Desoxyribonucleic Acids

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The action of desoxyribonuclease (DRNase) from OX-pancreas on desoxyribonucleic acid (DRNA) isolated from calf thymus gland, can be differentiated into two stages[1,2]. An initial depolymerisation (disaggregation) is followed by splitting of the disaggregated nucleic acid into acid-soluble oligonucleotides of varying size down to dinucleotides [1,3,4,5] together with a non-diffusible residue or "core" [1,3,4]. Zamenhof and Chargaff [4] have shown that the quantity of this non-dialysable residue appears to decrease asymptotically with increasing duration of enzyme digestion and dialysis. The dialysis residue is characterised by an increased ratio of purines to pyrimidines and by a greater resistance to further enzymic degradation than the parent nucleic acid, and it was suggested by Chargaff [6] that possibly this finding could be explained by a dissymmetry of the nucleotide sequence in the nucleic acid moelcule.

As part of an extensive programme of investigation of the properties of DRNA's isolated from a variety of sources [7-11] and of their degradation by physical [12], chemical and enzymic [1,13] methods, which our Group has undertaken, some aspects of the enzymic method have been studied. In particular the first stage (disaggregation) of the enzyme action on DRNA from various animal, plant and microbial sources, has been analysed, and also the rate of formation of acid-soluble polynucleotides has been examined. The results obtained are described in this paper.

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MATERIALS AND METHODS

Stage 1. Initial disaggregation. — DRNA's isolated from the following sources were used in this investigation: calf thymus gland [14], herring testis [8], wheat germ [10], mouse sarcoma [8], virulent and avirulent *H. pertussis* [11] and mouse leukaemic tissue [15]. The methods of isolation employed were as described in the references quoted.

The DRNase was prepared from OX-pancreas according to the procedure of McCarty [16] as modified by Overend and Webb [1]. (For the freeze-dried preparation, Found: N 15.8; P 2.0 %.) Previous investigation has shown that enzyme samples prepared in this way behave essentially the same as the crystalline DRNase described by Kunitz [2].

The depolymerisation of the DRNA by DRNase was investigated viscosimetrically in Ostwald viscometers (flow times for water (4.5 ml.) of between 80 and 90 seconds), at $34.5 \pm 0.25°$. Approximately 2 per cent aqueous solutions of the nucleic acid specimens (dried in the frozen state) were analysed for total phosphorus content (method of Allen [17] as modified ny Jones, Lee and Peacocke [18]) and were then suitably diluted to make a final concentration of 0.116 mg of nucleic acid phosphorus per ml.

The buffer solutions used in the following experiments were made 0.01 M with respect to Mg^{++} and were used in such proportion that the final enzyme-substrate-buffer mixture contained sufficient Mg^{++} for optimum enzyme activity.

A mixture of the DRNA solution (3 ml) and 0.01 M-magnesium sulphate in 0.1 M-veronal buffer (pH 7.1) (1.0 ml) was introduced into the viscometer at 34.5° and the temperature allowed to equilibrate. Thereafter the viscosity was determined. The experiment was carried out with each of the samples of DRNA listed above. The relative viscosities of these specimens were as follows:

Source of DRNA $\eta r.$	Thymus gland 3.04	Herring testis 1.83	Wheat germ 1.53	Mouse sarcoma 4.60	Virulent *H. pertussis* 3.26

Continued:	Avirulent *H. pertussis* 2.31	Mouse Leukaemic Tissue 4.61

In each case, after determination of the initial relative viscosity, a freshly prepared solution of DRNase (0.002 %, 0.5 ml) preheated to 34.5° was added to the sample. Viscosity measurements were made at frequent intervals until the viscosity became constant. Representative results are shown in Fig. 1.

Stage 2. (a) *Formation of acid-soluble polynucleotides* — A solution of DRNase (0.01 %, 1 ml), preheated to 37°, was added to each of a series of centrifuge tubes containing the substrate solution (2 ml) and 0.01 M-magnesium sulphate in 0.1 M-veronal buffer (pH 7.1) (1 ml) at 37°. Tubes were removed at intervals, cooled to 0° and 5 N-hydrochloric acid (0.1 ml) added to precipitate the acid-insoluble polynucleotides. The precipitates were removed at the centrifuge and aliquots (2 ml) of the clear supernatent solutions analysed for total phosphorus. Results are shown in Fig. 3. Control experiments showed no measurable liberation of "acid-soluble" phosphorus occurred when either the enzyme solution or the nucleic acid solutions were incubated alone at pH 7.1 for 4 hours at 37°.
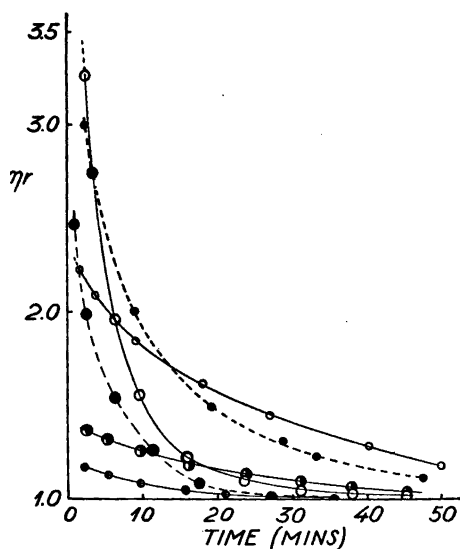
Fig. 1. The depolymerisation of the DRNA's from calf thymus gland (°——°), mouse sarcoma tissue (O——O), virulent H. pertussis (●--●), herring testis (◑——◑), wheat germ (◐——◑) and mouse leukaemic tissue (●--●) by DRNase.
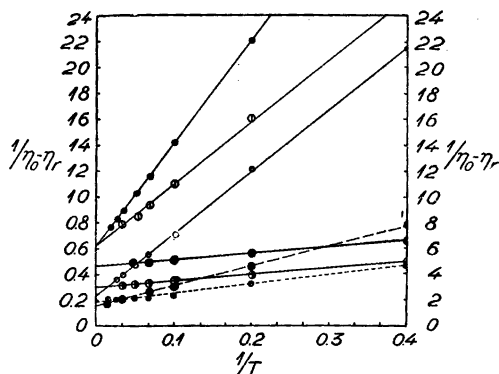
Fig. 2. Application of the modified Langmuir Isotherm $\dfrac{1}{\eta_0-\eta_r}=\dfrac{B}{A}\cdot\dfrac{1}{t}+\dfrac{1}{A}$ to the viscosimetric data obtained from the enzymic depolymerisation of the DRNA's from wheat germ (◑——◑), herring testis (O——O) (Right hand axis), avirulent H. pertussis (●——●), virulent H. pertussis (◑——◑), calf thymus gland (●——●), mouse sarcoma tissue (◑--◑) and mouse leukaemic tissue (●--●) (Left hand axis).

(b) *Dialysis of enzyme degradation products* — A mixture of DRNA solution (2X ml, where X = 15—25 ml), 0.1 M veronal buffer containing 0.01 M Mg^{++} at pH 7.1 (X ml) and 0.01 % DRNase (X ml) was incubated at 37° for 15 hours. At the end of this period the addition of 5 N-hydrochloric acid (0.05 ml) to an aliquot (2 ml) of this solution (pH 5.7—6.0) gave no precipitation. The solution was then dialysed in cellophane against distilled water (10 vols.) at 4°. After 7, 24, 48, 72, 110 and 144 hours the dialysis sac was transferred to fresh distilled water and the diffusate was concentrated under diminished pressure to small volume. Thereafter it was transferred to a graduated flask and diluted to volume. Aliquots of this solution were analysed for total phosphorus content. Results are recorded in Fig. 4.

RESULTS

The initial measurements of the relative viscosities of the buffered nucleic acid solutions (*i.e.* before the addition of enzyme) were taken as an approximate indication of the degree of polymerisation of the nucleic acids. Representative results of changes in viscosities after addition of the enzyme are shown in Fig. 1. (Time intervals are recorded as the periods elapsing between the addition of the enzyme (zero time) and the mid-point of the flow-time in each

determination.) In an attempted interpretation of these results, the curves (Fig. 1) were extrapolated to t = 0 to obtain rough approximations of η_0 ($\eta_r = \eta_0$ at $t = 0$). From the values so derived, linear plots of $\dfrac{1}{\eta_0 - \eta_r}$ against $1/t$ were obtained, indicating that the results followed the modified Langmuir isotherm $\left(\eta_r = \eta_0 - \dfrac{At}{B + t} \text{ or } \dfrac{1}{\eta_0 - \eta_r} = \dfrac{B}{A}\dfrac{1}{t} + \dfrac{1}{A}\right)$. Mean values of the constants A and B were calculated for each set of results from experimental values of η_r and t. From these figures true values of η_0 were derived and using the latter the linear relationships in Fig. 2 were obtained. Values of A were derived from the intercepts of the graphs on the ordinate at $1/t = 0$ (when $\dfrac{1}{\eta_0 - \eta_r} = \dfrac{1}{A}$) and the constants B were calculated from the slope of the lines. Results so obtained are summarized in Table 1. From the previous equation it follows that at infinite time (t_∞), $A = \eta_0 - \eta_r$. Since on prolonged incubation the relative viscosity (η_r) of the enzyme-substrate mixture tends towards unity, it follows that $A = \eta_0 - 1$. That the values calculated for $\eta_0 - 1$ are in reasonable agreement with the values of A derived from Fig. 2 is apparent in Table 1.

On the assumption that at any given time during the enzymic depolymerisation, the relative viscosity of the enzyme-substrate mixture was proportional to the concentration of the unattacked DRNA, Maver and Greco [19] found that the hydrolysis of thymus DRNA by the DRNases of certain cathepsin preparations, follows the equation of a first order reaction. It may be pointed out, however, that for the enzyme-substrate systems now studied, Fig. 1 does not fit the equation $K = \dfrac{1}{t} \log \eta_0/\eta_t$

The second stage of the enzymic hydrolysis of the DRNA's, namely the splitting into acid-soluble polynucleotides was followed by the methods

Table 1.

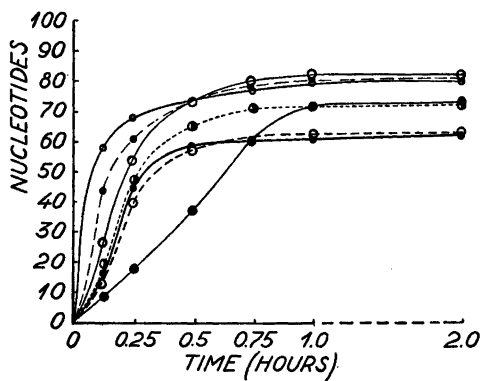| Source of nucleic acid	η_0	$\eta_0 - 1$	A	B/A	B
Avirulent *H. pertussis*	2.99	1.99	2.10	0.46	0.97
Virulent *H. pertussis*	4.02	3.02	3.21	0.47	1.50
Thymus gland	2.47	1.47	1.59	7.90	12.56
Mouse sarcoma	5.58	4.58	5.26	0.73	3.84
Mouse leukaemic tissue	7.40	6.40	6.67	1.55	10.34
Herring testis	1.44	0.44	0.42	48.0	20.16
Wheat germ	1.14	0.14	0.16	48.0	7.68

Fig. 3. The hydrolysis of the DRNA's from calf thymus gland (○——○), mouse leukaemic tissue (●---●), mouse sarcoma tissue (○——○), wheat germ (◐---◐), avirulent H. pertussis (●——●), virulent H. pertussis (○······○) and herring testis (●——●) to acid-soluble nucleotides by DRNase.
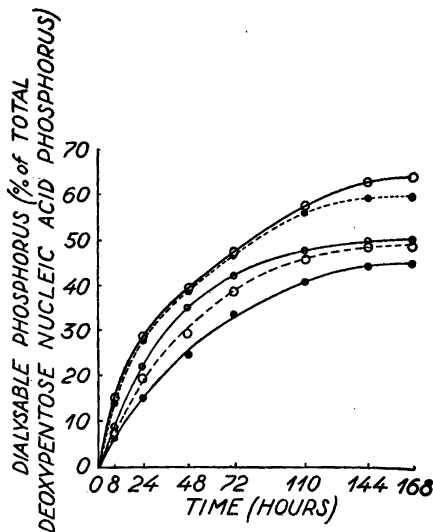
Fig. 4. Dialysis of the enzymic degradation products of the DRNA's from calf thymus gland (○——○), wheat germ (○---○), mouse sarcoma tissue (○——○), virulent H. pertussis (●······●) and herring testis (●——●).

described in the Experimental section. The results shown in Fig. 3 are expressed to illustrate the relationship between the percentage hydrolysis of the substrate and the time of incubation with the enzyme. The curves (Fig. 3) show in each case an initial lag in the formation of acid-soluble polynucleotides which corresponds to the period of disaggregation of the nucleic acid. The curves showing the hydrolysis of the nucleic acid specimens from virulent and avirulent strains of H. pertussis so closely parallel one another that it seems likely that the two specimens are similar (see also Fig. 2). It appears that the DRNA from herring testis is more resistant than the other DRNA's to both enzymic hydrolysis (Fig. 3) and disaggregation (Figs. 1 and 2, and Table 1).

Under the conditions of the present experiments, hydrolysis ceases when about 60—80 percent of the nucleic acid has been rendered acid-soluble.

Preliminary quantitative measurements were made on the amounts of enzymic degradation products which underwent dialysis and the amounts of non-dialysable »cores». Fig. 4 shows that the quantity (based on phosphorus estimations) of non-dialysable nucleotide material varies from 45—65 per cent

according to the sample of DRNA used. The amounts of these non-dialysable fragments are greater than those reported by Chargaff and Zamenhof[4]. The differences are due probably to changes in the dialysis procedure rather than to large differences in the sizes of the polynucleotide fragments obtained in our experiments and those of Chargaff and Zamenhof. In the present experiments dialysis was carried out against repeated changes of distilled water and not against buffer solution as reported by Chargaff and Zamenhof[4]. This procedure served to remove effectively from the system metallic ions in the presence of which polynucleotides are known to dialyse to a greater extent[21]. It follows that dialysis against distilled water results in a larger amount of the nucleic acid remaining in the dialysis sac.

From the above results it is clear that for all the samples of DRNA studied, the mode of disaggregation and hydrolysis by pancreatic DRNase follows closely the pattern previously observed with DRNA from calf thymus gland. Furthermore, it seems that for individual samples of DRNA, the rates of disaggregation and hydrolysis do not run parallel.

SUMMARY

The enzymic degradation (by pancreatic DRNase) of DRNA's isolated from various sources has been studied. The initial stage of disaggregation has been followed viscosimetrically and a preliminary analysis made of the results obtained. These have been shown to follow the modified Langmuir Isotherm. It was illustrated that the rates of disaggregation and subsequent hydrolysis of the DRNA's by the enzyme do not run parallel.

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