DEOXYRIBONUCLEASE II FROM AVIAN LEUKEMIC MYELOBLASTS. I. ISOLATION AND BASIC ENZYMOLOGICAL CHARACTERIZATION

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A protein with properties of DNase I* was isolated from chick leukemic myeloblasts by fractionation with ammonium sulfate and by chromatography on DEAE-cellulose and hydroxyapatite. By comparing the properties of myeloblastic DNase II with those of classical DNase II from hog spleen, it was possible to establish the identity of relationships of enzymic activities on the concentration of univalent ions during the first and second phases of substrate cleavage, as well as on the concentration of divalent ions. Differences were found in pH optima which, moreover, are shifted with the myeloblastic enzyme by changing the concentration of univalent ions. Further features showed that the avian myeloblastic enzyme is not an SH-protein, in common with the spleen DNase II; the mechanism of substrate cleavage is diplotomic and the temperature optimum lies at 50°C.

Living systems are known to contain a number of enzymes capable of catalyzing hydrolytic cleavage of nucleic acids. One of the nucleases, splitting DNA and found only in animal tissues[1-5] is DNase II. Its detailed characterization was possible on the basis of studies of the enzyme isolated from hog spleen[6]. Data on the activity in tumour-altered cells which display a permanent genetically fixed stimulation of DNA synthesis[7] and hence also a change of the equilibrium between anabolic and catabolic pathways in favour of DNA synthesis, are only infrequent[8]. The present communication deals with the isolation and characterization of DNase II from avian leukemic myeloblasts which represent cells transformed neoplastically by the oncogenic RNA virus of BAI strain A (AMV).

* Abbreviations used: A_{260}, absorbance of sample at 260 nm; AMV, avian myeloblastosis virus; DEAE-cellulose, O-(diethylaminoethyl)cellulose; DNA, deoxyribonucleic acid; DNase, deoxyribonuclease; EDTA, ethylenediaminetetraacetic acid; PCMB, *p*-chloromercuribenzoate; RNA, ribonucleic acid.

EXPERIMENTAL

Isolation of leukemic myeloblasts. Leghorn white chicks, susceptible[9] to the avian myeloblastosis virus (BAI strain A)[10-12] aged 24—30 h, were injected intravenously into the tibial vein with 0·1 ml fresh blood plasma obtained from chicks with a fully developed leukemic process. The chicks were maintained under standard conditions at 27—30°C (see[9]).

The starting material was fresh heparinized blood of chicks 2—3 weeks after the injection, with fully developed myeloblastosis (20—40% v/v myeloblasts)[9]. The myeloblasts were separated from erythrocytes by centrifugation (100 *g*, 15 min) of blood layered over an equal volume of 0·88M sucrose[13]. The upper layer containing a suspension of myeloblasts in plasma was drawn off by suction from the lower sucrose layer containing erythrocytes, and centrifuged again (1 700 *g*, 15 min). The myeloblast sediment was purified by suspending it in an isotonic solution of sodium chloride and by recentrifugation under identical conditions. The entire procedure was carried out at 0—4°C. The resulting sediment was formed by intact myeloblasts only minutely contaminated with red blood cells. For isolating the enzyme, myeloblasts were frozen and maintained under dry ice (for about 2 months) were used.

The isolation of DNase II from the myeloblasts was carried out according to Bernardi[14], using the fractionation procedure without the pH 2·5 step, combined with chromatographic isolation according to Cordonier[3]. The final preparation was freeze-dried and maintained at —17°C. The enzyme activity was stable for at least 7 months. The DEAE-cellulose column for chromatography was prepared according to the Whatman Laboratory Manual. Hydroxyapatite was prepared according to Tiselius[15].

Hog spleen DNase II was isolated by extraction and fractionation with ammonium sulfate according to Bernardi's method I (see[14]). The purified enzyme preparation obtained was freeze-dried and maintained at —17°C.

Isolation of DNA. To determine the activity of DNase II by spectrophotometry we used DNA isolated from calf thymus[16]. The purity of the preparation was determined spectrophotometrically ($A_{230} : A_{260} : A_{280} = 0{\cdot}457 : 1 : 0{\cdot}56$) and from the P/N ratio which was equal to 1·7 (see[17,18]). Contamination with RNA was 2% on the basis of the deoxyribose and ribose ratios determined colorimetrically[19]. The amount of DNA used during the individual determinations was estimated spectrophotometrically[20]. After isolation, the DNA was stored at a final concentration of 5 OD_{260} units per 1 ml 0·015M sodium chloride and 0·0015M sodium citrate, pH 7, with several drops of chloroform, at 4°C.

Labelled DNA was isolated[21] from *E.coli* 13 T^-, grown in a medium with thymine-C^3H_3 of specific activity equal to 10 Ci/mmol. The bacteria were grown after inoculation of a culture growing in Spizizen's medium[22] into a SYB medium[23] containing 1 mCi thymine-C^3H_3 and 2 mg nonlabelled thymine in 1 liter medium, at 37°C. Growth was terminated at a cell count of $5 \cdot 10^8 - 10^9$ cells/ml by cooling in ice. Isolated labelled DNA was frozen at a concentration of 0·25 mg/ml 0·015M sodium chloride and 0·0015M sodium citrate, pH 7, in a bath of dry ice with ethanol, and stored under dry ice.

The sedimentation coefficient, $S_{20,w}$, of DNA dissolved in 0·015M sodium chloride and 0·0015M sodium citrate, pH 7, was determined in a Spinco Model E ultracentrifuge at a concentration of 15 μg/ml at a rate of 42 040 r.p.m., by using UV optics. The sedimentation coefficient was 20·1. Molecular weight was determined on the basis of the empirical formula[24] $S_{20,w} = 0{\cdot}063\ M_W^{0.37}$ and was found to be $5{\cdot}632 \cdot 10^6$.

The activity of the enzyme in viscometric units was determined by a flow method using Ostwald's viscometer in a water bath of 40°C. The time of flow of 4 ml basic substrate, formed by a solution of 0·02% DNA from calf thymus in 0·05M potassium acetate–0·01M EDTA, pH 5, was 70·4 s, that of water 53 s. The viscometric unit was selected arbitrarily as such enzyme activity as will

cause the value of log η_{sp} to drop by 0·1 in 150 s. The determination of activity in viscometric units was done on the basis of a graphic plot of the dependence of log η_{sp} on the duration of the enzyme reaction.

Spectrophotometry of DNase II activity was done in defined units[1]. Determination of DNase II activity according to the number of cleavage products of labelled DNA not precipitable with acid in the cold (isotopic method) was done in principle according to Kates[2] and the reaction mixture in our case contained in a total volume of 350 µl: 12·5 µg labelled DNA, 3·5 µmol potassium acetate and 3·5 µmol EDTA. The reaction was stopped by cooling in ice and by precipitation[2]. After centrifugation, an equivalent of the supernatant (0·8 ml) was used for the determination of radioactivity. A unit was defined as such an amount of enzyme as will decompose in 10 min 1 µg labelled DNA under the given conditions. When measuring the enzyme activity of fractions during chromatographic purification of myeloblastic DNase II, the substrate used was labelled DNA which produced 10 600 c.p.m. in the supernatant when fully decomposed enzymatically under the present conditions. The specific activity was then defined as the number of units of activity of DNase II in mg protein of enzymatically active preparation[25].

The radioactivity of samples was determined in a liquid scintillation spectrometer Tri-Carb (Packard Instrument Co., model 3375). Measurement was done in glass vials with 10 ml scintillation solution[26]. The mean efficiency of detection of ^3H disintegration in a solution thus prepared, using an external gamma standard, was 7%.

Graphical determination of the K_m constant[27] was done on the basis of dependence of the rate of enzyme reaction estimated isotopically at pH 5 and 6, on the concentration of substrate in the reaction mixture, between 2·5 and 77·5 µg DNA which corresponds to $1{\cdot}28 \cdot 10^{-9}$M – $3{\cdot}96 \cdot 10^{-8}$M-DNA.

RESULTS

Purification of Enzyme

The procedure for purifying the myeloblastic enzyme following from Table I was checked by estimating the specific activity of the preparation. The supernatant of the myeloblast homogenate after fractionation with ammonium sulfate yielded a prepara-

TABLE I

Purification of Myeloblastic DNase II

The enzyme activity was determined by the isotopic method at pH 5.

Fraction	Weight	Enzyme units	Specific activity
Starting material	49·0 g	—	17·8[a]
Precipitate with $(NH_4)_2SO_4$	927·8 mg	103 171	111·2
DEAE-cellulose	266·0 mg	185 383[b]	696·8
Hydroxyapatite	70·5 mg	165 400	2 720·0

[a] Determined after sonication of isolated chick leukemic myeloblasts. [b] See ref.[14].

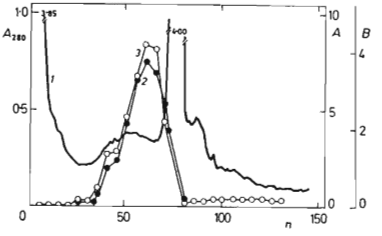

FIG. 1

Elution Profile of Myeloblastic DNase II from a DEAE-cellulose Column

1 Absorbance of fractions at 280 nm; *2* activity of enzyme *A* (enzyme units determined isotopically in 5 µl of fraction); *3* specific activity B (. 10^3); *n* number of fraction. 927·8 mg enzyme extract in 60 ml 0·005M potassium phosphate, pH 8, specific activity 111·2 placed on a 2 × 20 cm DEAE-cellulose column. A pH and molarity gradient (limiting buffers, 500 ml of 0·005M phosphate, pH 8, and 500 ml of 0·15M acetate, pH 5, respectively) was then applied to elute the proteins, which had been retained by the column, in a cold room. Rate of flow 25 ml/h, fractions taken after 15 min.

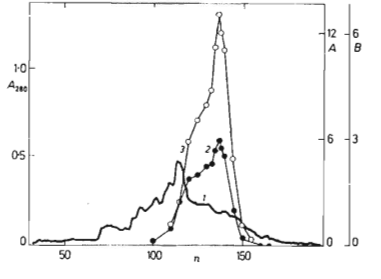

FIG. 2

Elution Profile of Myeloblastic DNase II from a Column of Hydroxyapatite

Designation as in Fig. 1. Combined fractions 35—77 from a DEAE-cellulose column of 273 ml volume were dialyzed for 16 h against water and then 24 h against 0·05M potassium phosphate, pH 6·8. After placing on a 1·5 × 60 cm column it was eluted with 0·05M potassium phosphate, pH 6·8 (fractions 1—69) at a rate of flow of 25 ml/h. Fractions taken at 15 min intervals. From fraction 70 on, elution was done using a linear molarity gradient of phosphate buffer, pH 6·8 (0·05—0·5M) of a total volume of 1 000 ml. Rate of flow 35 ml/h, fractions at 15 min intervals.

tion of DNase with specific activity of 111 enzymatic units. At this stage, the specific activity was increased by more than 6-fold, as follows from a comparison of the attained specific activity and that of DNase II in a sonicate of chick leukemic myeloblasts. Further increase of specific activity was gradually achieved by chromatography of the enzyme on a column of DEAE-cellulose and hydroxyapatite. The enzyme with DNase II properties was liberated from the DEAE-cellulose column (Fig. 1) in a range of molarities of the elution solution between 0·036M and 0·088M. At this stage of purification the specific activity appeared as a 38-fold increase. Further increase of specific activity was achieved by chromatography of the DEAE-cellulose preparation of DNase II on hydroxyapatite (Fig. 2). At this stage of purification the specific activity of the enzyme was increased by as much as 7-fold. The mean specific activity of combined fractions was comparable with that of the preparation of spleen DNase II obtained by fractionation with ammonium sulfate.

The enzyme activity of DNase II was evaluated in the second phase of substrate cleavage[6] by determining the amount of labelled or nonlabelled products of enzymic cleavage, soluble in cold perchloric acid. In the case of the preparation of myeloblastic DNase II it was found in contrast with literature data[2], that the enzyme activity may be followed even during the first stage of substrate cleavage with DNase II[6]. In this case, the measurement must be done in the range of enzyme concentrations in the reaction mixture between 0·18 and 2·5 viscometric units when the relationship

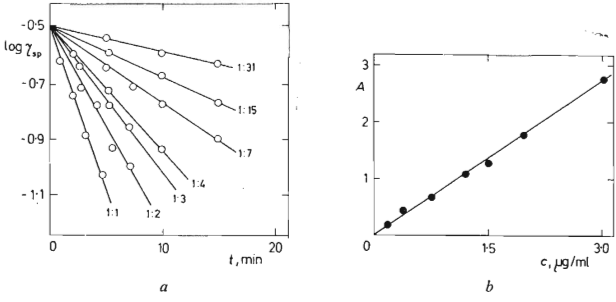

FIG. 3

Decrease of Viscosity during Cleavage of DNA with DNase II (*a*) and Its Dependence on Enzyme Concentration (*b*)

a The reaction mixture contained the basic substrate and 5 µl solution of DNase II at the dilution shown, the stock solution containing 4 µg protein/5 µl. *b* Data taken from Fig. 3*a*; *A* viscometric units.

between enzyme concentration and reaction rate of substrate cleavage was found to be linear (Fig. 3*a*,*b*).

Enzymological Properties

The study of the dependence of enzyme activity of myeloblastic DNase II on the medium pH measured viscometrically showed that the enzyme has a pH optimum at 6 in contrast with the spleen DNase II which, under the same conditions of substrate cleavage, showed highest activity at pH 5 (Fig. 4). The value of the pH optimum for the myeloblastic DNase was supported by determining the enzyme activity isotopically, *i.e.* by following the enzyme activity during the second phase of substrate cleavage[6].

The enzyme properties of the myeloblastic DNase II were further studied from the point of view of changes of concentration of univalent ions at pH 5 and 6 and compared with the properties of spleen DNase II exposed to the same experimental conditions. It was found that the rate of hydrolytic cleavage by myeloblastic DNase II under the conditions of its optimal pH (6) decreased with increasing concentrations of sodium chloride. The spleen DNase II under its optimal pH conditions (5) increased in activity with rising concentrations of sodium chloride in the medium with a maximum at 0·1M-NaCl. At pH 5· *i.e.* under conditions where the myeloblastic enzyme shows only 36% of its maximum activity, it is influenced by rising concentrations of sodium chloride, similarly to the spleen enzyme under its optimum pH conditions (pH 5; Fig. 5). The relationships were confirmed by spectrophotometric determinaion of the reaction rate of cleavage by DNase II in a medium containing different concentrations of acetic acid. It was found here, too, that at lower

TABLE II

Dependence of the Reaction Rate of DNase II during the 2nd Phase of Cleavage on Buffer Concentration in the Reaction Mixture

The reaction mixture was supplemented with myeloblastic DNase II (2 μg) or with spleen DNase II (4·4 μg). Activity *A* was expressed in spectrophotometric units.

Buffer concentration, M	Ion	Myeloblastic enzyme		Spleen enzyme	
		pH 5	pH 6	pH 5	pH 6
0·15	Na	0·12	0·0013	0·053	0·0013
	K	0·079	0·0093	0·048	0·0037
0·05	Na	0·052	0·046	0·052	0·0051
	K	0·051	0·066	0·054	0·0073

concentrations of potassium acetate (0·05M) the myeloblastic enzyme showed maximum activity at pH 6, the higher concentration (0·15M) at this pH suppressing the activity of the enzyme while at the lower pH 5 it stimulated it. The above relationship between the pH optimum of enzyme activity and the concentration of univalent ions was confirmed by results obtained on using different concentrations of potassium and sodium acetate (Table II). The fact that the observed relationships followed from a viscometric and spectrophotometric determination of activity indicates that the effect of univalent ions influenced by the pH of the medium, is reflected both during the initial and during the middle phases of DNA cleavage by myeloblastic and spleen DNase II.

It is known that low concentrations of magnesium ions may bring about as much as a 200% activation of DNase II[28-30]. Hence we compared the dependence of the

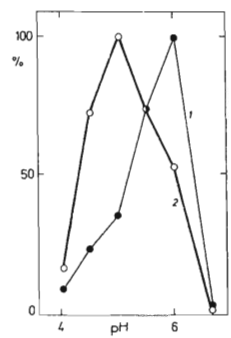

FIG. 4

Dependence of the Rate of DNase II Reaction on pH

1 Myeloblastic enzyme, *2* spleen enzyme. The reaction mixture contained 0·02% DNA in 0·05M-CH_3COOK of the given pH, and 0·01M EDTA. After addition of the enzyme (9 μg myeloblastic DNase II or 2·2 μg spleen DNase II) the rate of the enzyme reaction was measured viscometrically at 40°C. The values of the reaction rates for individual pH values are expressed in per cent of the pH optimum.

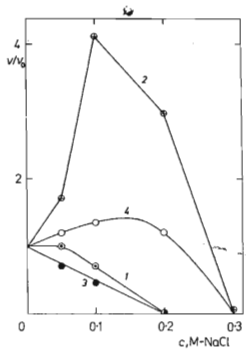

FIG. 5

Dependence of the Rate of DNase II Reaction on the Concentration of Univalent Ions

The rate of enzyme cleavage by myeloblastic DNase II: *1* pH 6, *2* pH 5; by spleen DNase II: *3* pH 6; *4* pH 5. The reaction mixture contained 0·02% DNA in 0·01M-CH_3COOK of the given pH, and 0·01M EDTA. NaCl was added to the final concentrations shown. After addition of the enzyme (1 μg myeloblastic DNase II or 2·2 μg spleen DNase II) the measurement of the rate of the enzyme reaction was done viscometrically (v). The rate without NaCl is shown as v_0.

two activities on magnesium concentration. It follows from Fig. 6 that both DNase II showed marked differences in their activation by magnesium ions. These differences, similarly as in the case of the effect of univalent ions, were related to the pH of the medium. It was found that myeloblastic DNase II in the presence of 0·01M magnesium ions, in contrast with spleen DNase II, showed a marked decrease of activity in the region of its pH optimum (*i.e.* pH 6) while spleen DNase II showed a 20% activation at its pH optimum (*i.e.* pH 5). This enzyme showed a pronounced drop of activity at the pH optimum of myeloblastic DNase while myeloblastic DNase II displayed a clear activation outside the region of its pH optimum (by 4-fold at 0·0125M magnesium). A comparison of the activation properties of magnesium cations at higher ionic strengths (0·1M sodium chloride) showed (Table III) that the optimal effect of magnesium ions on both enzymes requires univalent ions only at the concentration found in the reaction medium and that further increase of concentration of univalent ions may fully eliminate the activating effect of magnesium ions which

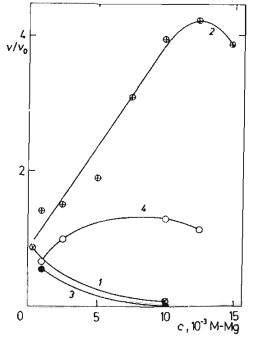

FIG. 6

Dependence of the Rate of DNase II Reaction on Magnesium Concentration

Designation as in Fig. 5. The reaction mixture contained 0·03% DNA in 0·01M-CH_3COOK. $MgCl_2$ was added up to the final concentrations shown. After adding the enzyme (1 μg myeloblastic DNase II or 2·2 μg spleen DNase II) the rate of the enzyme reaction was measured viscometrically (v). The rate without $MgCl_2$ is shown as v_0.

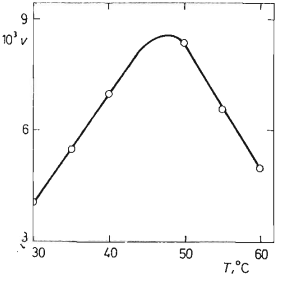

FIG. 7

Dependence of the Rate of DNase II Reaction on the Temperature of the Reaction Mixture

The reaction mixture contained in a total volume of 350 μl: 12·5 μl labelled DNA; 3·5 μmol CH_3COOK of pH 6; 0·3 μg myeloblastic DNase II. The rate of enzyme cleavage of substrate v was measured isotopically and is expressed in counts/min.

occurs at 0·1M sodium chloride, *i.e.* at the concentration when maximum activation of both enzymes by univalent ions takes place.

The enzyme characterized from the point of view of its temperature optimum cleaved the substrate at its maximum rate (during the 2nd phase of reaction) under optimal pH conditions between 47·5 and 50°C (Fig. 7).

It is known that the SH groups are not essential for the function of spleen DNase II[6] or DNase I[31]. In the case of myeloblastic DNase II the problem has been approached by a study of the effect of mercaptoethanol and *p*-chloromercuribenzoate on enzyme activity. As follows from Table IV, the presence of mercaptoethanol, an agent protecting the free SH groups, just as the presence of *p*-chloromercuribenzoate which is a powerful inhibitor of SH groups, did not appreciably affect the activity of myeloblastic DNase II.

A fundamental characteristic of every enzyme preparation is the relationship between the rate of reaction and the concentration of substrate expressed by the K_m value. In the case of DNase II it was necessary to select a suitable concentration range as the dependence between reaction rate and substrate concentration was

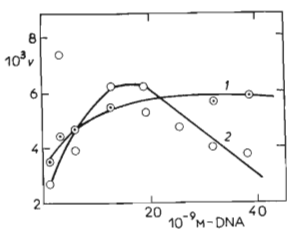

FIG. 8

Dependence of the Rate of DNase II Reaction on Substrate Concentration

Rate of enzyme cleavage: *1* at pH 5; 2 at pH 6. The reaction mixture contained in a volume of 350 µl: labelled DNA; 3·5 µmol CH_3COOK; 0·3 µg myeloblastic DNase II. The reaction mixtures were incubated for 10 min at 37°C and the reaction rate is expressed in counts/min.

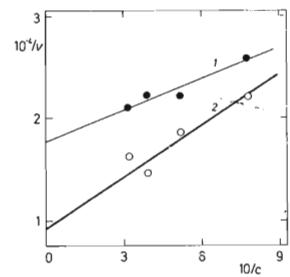

FIG. 9

Dependence of the Rate of DNase II Reaction on Substrate Concentration

The rate of enzyme cleavage of substrate and composition of the reaction mixture are as in Fig. 8.

anomalous above $3{\cdot}2 \cdot 10^{-9}$M (Fig. 8). The K_m was found to be $6{\cdot}1 \cdot 10^{-10}$M at pH 5 and $1{\cdot}9 \cdot 10^{-9}$M at pH 6 (Fig. 9). Hence it follows that at its optimal pH the enzyme showed a lower affinity for its substrate than outside the pH optimum.

One of the most important features distinguishing between DNase II and DNase I from the pancreas is the way the DNA molecule is attacked. It was found that myeloblastic DNase cleaves the substrate diplotomically[6] without lag phase which is characteristic for the mechanism of cleavage by DNase II. Both at pH 6 and at pH 5 the course of diplotomic cleavage by myeloblastic DNase differed from the course of cleavage by DNase I (Fig. 10) where the lag phase at the beginning of cleavage[32] is well defined.

DISCUSSION

The study of the properties of DNase II present in chick leukemic myeloblasts proceeded from a single literature reference[33] and led us to a gradual verification of isolation procedures used in the literature for isolating DNase II that would produce preparations of the highest possible specific activity. Although the preparations of DNase from these cells have the advantage of cytological homogeneity of the material the amount of starting material is rather limited as compared with hog spleen. Moreover, it was found here that during isolation of myeloblastic DNase II one cannot use the step of precipitation with ammonium sulfate which is applied at pH 2·5 since the activity of the myeloblastic enzyme at this pH was irreversibly decreased. Damage to DNase II at low pH might be explained in this case by a deamidation of the enzyme rich in amide groups[6]. From this point of view, myeloblastic DNase II is much more sensitive than the spleen enzyme. The purification procedure used here yielded a preparation which showed in the individual fractions an as much as 366-fold increase of activity as compared with the specific activity of DNase II found in the sonicate of chick leukemic myeloblasts. In fractions combi-

TABLE III

The Effect of Increasing the Mg^{2+} Concentration on DNase II Activity in the Presence of 0·1M-NaCl

The reaction mixture contained 0·02% DNA in 0·05M-CH_3COOK, pH 5; further 0·1M-NaCl, and $MgCl_2$ at the concentrations shown. DNase II activity was expressed in viscometric units.

Concentration of Mg^{2+}, M	0	$5 \cdot 10^{-5}$	10^{-4}	$5 \cdot 10^{-4}$	$5 \cdot 10^{-3}$	10^{-2}	$2 \cdot 10^{-2}$
A	0·377	0·31	0·384	0·384	0·375	0·254	0·286
%	100	83	102	102	100	67	76

ned on the basis of their elution profile a 153-fold increase of activity was observed. In this way, an approximately identical increase of purity was achieved as by fractionating the homogenate of hog spleen with ammonium sulfate. This is in agreement with the decrease of DNase II activity in the course of the leukemic process[33]. In solid tumours, the activity of DNase II was found to rise[3].

The attempt to define in greater detail the enzymological properties of preparation of myeloblastic DNase II led us to using and comparing the spectra of analytical procedures for determination of enzyme activity which would make it possible to follow the individual stages of operation

TABLE IV

The Effect of Mercaptan and of Sulfhydryl Reagent on DNase II Activity

The reaction mixture contained 0·02% DNA in 0·05M-CH_3COOK, pH 5, and 0·1M-NaCl. Myeloblastic DNase II (0·024% solution) was heated for 10 min in the presence of the reagent and the enzyme activity, determined after cooling, was expressed in viscometric units.

Temperature °C	H_2O	$5 . 10^{-4}$M C_2H_5SH	$5 . 10^{-3}$M C_2H_5SH	10^{-5}M PCMB
40	0·565	0·565	0·600	0·555
55	0·565	0·565	0·525	0·525

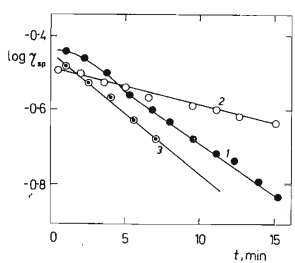

FIG. 10

Cleavage of DNA by DNase I and II

The drop of viscosity of DNA cleaved *1* by DNase I, *2* by myeloblastic DNase at pH 5, *3* by myeloblastic DNase at pH 6. The reaction mixture for DNase I contained 0·02% DNA in 0·01M CH_3COOK, 0·01M veronal, 0·025M-$MgSO_4$, pH 7, 0·023 μg DNase I. For DNase II 0·02% DNA in 0·05M-CH_3COOK, pH 5 and 6, 0·01M EDTA; 1 μg myeloblastic DNase II. The enzyme reaction rate was measured viscometrically at 40°C.

of the isolated enzyme. From the point of view of sensitivity as well as from the point of view of economy defined by the limited amount of material the least suitable method was that of spectrophotometry of the acid-soluble products of DNA cleavage by DNase II (for a single determination one needs 12 μg enzyme protein). Viscometric determination of activity which monitors only the first phase of cleavage is more sensitive than spectrophotometry by two orders of magnitude so that the presence of 0·1 – 2·0 μg enzyme could be detected. The most sensitive of all the methods is the isotopic method where the hydrolysis of substrate could be detected by using as little as 0·05 μg enzyme protein.

The first phase of cleavage, in contrast with cleavage by DNase I shows no lag phase and this is typical of the diplotomic manner of DNA cleavage[2].

Identical properties were also found from the point of view of action of SH-group protectors and inhibitors. Mercaptoethanol and *p*-chloromercuribenzoate showed no effect on DNase II activity which indicates that myeloblastic DNase II, similarly to spleen DNase II, is not an SH-enzyme.

Pronounced differences were found in other enzymic properties. A comparison of enzyme activities in dependence on pH showed a striking difference which was confirmed when studying the effect of univalent and bivalent ions.

The differences were particularly expressed in the activity of the two enzymes at pH 5 and 6 in the presence of magnesium ions, the effect of which on the activity of nucleolytic enzymes represents one of the criteria of classification of animal nucleolytic enzymes[28-30,34]. Even if the enzyme activity of DNase II as one of the enzymes of the 2nd group of nucleolytic enzymes[34] should not depend on the concentration of bivalent cations and although the activity is usually determined in the presence of EDTA[1], the stimulation of its activity by certain low concentrations of bivalent cations has already been established[28-30]. The myeloblastic enzyme is more sensitive to the presence of magnesium ions, displaying, in contrast with the spleen enzyme, a pronounced stimulation by 0·0125M magnesium ions at pH 5. When studying the effect of these ions on enzyme activity of DNase II the possible additional effect, whether positive or negative, of univalent cations on DNase II activation with magnesium was taken into account[29], such an effect being known *e.g.* with ATPase[35]. From a systematic comparison of activation of the myeloblastic enzyme by magnesium ions in the presence of different concentrations of sodium ions one may judge at the degree of dependence of activation by magnesium ions on the concentration of univalent ions in the reaction mixture which may explain the previous contradictory evidence on the activating or inhibiting effect of magnesium ions on DNase II. It seems to be probable that the activation of DNase II by univalent and bivalent ions, in spite of certain similarities, differs from that due to a mere increase of the ionic strength of the medium. This follows from the comparison of ionic strength of 0·1M sodium chloride ($I = 0{\cdot}1$) and 0·0125M magnesium chloride ($I = 0{\cdot}0375$) which bring about an identical, almost four-fold, activation of the myeloblastic enzyme at pH 5 (Fig. 5 and 6).

Myeloblastic DNase II displayed maximum activity between 47·5 and 50°C which

is lower than the optimum for the phosphodiesterase firmly associated with the AMV virion membrane (55°C) [36]. The temperature optimum of the myeloblastic DNase II might account for the previously observed drop of activity of the enzyme in the course of the leukemic process in chicks which is accompanied by a constant decrease of body temperature of the chicks from 41°C to 38°C (ref.[33]).

The dependence of the reaction rate on substrate concentration showed that the affinity of the myeloblastic enzyme for substrate is higher at pH 5 than at pH 6. In this case, however, it was necessary to determine the K_m constants in the optimum concentration range of substrate ($1{\cdot}28 \cdot 10^{-9}$M – $3{\cdot}2 \cdot 10^{-9}$M DNA) when no substrate inhibition of the reaction took place. This phenomenon had been described for DNase II isolated from HeLa cells for DNA concentrations greater than 5μg DNA/350 μl reaction mixture[2]. The value of the K_m constant of this enzyme (4·5 μg) resembles rather closely the K_m found here for myeloblastic DNase II at the optimum pH (6) since $1{\cdot}92 \cdot 10^{-9}$M corresponds to 3·74 μg at this dilution although the question of the true K_m constants, referring to the giant DNA molecule, still remains unanswered[37].

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REFERENCES

1. Bernardi G., Bernardi A., Chersi A.: Biochim. Biophys. Acta *129*, 1 (1966).
2. Kates J. R., McAuslan B. R.: Biochim. Biophys. Acta *132*, 419 (1967).
3. Cordinnier C., Bernardi G.: Can. J. Biochem. *46*, 989 (1968).
4. Lesca P.: Nature *220*, 76 (1968).
5. Rosenbluth R., Shan-Ching Sung: Can. J. Biochem. *47*, 1081 (1969).
6. Bernardi G. in the book: *Advances in Enzymology* (F. F. Nord, Ed.), Vol. 31, p. 1. Wiley, New York 1968.
7. Říman J.: Chem. listy *62*, 1066 (1968).
8. Georgatsos J. G.: Biochim. Biophys. Acta *142*, 128 (1967).
9. Říman J.: Folia Biol. (Prague) *10*, 331 (1964).
10. Eckert E. C., Beard D., Beard J. W.: J. Natl. Cancer Inst. *12*, 447 (1951).
11. Beard J. W.: Ann. N. Y. Acad. Sci. *68*, 473 (1957).
12. Beard J. W.: Progr. Hematol. *3*, 105 (1962).
13. Trávníček M., Vepřek L., Říman J.: Int. J. Cancer *2*, 448 (1967).
14. Bernardi G., Griffe M.: J. Biol. Chem. *3*, 1419 (1964).
15. Tiselius A., Hjerten S., Levin Ö.: Arch. Biochem. Biophys. *65*, 132 (1956).
16. Kay E. R. M., Simmons N. S., Dounce A. L.: J. Am. Chem. Soc. *74*, 1724 (1952).
17. Lowry O. H., Lopez J. A.: J. Biol. Chem. *162*, 421 (1946).
18. Conway E. J., O'Malley E.: Biochem. J. *36*, 655 (1942).
19. Dische Z.: in the book: *The Nucleic Acids* (E. Chargaff, J. E. Davidson, Eds, Vol. 1, p. 285. Academic Press, New York 1955.
20. Sueoka N., Cheng T.: J. Mol. Biol. *4*, 161 (1962).
21. Marmur J.: J. Mol. Biol. *3*, 208 (1961).
22. Spizizen J.: Proc. Natl. Acad. Sci. US *44*, 1072 (1958).

23. Šorm F., Grünberger D.: This Journal *19*, 167 (1954).
24. Doty P., McGill B. B., Rice S. A.: Proc. Natl. Acad. Sci. US *44*, 432 (1958).
25. Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J.: J. Biol. Chem. *193*, 265 (1951).
26. Bray G. A.: Anal. Biochem. *1*, 279 (1960).
27. Lineweaver H., Burk D.: J. Am. Chem. Soc. *56*, 658 (1934).
28. Shack J.: J. Biol. Chem. *226*, 573 (1957).
29. Kurnick N. B., Sandeen G.: Arch. Biochem. Biophys. *85*, 323 (1959).
30. Laskowski M. in the book: *Advances in Enzymology* (F. F. Nord, Ed.), Vol. 29, p. 165. Wiley, New York 1967.
31. Zimmerman S. B., Sandeen G.: Anal. Biochem. *14*, 269 (1966).
32. Young L. T., Sinsheimer R. L.: J. Biol. Chem. *240*, 1274 (1965).
33. Říman J., Seifert J., Korb J.: National Cancer Institute Monograph (J. W. Beard, Ed.) *17*, 747 (1964).
34. Razzell W. E.: Experientia *23*, 321 (1967).
35. Yoshida H., Fujisawa H., Ohi Y.: Can. J. Biochem. *43*, 841 (1965).
36. Trávníček M., Říman J.: Neoplasma *13*, 377 (1966).
37. Dixon M., Webb E. C.: *Enzymes*. Academic Press, New York 1964.

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