid, peak 4 – guanylic acid.

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