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Mode of Action of Steroid Hormones, Catecholamines and Hexose Oximes on DNA

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The activity of deoxyribonuclease was not influenced by steroid hormones, catecholamines and hexose oximes. Decomposition tests with deoxyriboapyrimidinic or deoxyriboapurinic acid showed that cortisone broke specifically purine clusters, and that dopamine and noradrenaline split pyrimidine clusters more preferentially than purine clusters. Glucosoxime and galactosoxime clove pyrimidine and purine clusters to the same extent. The decomposition of the acids by these compounds was remarkably enhanced by cupric ions. It is assumed that the induction of pupation by these substances may be initiated by the breakage of purine nucleotide clusters in chromosomal DNA molecules.

INTRODUCTION

In the preceding study (Yamafuji ***et al.,*** 1971 c), we demonstrated that β-ecdysone, cortisone, dopamine, noradrenaline, glucosoxime or galactosoxime induced the pupation of silkworm, ***Bombyx mori.*** It was also proved (Murakami and Yamafuji, 1970; Yamafuji ***et al.,*** 1971a, b, c) that these pupation-inducing steroids, amines or oximes have the ability to break single and double strand **DNA.** To explore the mechanism of the pupation, we have now investigated the effect of these compounds on DNase, apyrimidinic or apurinic acid.

MATERIALS AND METHODS

Influence of steroids, catecholamines and **oximes on DNase activity**

DNase I (crystallized from beef pancreas, ***2400*** Kunitz units/mg) and II (purified from hog spleen, 1600 Kunitz units/mg) were purchased from Sigma Co. Ecdysterone was a gift of Takeda Pharmaceutical Co. Cortisone and catecholamines were purchased from Sigma Co. and Wako Junyaku, respectively. Sugar oximes were prepared in our laboratory.

To investigate the effect of the above mentioned reagents on DNase I, 1.5 ml of calf thymus DNA solution (0.6 mg/ml) was mixed with 1 ml of 0.1 M phosphate buffer (pH 7.0), 0.5 ml of 30 mM $MgSO_4$, 1 ml of the reagent dissolved in the same buffer, 0.5 ml of 0.1% gelatin and 0.5 ml of the pancreas en-

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zyme (*50* ng/ml). In the case of DNase II, *0.25* M acetate buffer (pH 5.2), 0.75 M KC1 and spleen enzyme (*0.2* μg/ml) were used instead of phosphate buffer, $MgSO_4$ and the pancreatic enzyme, respectively. The viscosity of the mixture was immediately measured at 37°C in an Ostwald viscosimeter. In the control, phosphate buffer was added instead of the reagent.

Preparation and decomposition of apyrimidinic (ApyA) and apurinic acid (ApuA)

Preparation of DNA from calf thymus was conducted according to the method described before (Yamafuji *et al.*, 1966). From DNA obtained, ApyA and ApuA were prepared by the method of Takemura (1959) and of Tamm *et al.* (1952), respectively.

To examine the decomposition of the acids by the reagent, the acid (1 mg/ml) was incubated with steroid, catecholamines or hexose oximes of adequate molar concentration at 37°C for *24* hrs, with or without addition of *250* μM Cu^{2+}. The total volume of the reaction mixture was 4 ml. The mixture in cortisone tests, however, contained 4 % methyl alcohol in order to dissolve the hormone. Subsequent separation and estimation of the acids were carried out with Sephadex G-50 fine column according to the procedures stated previously (Yamafuji *et al.*, 1970).

RESULTS

Action on DNase

It has been demonstrated in our laboratory that hormonal steroids, biological reductones and sugar oximes possess the DNA-depolymerizing ability (Yamafuji *et al.*, 1970; Murakami and Yamafuji, 1970; Yamafuji *et al.*, 1971a, b, c). There might, however, be the possibility that DNA-breakages would be caused by the action of DNase enhanced through the function of reagents. Therefore, we examined the effect of β-ecdysone, cortisone, dopamine, noradrenaline, glucosoxime and galactosoxime on DNase activity under varying conditions. It was observed, however, that the DNase action was not affected by these reagents as shown in Table 1.

Action on ApyA or ApuA

To find a site of DNA-breakage by these kinds of compounds, ApyA or ApuA was now treated with the reagents for rather a long time. ApyA was first incubated with *250* μM cortisone with or without Cu^{2+}. As illustrated in Fig. 1, the acid was decomposed by the hormone alone. The decomposing capacity was enhanced by the co-existence with *250* μM Cu^{2+}. No decomposition was brought about by *250* μM Cu^{2+} alone, ApuA was also treated in the same way. It was observed, however, that the decomposition of the acid was not caused even by the combined action of cortisone and Cu^{2+}. Another typical steroid hormone, β-ecdysone also decomposed only ApyA (Yamafuji *et al.*, unpublished). It is, therefore, concluded that, at least under these conditions, steroid hormones disintegrate specifically only purine clusters in DNA-threads.

It has been clarified that o-diphenol such as dopa, dopamine, noradrenaline and adrenaline decomposed the DNA-threads specifically at the site of pyrimidine

Table 1. Effect of steroid hormones, catecholamines and hexose oximes on DNases.

Reagent[b]	DNase I[a]: Decrease of η_{sp}[c] in first 30 min		Reagent[b]	DNase II[a]: Decrease of η_{sp}[c] in first 30 min	
	Test	Control		Test	Control
Ecdysone, 10 μM	0.42	*0.40*	Ecdysone, 10 μM	0.34	0.32
Cortisone, 100 μM	0.43	0.41	Cortisone, 100 μM	0.37	0.35
Dopamine, 100 μM	0.38	0.39	Dopamine, 100 μM	0.32	0.34
Noradrenaline, 2 mM	0.41	0.40	Noradrenaline, 2 mM	0.33	0.35
Glucosoxime, 100 μM	0.38	0.40	Glucosoxime, 100 μM	0.34	0.35
Galactosoxime, 2 mM	0.40	0.39	Galactosoxime, 2 mM	0.33	0.34

a) The viscosimetric estimation of DNases was performed as described in MATERIALS AND METHODS.
b) All reagents were dissolved in water, except that 100 μM cortisone contained 3 % methyl alcohol, in order to keep the hormone in soluble state.
c) Specific viscosity.

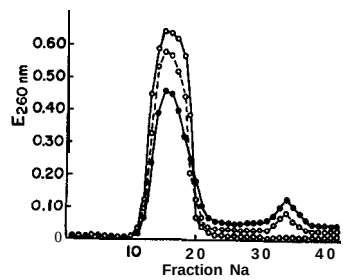

Fig. 1. Gel-filtration pattern of apyrimidinic acid after treating with cortisone and copper. The reaction was performed as stated in MATERIALS AND METHODS. The reaction mixture was then filtered through Sephadex G-50 fine (102x1.6 cm) and eluted with distilled water. Nucleotides were detected by U. V. absorbancy at 260 nm. ○–○, control ; ○···○, 250 μM cortisone; ●–●, 250 μM cortisone+250 μM Cu^{2+}.

nucleotide clusters in combination with Cu^{2+} (Yamafuji *et al.*, 1970). We have now examined the action of catecholamines on both clusters with higher molarity of 2.5 mM.

Experiments with dopamine disclosed that 2.5 mM amine degraded ApuA more intensively than ApyA (Fig. 2). The decomposition of both acids was strongly accelerated by cupric salt. It is inferred that dopamine has the ability to break pyrimidine clusters more preferentially than purine clusters.

Treatment with 2.5 mM noradrenaline similarly resulted in a stronger disintegration of ApuA than ApyA and an enhancement of its decomposing activity by Cu^{2+} (Fig. 3). It has thus been confirmed that the mode of action

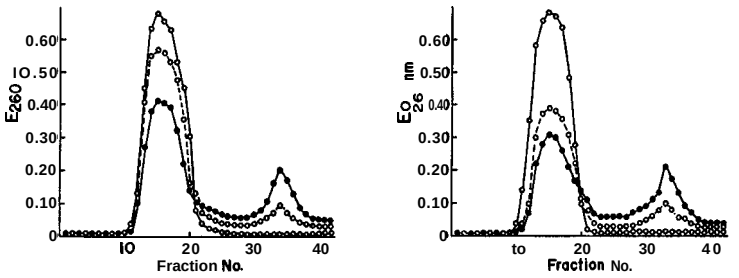

Fig. 2. Gel-filtration pattern of apyrimidinic acid (A) or apurinic acid (B) after treating with dopamine and copper. The assay was performed as described in Fig. 1. ○–○, control; ○···○, 2.5 mM dopamine; ●–●, 2.5 mM dopamine+250 μM Cu^{2+}.

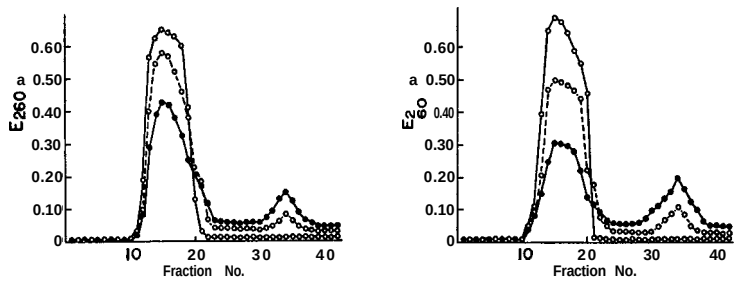

Fig. 3. Gel-filtration pattern of apyrimidinic acid (A) or apurinic acid (B) after treating with noradrenaline and copper . The assay was performed as described in Fig. 1. ○–○, control ; ○···○, 2.5 mM noradrenaline ; ●–●, 2.5 mM noradrenaline + 250 μM Cu^{2+}.

of these two catecholamines on purine or pyrimidine nucleotides is the same.

Tests with 250 μM glucosoxime further revealed that ApyA or ApuA was somewhat decomposed by the oxime alone, and that the decomposition was considerably increased by cupric ions (Fig. 4). It was observed that the degree of the decomposition of both acids was about the same.

Similar observation was obtained in the experiments with 2.5 mM galactosoxime (Fig. 5). It has thus been demonstrated that these two oximes can split purine and pyrimidine clusters to the same extent.

DISCUSSION

Recently we have proposed the hypothesis that the initiative process of cellular differentiation and anomalization may be appropriate DNA-breakages (Yamafuji, 1969, 1970; Yamafuji et al., 1971a, b). In the present investigation, it has been corroborated that the DNA-breaking capability of β-ecdysone, cortisone, dopamine, noradrenaline, glucosoxime and galactosoxime is not to be attributed to the enhancement of DNase activity by these substances.

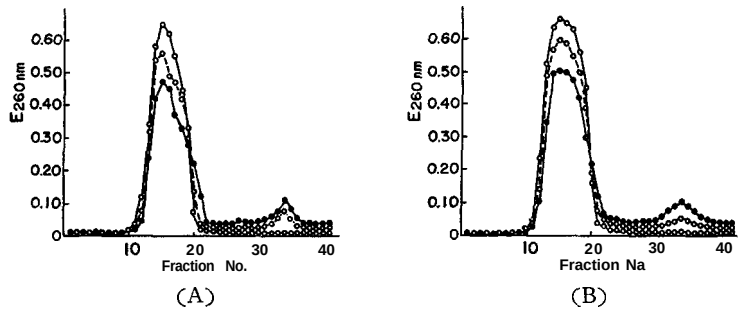

Fig. 4. Gel-filtration pattern of apyrimidinic acid (A) or apurinic acid (B) after treating with glucosoxime and copper, The assay was performed as described in Fig.1. O-O, control ; ○···○, 250 μM glucosoxime ; ●—●, 250 μM glucosoxime + 250 μM Cu^{2+}.

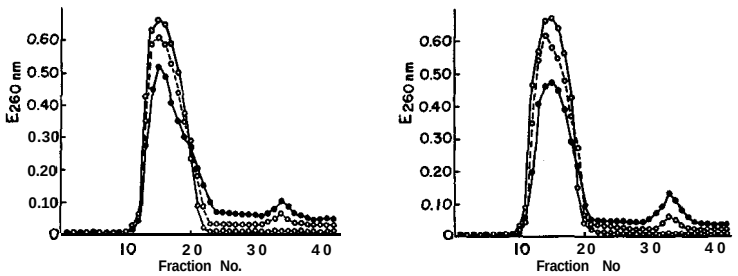

Fig. 5. Gel-filtration pattern of apyrimidinic acid (A) or apurinic acid (B) after *treating* with galactosoxime and copper. The ***assay*** was performed as described in Fig. 1. o-o, control; ○···○, 2.5 mM galactosoxime ; ●—●, 2.5 mM galactosoxime +250 μM Cu^{2+}.

Our previous experience (Yamafuji et al., 1971c) demonstrated that these reagents can induce the pupation in *Bombyx mori*. Quite recently, McMahon (1974) reported that adrenaline, noradrenaline and dopamine regulate the early development of the sea urchin. Moreover, it has been also proved that the proper DNA-breakages increase the RNA polymerase activity (Yamafuji *et al.*, 1972; Iiyama *et al.*, 1973; Omura *et al.*, 1973), and that purine or pyrimidine clusters exist in DNA-strands (Habermann *et al.*, 1963; Mushynski et *al.*, 1970). Our studies have shown that ApyA or ApuA from DNA is decomposed by steroid hormone, catecholamines and hexose oximes in peculiar ways. In view of the peculiarities and the pupation-inducing potencies, it seems reasonable to assume that the induction of pupation by these compounds (Yamafuji ***et al.***, 1971c) is initiated by the decomposition of purine clusters in cellular DNA molecules.

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