BIOLOGICAL ACTIVITY OF PHENYLETHANOL AND ITS DERIVATES. INFLUENCE ON ISOLATED DNA NUCLEOTIDYLTRANSFERASE AND DNAase*

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1. Introduction

Berrah et al. [1] first reported that PEA† strongly inhibited DNA synthesis in *E. coli* at concentrations which did not affect RNA and/or protein synthesis. In mammalian tissue culture cells, both RNA and DNA synthesis are influenced at comparable PEA levels [survey 2]. Results of *in vivo* experiments with mouse lymphoma cells of our group suggest that the primary effect of PEA might be inhibition of DNA synthesis [3–5].

It is the purpose of this paper to clarify an aspect of the mechanism by which PEA is believed to inhibit DNA synthesis in cells of higher organisms: PEA is shown to inhibit DNA nucleotidyltransferase (E.C. 2.7.7.7) preparations from mouse lymphoma cells in a non-competitive fashion at concentrations that favorably compare to the action on intact cells.

2. Material and methods

The deoxynucleoside triphosphates were obtained from Schwarz Bioresearch, USA. In the experiments mouse lymphoma cells L_{5178Y} [6] were used, growing in suspension in a half-synthetic medium (Fischers medium, Grand Island Biological Co., Grand Island, USA; 10% horse serum, Microbiological Associates, Bethesda, USA). For incubation with PEA, the cells were transfered into Gey's balanced salt solution [7].

DNA nucleotidyltransferase assay was performed according to Bollum [8] modified by doubling the molar concentrations of the deoxyribonucleoside triphosphates. The acid-insoluble radioactivity was determined by the "filter paper disc technique" [9]. The DNAase† reaction mixture (0.5 ml) contained 50 mM K-Phosphate buffer pH 7.25, 12.5 mM $MgCl_2$ 1.25 mM $CaCl_2$, 2.5 mM EDTA, 5 mM 2-mercapto-ethanol and native herring DNA 40 μg/ml reaction mixture. The reaction was intiated by the addition of the enzyme (0.1 ml) and terminated after 12 min (25°). The enzyme activity was measured spetrometrically according to Kuntz [10]. For protein determination the biuret reagent was used [11].

In the preparation the two enzymes nucleotidyltransferase and DNAase have not been separated. All steps are carried out at 0–4°. Buffer A contains 50 mM K-phosphate buffer pH 7.4, 50 mM NaCl and 3 mM 2-mercaptoethanol; buffer B consists of 50 mM K-phosphate buffer pH 7.25, 2.5 mM EDTA and 5 mM 2-mercaptoethanol. Cell suspensions were centrifuged in a Sorvall centrifuge (rotor SS-34) at 2000 *g* for 30 sec. The sediment obtained from 10^8 cells is washed three times with physiological saline and subsequently suspended in 1.2 ml buffer A. The cells are lysed by rapid freezing and thawing three times. The lysate is centrifuged at 10 000 *g* for 5 min at 4° and the supernatant is decanted and dialysed (Tubes from: Visking Corp., Chicago, USA) for 12 hr against 500 vol. buffer B. The resulting preparation contains DNA nucleotidyltransferase (specific activity 21 units/mg proteins [8]) and DNAase (specific activity of 4.1 Kunitz units/mg protein).

* This is part IV of this series.

† *Abbreviations*
PEA: 2-phenyl-ethanol.
DNAase: deoxyribonuclease (E.C. 3.1.4.5).

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3. Results and discussion

Inhibition of DNA synthesis theoretically could be brought about by changing the amount and/or the specific activity of either nucleotidyltransferase or DNAase during incubation of the cells with PEA, the 50% inhibitory concentration of cell division rate (ED_{50}) was found to be 0.055% PEA [5] for a 4 hr incubation. After such an incubation the cells were freed of PEA by washing with saline containing 40 μg/ml cycloheximide (Serva, Heidelberg). This antibiotic is supposed to stop any further protein synthesis immediately [12] without influencing the activity of both enzymes *in vitro*.

As can be seen from table 1, incubation of cells with PEA at ED_{50} alters neither DNA nucleotidyltransferase activity nor DNAase activity to any significant extent. Though these results do not exclude the possibility that PEA might produce a factor that alters the intracellular activity of these enzymes and which might be lost during enzyme preparation, the conclusion may safely be drawn that under these conditions no short term decrease in the amount of DNA

Table 1

The activity of DNA nucleotidyltransferase and DNAase of L_{5178Y} mouse lymphoma cells after incubation with PEA. 7×10^6 cells/ml were incubated for 4 hr in Gey's solution containing 0.055% PEA.

a) *DNA nucleotidyltransferase*

At zero time, 1–4 hr aliquots of 10^8 cells were withdrawn for enzyme preparation and activity determination.

	Incubation time (hr)	Relative dATP-^{3}H incorporation into DNA by enzyme preparation
Control cells	0	100
	1	104
	2	93
	3	98
	4	103
Cells incubated with 0.055% PEA	0	101
	1	108
	2	95
	3	102
	4	104

b) *DNAase*

At zero time and after 4 hr incubation aliquots of 10^8 cells were used for enzyme preparation and activity determination in a 12 min (linear) test period.

	Incubation period of cells in Gey's solution (hr)	Number of experiments	Activity of enzyme preparation in percent Mean ±	standard deviation
Control cells	0	3	100	± 28
	4	3	104	± 28
Cells incubated with 0.055% PEA	0	3	117	± 28
	4	3	104	± 31

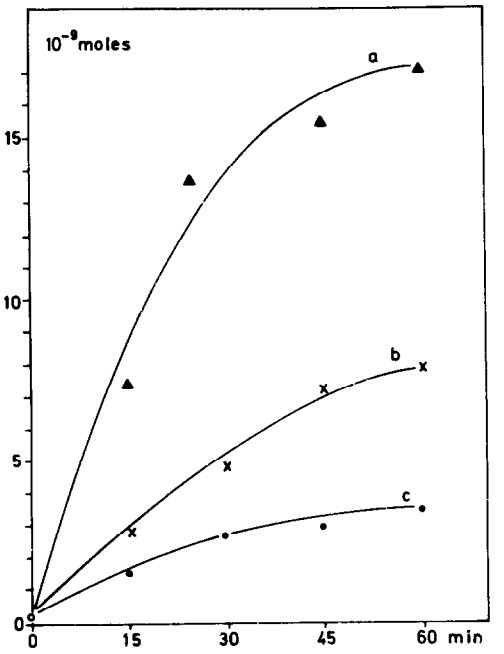

Fig. 1. Inhibition of isolated L_{5178Y} DNA nucleotidyltransferase by PEA. Reaction mixture as described under methods.
abscissa : incubation time in minutes;
ordinate: amount of dATP-^{3}H incorporated into DNA in 10^{-9} moles/ml.
The reaction mixture contained 4×10^{-4} g denatured DNA per ml as template (condition of template saturation).
curve a: control; initial incorporation rate is $V_a = 5.88 \times 10^{-10}$ moles dATP/min. ml = 100.0%;
curve b: 0.2% PEA added; initial incorporation rate is $V_b = 2.66 \times 10^{-10}$ moles dATP/min. ml = 45.2%;
curve c: 0.6% PEA added; initial incorporation rate is $V_c = 1.03 \times 10^{-10}$ moles dATP/min. ml = 17.5%.

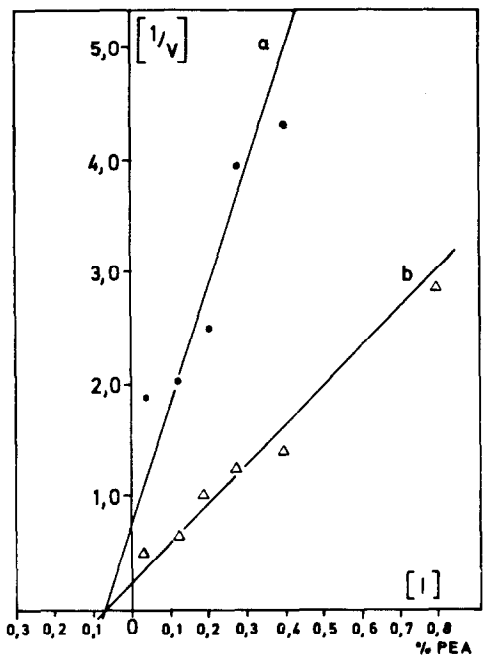

Fig. 2. The inhibition of L_{5178y} DNA nucleotidyltransferase in relation to varying PEA concentrations. Reaction mixture as described under methods. The reaction was terminated after 60 min at 37°.
abscissa : [I] Inhibitor concentrations in % PEA;
ordinate: [1/V] reciprocal of the initial reaction velocity in 60×10^9 min. ml/moles dATP under nonsaturating template concentrations. Plot according to Dixon et al. [16].
curve a: template concentration: 8×10^{-6} g DNA per ml;
curve b: template concentration: 4×10^{-6} g DNA per ml;
"K_I" = 0.082 ± 0.025% PEA.

nucleotidyltransferase through inhibition of RNA messenger synthesis has occurred; this disproves the mechanism suggested by Rosenkranz et al. [13].

On the other hand no increase in DNAase activity and/or amount could be detected. This is hard to reconcile with results of Leach et al. [14] and Higgins et al. [15]. Their results indicate DNA losses from DNA-containing cellular substructures under PEA incubation. Our results imply that this is not primarily related to PEA action.

Beyond this negative evidence, we can restate our earlier conclusion indicating the enzyme DNA nucleotidyltransferase to be the primary target of PEA. PEA strongly reduced the initial rate of DNA synthesis in tissue culture extracts (fig. 1).

It has been possible to show that the PEA inhibition is noncompetitive by running the tests at two different non-saturating template levels against different inhibitor concentrations (fig. 2). An inhibitor constant (being aware of necessary restrictions) may be derived as K_I = 0.082 ± 0.025%. Thus the DNA nucleotidyltransferase activity of lymphoma cells is reduced to 50% at 0.082 ± 0.025% (6.72 ± 2.0 mM) PEA which is in rather good agreement with the ED_{50} = 0.055 ± 0.003% PEA in intact lymphoma cells [5]. This correspondence of active concentrations leads to the conclusion that PEA, both *in vivo* and *in vitro*, acts by noncompetitive inhibition of DNA nucleotidyltransferase, which in our hypothesis is the primary target of PEA in cells of higher organisms.

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