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Epinephrine-Oxidizing Activity in Calf Thymus Nucleosome

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We characterized epinephrine-oxidizing activity in calf thymus nucleosome. The activity was detected in the nucleosome fraction extractable with 10 mM acetate buffer (pH 7.0) and the fraction insoluble in the buffer. Both activities were extremely unstable in the buffer, but stabilized by the addition of $MgCl_2$ or $CaCl_2$. The activity disappeared in the presence of 0. l-O.6 M NaCl. Calf thymus nucleosome oxidized epinephrine rapidly, but the activity was negligible against norepinephrine, dopa and dopamine. These results indicate that calf thymus chromatin contains ***some*** substance (s) which oxidizes epinephrine specifically.

INTRODUCTION

Catecholamines (CA), such as epinephrine (Ep) are adrenal medullar hormones and neurotransmitters at the terminals of sympathetic nerves. In addition to the physiological functions, CA break DNA in the presence of Cu^{2+} in *vitro* (Murakami *et al.*, 1978b, 1979 ; Yamada *et al., 1985)* and in cultured mammalian cells (Murakami *et al.*, 1975, 1978b; Yamada *et al., 1985).* It has been believed that CA express their functions through their binding to the cell membrane and activation of adenyl cyclase. However, we revealed that ^{14}C-Ep added into culture media was incorporated into rat fetal lung (RFL) cells and about 50 % of the incorporated Ep bound to chromosomal DNA (Murakami *et* al., 1978a). Moreover, cellular DNA breaking activity of Ep correlates with the amount of Ep bound to DNA in various cell lines (Yamada *et al., 1979).* These findings suggest that CA break cellular DNA through direct binding with DNA. The DNA breaking reaction is coupled with oxidation of CA catalyzed by Cu^{2+} *in vitro* (Shirahata *et al., 1978* ; Murakami *et al., 1979).* On the other hand, the cellular DNA breakage by CA need not addition of Cu^{2+} in culture media. Recently, we found that calf thymus chromatin had Ep-oxidizing activity (Yamada *et al.,* 1984). It is possible that the activity plays an important role in the cellular DNA breakage by Ep. In the present paper, we characterized the Ep-oxidizing activity to clarify the mechanism of cellular DNA breakage by CA.

MATERIALS AND METHODS

Preparation of nucleosome

Calf thymus chromatin was prepared by the method of Busch (1968) and nucleosome by the method of Oosterhof *et al.* (1975), with slight modifications as de-

scribed previously (Yamada et al., 1978, 1984). In short, chromatin (200 μg/ml as DNA) was digested with 10 units/ml DNase II (Miles Laboratories) at 37°C in a digestion buffer (D buffer ; 5 mM sodium bisulfite and 0.1 mM Na_2EDTA, adjusted to pH 7.0 with solid tris). The solution was centrifuged at 10,000 g for 30 min to remove insoluble materials. Amount of acid soluble and insoluble substances were determined after precipitation with 4 % perchloric acid (final concentration) (Yamada *et al.*, 1978). Nucleosome in the supernatant was precipitated by the addition of 2 M NaCl (final concentration, 0.14 M) and kept at 0°C for 30 min. The solution was centrifuged at 6,000 g for 30 min to obtain soluble fraction (S fraction). The pellet thus obtained was extracted with 10 mM acetate buffer (pH 7.0) to recover nucleosome fraction (N fraction). The insoluble material obtained after centrifugation at 6,000 g for 30 min (P fraction) was suspended in the same buffer and used for Ep oxidation.

Oxidation of CA by nucleosome

Basically, reaction mixtures contain 4 ml of nucleosome fractions, 0.4 ml of 20 mM Ep, 0.4 ml of 100 mM acetate buffer (pH 7.0), and 3.2 ml of deionized water. The mixtures were incubated at 37°C and coloring of Ep at 470 nm was determined with a Tokyo Koden ANA-l Electrophotometer. To compare the CA-oxidizing activity of nucleosome fraction, final CA concentrations were adjusted to 10 mM.

RESULTS AND DISCUSSION

1. Effect of digestion time on recovery of Ep-oxidizing activity

Calf thymus chromatin was digested with DNase II to obtain nucleosome fractions. As shown in Fig. 1, most chromatin was solubilized within 2 hr. Amount of acid soluble fraction increased linearly for the first 8 hr and then ceased to increase. Amount of S fraction, which may be corresponding to acid soluble fraction, increased with digestion time. Nucleosome fraction thus obtained was divided into two fractions, N fraction being soluble in 10 mM acetate buffer and P fraction being insoluble in the same buffer. Amount of N fraction increased slightly by 4 hr digestion with DNase II and then decreased with digestion time. Amount of P fraction decreased with elongation of DNase II digestion.

When the two fractions were incubated with 1 mM Ep, P fraction oxidized Ep after a short lag period (Fig. 2). N fraction also oxidized Ep with a similar rate after a longer lag period. When the two fractions were prepared from chromatin treated with DNase II for various periods, specific activity of P fraction decreased linearly with digestion time (Fig. 3). Specific activity of N fraction was lower than that of P fraction, but the activity was more stable than the latter. When the two fractions prepared from a 2-hr digest were applied to Ep oxidation after incubating them at 37°C for 3 or 6 hr, the activity decreased rapidly with half-lives by about 1.5 hr in P fraction and about 3 hr in N fraction, respectively. Thus, we saught some factors stabilizing the Ep-oxidizing activity of the nucleosome fractions.

2. Effect of dications on stability of Ep-oxidizing activity

When nucleosome pellets obtained after 0.14 M NaCl precipitation were suspended

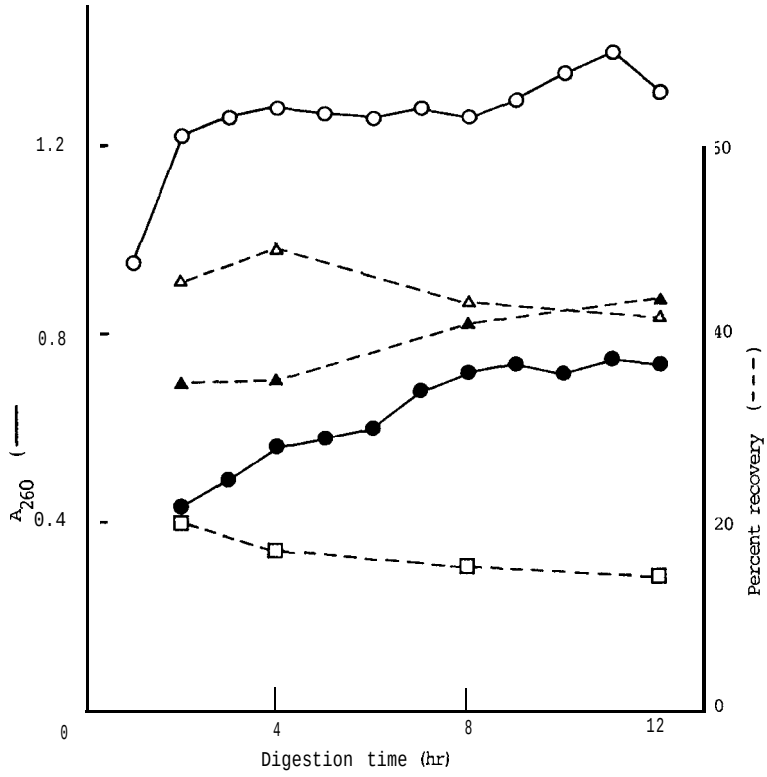

Fig. 1. Course of DNase II Digestion of Calf Thymus Chromatin. Increases in A_{260} of soluble (○) and acid soluble (●) fractions with digestion time were shown by solid lines. Changes in percent recoveries of S (A), N (a), and P (□) fractions were shown by dotted lines.

in 10, 100 and 1,000 mM acetate buffers (pH 7.0), the Ep-oxidizing activity was highest in the 10 mM buffer (data not shown). When the same pellets were suspended in the 10 mM acetate buffer containing $CaCl_2$ or $MgCl_2$, the activity was 50 % higher in the presence of 1 mM $CaCl_2$ than that of control (Table 1). In the presence of 1 mM $MgCl_2$, the activity increased by 10 %. In the presence of 10 mM dications, the activity was very low. Nucleosome suspended in the buffer containing 1 mM $MgCl_2$ or $CaCl_2$ retained 85 % of the activity after incubating them at 37°C for 100 min. These results indicate that these dications stabilize the Ep-oxidizing activity in the nucleosome fractions.

To determine the optimum concentration of $CaCl_2$, nucleosome pellets were extracted with 10 mM acetate buffer containing various concentrations of $CaCl_2$ and remaining activities after a 37°C incubation for 2.5 hr were determined. As shown in Fig. 4, the activity was highest at 0.5 mM $CaCl_2$. Thus, we used the 10 mM acetate buffer (pH 7.0) containing 0.5 mM $CaCl_2$ (referred as 0 buffer) thereafter. When N and

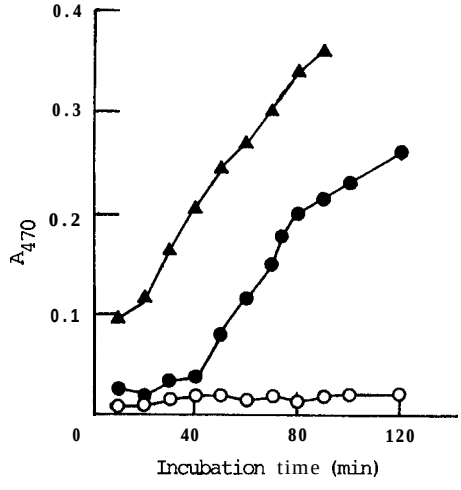

Fig. 2. Course of Ep Oxidation by Nucleosome Fractions. N and P fractions were incubated with 1 mM Ep at 37°C and coloring at 470 nm was determined. Control (○); N fraction (●); P fraction (▲).

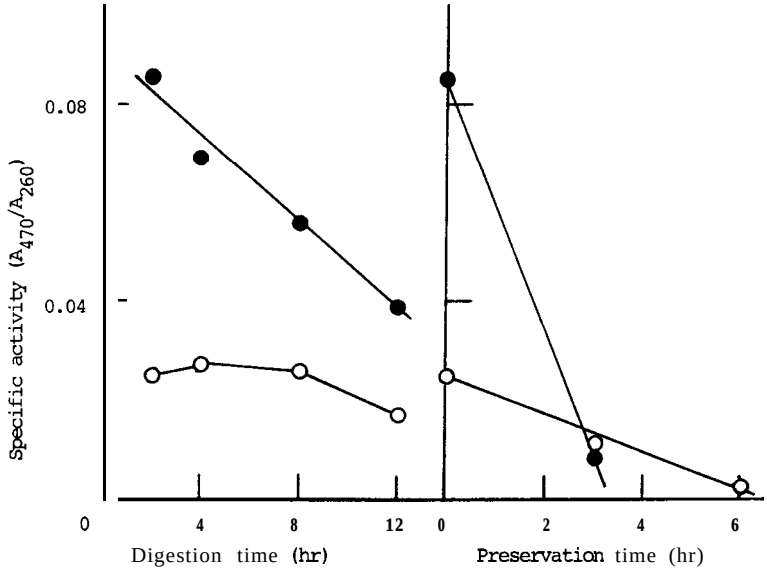

Fig. 3. Effect of Digestion Time and Preservation Time on Ep Oxidation by Nucleosome Fractions. N and P fractions were prepared from chromatin digested with DNase II for indicated periods. The activities in the fractions prepared from a 2 hr-digest were determined every 3 hr after incubating them at 37°C. N fraction (○); P fraction (●).

Table 1. Effect of dications on stabilization of Ep-oxidizing activity in nucleosome.

Dications	Conc. (mM)	A_{470}*	
		0 min	100 min
$CaCl_2$	0	0.090	0.035
	1	0.135	0.115
	10	0.010	ND**
$MgCl_2$	1	0.100	0.085
	10	0	ND

* Data from 90 min reaction at 37°C.
** Not determined.

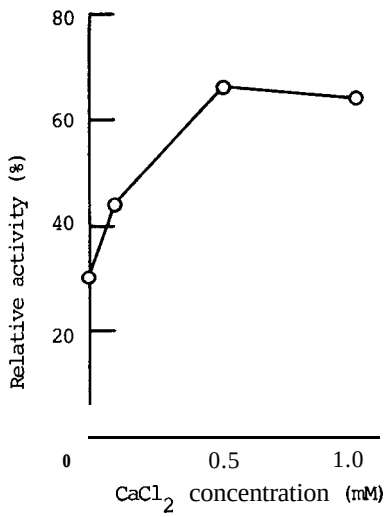

Fig. 4. Optimum Concentration of $CaCl_2$ on Stabilization of Ep-oxidizing Activity in N Fraction. N fraction was extracted with 10 mM acetate buffer containing various concentrations of $CaCl_2$ and incubated at 37°C for 2.5 hr. Remaining activities of the extracts were compared with the original activity.

P fractions were stored in the buffer at 4°C for 6 days, remaining activities were 78 % in N fraction and 37 % in P fraction, respectively.

3. Effect of NaCl on Ep oxidation by N fraction

Nucleosome is consists of DNA, histone, and small amount of nonhistone proteins. Among histone molecules, H1 dissociates from DNA at 0.4-0.5 M NaCl, HZ at 0.8-1.2 M, and H3 and H4 at 1.0-2.0 M (Bonner et al., 1968). To examine the role of histones on the Ep oxidation by nucleosome, the activity of N fraction was determined in the presence of NaCl. The activity decreased to 60 % of the control in the presence of

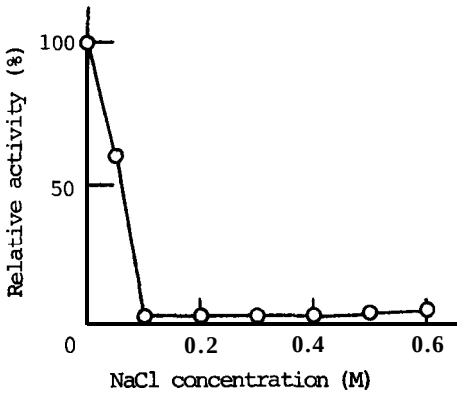

Fig. 5. Effect of NaCl on Ep Oxidation by N Fraction. Ep-oxidizing activity in N fraction was determined in the presence of various concentrations of NaCl.

0.05 M NaCl and was lost in the presence of 0.1-0.6 M NaCl. This means that the activity is lost independently with dissociation of histone molecules.

4. **Effect of Cu^{2+} on Ep oxidation by N fraction**

Cu^{2+} oxidizes Ep in the presence of nucleic acids and induces strand breakage in nucleic acids *in vitro* (Murakami et al., 1979). Thus, we examined the effect of Cu^{2+} in the Ep oxidation by nucleosome. As shown in Table 2, 1 mM Cu^{2+} oxidized Ep rapidly in the absence of nucleic acid.

Table 2. Effects of Cu^{2+} and EDTA on Ep oxidization by nucleosome.

		A_{470}*
1 mM $CuSO_4$		0.60
N fraction		0.115
	+1 mM $CuSO_4$	0.200
	+1 mM EDTA	0.070

* Data from 60 min reaction at 37°C.

Activity of N fraction was about one fifth of that in 1 mM Cu^{2+}. When Ep was treated with N fraction and 1 mM Cu^{2+}, oxidation rate of Ep was one third of that in 1 mM Cu^{2+}. These results indicate that nucleosome oxidizes Ep by itself but inhibits Ep oxidation by Cu^{2+}. When EDTA was added to N fraction to remove free Cu^{2+}, decrement of the Ep-oxidizing activity in N fraction was only 40 %. This suggests that the Ep oxidation by nucleosome need not free Cu^{2+}.

5. Oxidation of CA by N fraction

Binding of Ep to calf thymus nucleosome occurs highly specifically even in the presence of excess amounts of other CA (Yamada *et al.*, 1978). Thus, we compared the

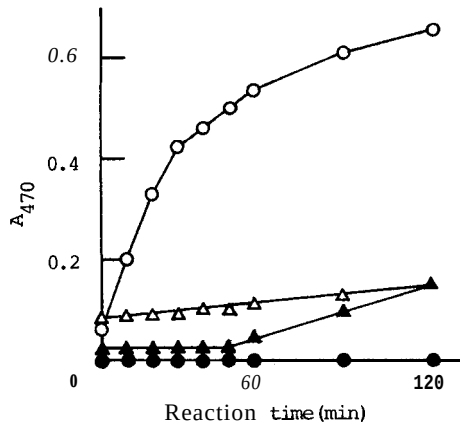

Fig. 6. Oxidation of CA by N fraction. N fraction was incubated with 10 mM CA and coloring at 470 nm was measured. Ep (○); Norepinephrine (▲); Dopamine (△); Dopa (●).

CA-oxidizing activity of N fraction. In the presence of 10 mM Ep, N fraction oxidized Ep rapidly without any lag period, as seen in the presence of 1 mM Ep. The fraction showed no or very weak activity against dopa, dopamine, and norepinephrine.

^{14}C-Ep added in the culture media is incorporated into RFL cells and the incorporated Ep binds to cellular protein and DNA (Murakami *et al.*, 1978). Among chromatin components, Ep binds to histone and DNA, but both components have no Ep-oxidizing activity in the absence of Cu^{2+} (Yamada *et al.*, 1978). Binding of Ep to nucleosome occurs highly specifically, in the presence of other CA (Yamada *et al.*, 1978) and we showed here calf thymus nucleosome oxidized Ep specifically. Cellular DNA breaking activity of CA varies with CA (Yamada *et al.*, 1985) and cell lines (Yamada *et al.*, 1979), and is sometimes different with their *in vitro* activity. For example, dopa shows strong DNA breaking activity *in vitro*, but does not break RFL cellular DNA (Yamada *et at.*, 1985). The difference in cellular DNA breaking activity with cell lines is partly due to the difference in intracellular incorporation of CA (Yamada *et al.*, 1979). Our results obtained here suggest strongly that the CA-oxidizing substance in cellular chromatin is also important for the induction of cellular DNA breakage.

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