INFLUENCES OF EDTA ON THE INDUCTION OF CHROMATID ABERRATIONS BY TRIETHYLENEMELAMINE AND ETHYL ALCOHOL

Arnd Michaelis, Hristo Nicoloff[+] and Rigomar Rieger

Institut für Kulturpflanzenforschung der Deutschen Akademie der Wissenschaften, Gatersleben Krs. Aschersleben

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Influences of pre- and simultaneous treatments with ethylenediaminetetraacetic acid (EDTA, Na-salt) on the yield of chromosomal aberrations induced by the polyfunctional alkylating agent 2,4,6-triethyleneimino-1,3,5-triazine (TEM) and by ethyl alcohol (EA) were studied. Both agents are able to induce preferentially localized chromatid aberrations in Vicia faba root tip meristem cells (Ockey 1957, 1960; Michaelis et al. 1959; Rieger and Michaelis 1960) possibly by different modes of action. Methods and preparation technique used were as described by Michaelis and Rieger (1961). Since repeat experiments brought identical results the repetitions are combined in the tables.

10^{-3} and 10^{-4} M EDTA alone for 20 h (18,24,27°C) gave a very slight, mostly insignificant increase of spontaneous aberration rate in Vicia main root meristems. The influence of pretreatment with EDTA (10^{-3}M; 20 h) on the percentage of metaphases with chromatid aberrations induced by $5 \cdot 10^{-5}$ and 10^{-4}M TEM (30 min.) is shown in table 1. Isolocus-breaks, chromatid translocations, triradials, duplication-deletions and deletions were scored. From the experimental results summarized in table 1 it is evident that EDTA pretreatment for 20 h sensitizes against the radiomimetic activity of TEM. The percentage of aberrant metaphases is approximately doubled with pretreatment temperature of 18 or 24°C. The EDTA sensitization is confined to the percentage of aberrant metaphases, the number of aberrations per damaged cell being increased only slightly. Pretreatment with

[+]Central Institute of Plant Industry, Sofia

10^{-3}M EDTA and 27°C was not followed by an increase of TEM-induced aberrations. Using concentrations of 10^{-4}M TEM instead of $5 \cdot 10^{-5}$M, a similar sensitization by EDTA was found when the pretreatment temperature was 24°C. In contrast to the experiment with $5 \cdot 10^{-5}$M TEM, EDTA-pretreatment at 27°C gave an increased aberration percentage, but this increase was less than in the series with pretreatment at 24°C. This difference connected with pretreatment temperature and depending on the TEM concentration used is unexplained up to now.

Table 1 The effect of EDTA-pretreatment (20 h, 10^{-3}M) on the radiomimetic activity of TEM (30 min., 24°C) with 24 h recovery time

Number of scored cells	Pretreatment temperature °C	TEM M	Metaphases with aberrations %	Number of aberrations	Aberrations per damaged cell
400	no pretreatment	$5 \cdot 10^{-5}$	11.75 ± 1.1	47	1.00
400	18	-	0.0	-	-
400	18	$5 \cdot 10^{-5}$	26.75 ± 2.1	113	1.06
400	no pretreatment	$5 \cdot 10^{-5}$	12.25 ± 1.1	49	1.00
400	24	-	2.75 ± 0.7	11	1.00
400	24	$5 \cdot 10^{-5}$	28.0 ± 1.6	115	1.03
400	no pretreatment	$5 \cdot 10^{-5}$	10.75 ± 1.4	43	1.00
400	27	-	1.75 ± 0.8	7	1.00
400	27	$5 \cdot 10^{-5}$	8.0 ± 1.3	32	1.00
600	no pretreatment	10^{-4}	30.7 ± 2.3	217	1.18
600	24	-	1.0 ± 0.6	6	1.00
600	24	10^{-4}	46.2 ± 3.1	302	1.09
400	no pretreatment	10^{-4}	36.5 ± 0.7	156	1.07
400	27	-	1.0 ± 0.5	4	1.00
400	27	10^{-4}	45.0 ± 2.2	181	1.01

In order to rule out the possibility of the different aberration percentages with and without pretreatment being only spurious and produced by different distribution of aberrations over the recovery period, a further experiment with 10^{-4}M TEM

(30 min.) and recovery times of 12, 18, 24 and 30 h was done. As may be seen from table 2, EDTA has a true sensitization effect on TEM because the percentage of metaphases with chromatid aberrations is higher with EDTA-pretreatment for all recovery times tested. Without pretreatment the aberration maximum was found for 24 h recovery, with EDTA-pretreatment it was delayed (30 h).

Table 2 The effect of 20 h EDTA-pretreatment (10^{-3}M, 24°C) on the radiomimetic activity of TEM (30 min., 10^{-4}M, 24°C)

Number of scored cells	Recovery time h	Metaphases with aberrations %	Number of aberrations	Aberrations per damaged cell
a) without EDTA				
200	12	6.5 ± 1.0	13	1.00
200	18	18.0 ± 1.1	38	1.05
200	24	31.5 ± 1.0	69	1.10
200	30	21.5 ± 1.5	45	1.05
b) with EDTA-pretreatment				
200	12	13.0 ± 1.3	26	1.00
200	18	26.5 ± 1.0	55	1.04
200	24	49.0 ± 2.6	112	1.14
200	30	56.0 ± 1.4	137	1.22

As shown in table 3 also with ethyl alcohol (EA) instead of TEM a sensitization effect of EDTA was found. Compared with the control experiment simultaneous treatment (24h) with 10^{-4}M EDTA and 10^{-1}M EA (24°C) increased the percentage of aberrant metaphases significantly. The same effect is clear for 20 h pretreatment with 10^{-3}M EDTA (24°C) before treatment with $2 \cdot 10^{-1}$M EA for 4 h (30°C). In the same way as with TEM, only the percentage of aberrant metaphases was influenced by EDTA and not the number of aberrations per damaged cell.

Although a significant sensitization effect of EDTA on the radiomimetic effectivity of TEM and EA is clear from the experimental results, the chemical nature of the sensitizing reaction remains unknown for the time being. The amount of sensitization is the same as found by Wolff and Luippold (1956) using combined treatments of Vicia faba with EDTA and X-rays.

Table 3 The effect of simultaneous-(10^{-4}) and pretreatment (10^{-3}M) with EDTA on the radiomimetic activity of ethyl alcohol

Treatment	Metaphases with aberrations %	Number Of aberrations	Aberrations per damaged cells
24 h 10^{-1}M EA 24°C	43.5 ± 1.5	94	1.08
24 h 10^{-1}M EA 24°C + 10^{-4}M EDTA (200 cells)	64.5 ± 3.0	141	1.09
4 h $2 \cdot 10^{-1}$M EA 30°C	13.5 ± 1.5	54	1.00
20 h 10^{-3}M EDTA 24°C 4 h $2 \cdot 10^{-1}$MAEA 30°C (400 cells)	35.5 ± 2.8	144	1.01

Since EDTA is a chelating agent removing metal ions from the cell, it is tempting to use this fact for an interpretation of the EDTA sensitization against TEM and EA. Mazia (1954) and Steffensen (1955) pointed out, that metal ions are forming stabilizing bonds in the chromosomes. By removal of these ions the chromosomes might become labilized, reacting more drastically on posttreatment with TEM or EA. In fact most of the experiments with EDTA have been interpreted in this way, when an increase of chromosomal aberrations, of crossing over or a sensitization against aberration production by other means was found (see Steffensen 1961). Since EDTA is able to tie up most of the di- and trivalent metals (heavy metals first), it is impossible to point out the nature of the metals being engaged in sensitization. Furthermore, it is to be expected that the binding of heavy metals inhibits many enzymes and enzyme inhibition instead of metal removal from the chromosomes may also play a role in the EDTA effects on the production of chromatid aberrations.

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