STUDIES ON THE FACTORS AFFECTING THE REJOINING OF CHROMOSOME BREAKS PRODUCED BY X-RAYS. II. EFFECTIVENESS OF DEOXYRIBONUCLEOSIDE TRIPHOSPHATES FOR REDUCTION OF X-RAY-INDUCED CHROMOSOME ABERRATIONS

SHIGEYUKI TANIFUJI, TAKASHI SAHO, AND MASAKI IWABUCHI

Botanical Institute, Faculty of Science, Hokkaido University, Sapporo

Received April 4, 1966

In our previous papers it was suggested that DNA synthesis might be necessary for rejoining of X-ray-induced chromosome breaks because mitomycin C raises the frequency of X-ray-induced chromosome breaks (Matsuura *et al.* 1963; Iwabuchi *et al.* 1966) and a supply of DNA (Matsuura *et al.* 1962) or the mixture of AR, GR, CR and TdR (Iwabuchi *et al.* 1966) results in the decrease of the breaks in frequency.

Taylor *et al.* (1962) obtained the evidence that rather lower concentrations of FUDR reduce the frequency of X-ray-induced bridges and this reduced frequency of bridges is restored to the level of the X-ray control by supplying TdR or BUDR. Thus they proposed a hypothesis that DNA replication is involved in reunion of X-ray-induced chromosome breaks.

However, some doubts about the presumed inhibitory effect of FUDR on thymidylate synthetase are recently indicated by several investigators. Bell and Wolff (1964) indicated that the effect of FUDR is independent of DNA synthesis because it can occur in G_2 (postsynthetic interphase) cells and the effect occurs under conditions that do not inhibit DNA synthesis. Moreover, Bell and Wolff performed experiments in which *Vicia* seeds soaked for 20 hours were subjected to fractionated doses of X-rays and indicated that in G_1 (presynthetic interphase) cells the presence of FUDR during the interval between the two doses do not prevent the rejoining to form interchange aberrations. Kihlman (1962) had paid his attention to the fact that the frequency of chromatid exchanges produced by X-rays was not reduced by pre- and post-treatments with FUDR, although that of chromatid fragments was increased. Later Taylor (1963) indicated that a delay in reunion could not be revealed by treatment with FUDR in the interval between the two doses of X-rays separated by 1 hour, that is, the yields of exchanges and isochromatid breaks with sister reunion (SR) were constant irrespective of the administration of FUDR. Therefore Taylor mentioned that the role of TdR and DNA

The following abbreviations were used in the present paper: RNA, ribonucleic acid; DNA, deoxyribonucleic acid; ATP, GTP, CTP and UTP, triphosphates of adenosine, guanosine, cytidine and uridine, respectively; dATP, dGTP, dCTP and dTTP, triphosphates deoxyadenosine, deoxyguanosine, deoxycytidine and thymidine, respectively; AR, adenosine; GR, guanosine; CR, cytidine; TdR, thymidine; FUDR, 5-fluorodeoxyuridine; BUDR, 5-bromodeoxyuridine.

replication in reunion of chromosomes following radiation is less clear and another component may be involved in most exchanges and SR breaks.

If DNA synthesis is necessary for rejoining of X-ray-induced breaks the presence of all four deoxyribonucleoside triphosphates would be required as substrates for DNA synthesis (cf. Bessman *et al.* 1958). Any procedures resulting in the increase of the amount of these four deoxyribonucleotides within cells would effectively enhance the rejoining of the induced breaks if the ordinary pool of these precursors is not large. Along this line of research the present experiment was undertaken using various deoxy-ribo- and ribonucleotides.

MATERIALS AND METHODS

Ends of freshly cut stems with flowers of *Trillium kamtschaticum* were immersed in phosphate buffer solution (5×10^{-4} M, pH=6.8) to which were added the various chemicals as desired. Seventeen hours after initiation of the chemical treatment the materials were transferred into beakers of tap water and 7 hours later, *i.e.* 24 hours after beginning of the chemical treatment, the materials were irradiated with 70 R of X-rays at an intensity of 10 R/min (160 kVp, 3 mA, 0.5 mm Cu+1.0 mm Al filters).

All nucleotides used — both deoxyribosyl and ribosyl compounds — are of 5'-triphosphate form (nucleosides and ribonucleoside triphosphates; Nutritional Biochemicals Corporation: deoxyribonucleoside triphosphate; Pabst Laboratories Division of Pabst Brewing Co.). Concentrations of chemical solutions were adjusted to nearly 5×10^{-4} M, as a whole, whether mixed or not.

Forty-five hours after irradiation the ovaries of the materials were immersed in 0.05% colchicine solution after removal of the carpels. After the treatment with colchicine for 3 hours the ovaries were fixed with La Cour 2BE, hydrolyzed with 1 N HCl and stained with leuco basic fuchsin.

Two hundred metaphase cells from 5 plants, 40 cells per plant, were scored for chromosomal aberrations in each experimental group except in the X-ray control in which 360 cells from 9 plants were observed. The aberrations scored at metaphase were chromatid gaps, true chromatid breaks (sB'), isolocus chromatid or isochromatid breaks (sB''), various types of exchanges, and minute fragments. Accurate distinction between true chromatid breaks and achromatic lesions or gaps is difficult, then the figures given for these types of aberrations must be regarded as approximative. The problem whether the gap is true aberration or not is in the midst of discussion among various investigators. Therefore the yield of gaps is represented as the supplemental item in summarized table. Attempt was also made to distinguish between isolocus breaks with sister reunion (NUp, NUd & SR) and without sister reunion (NUpd). However, this task was also difficult to make perfectly. In the summarized table, therefore, the sum of various types of isolocus breaks are represented as simple isolocus breaks (sB''). The

most frequent type of exchange was chromatid-chromatid translocation. A few chromatid-isochromatid translocations were observed but the occurrence of chromosomal translocations was rare. The breaks rejoined to form exchanges are represented as rB' or rB'' according to whether they are chromatid- or isolocus chromatid type breaks. Small yield of minute fragments is omitted from the analysis because the exact nature of this fragment is unknown.

The t-test was carried out for comparison of mean values of total simple breaks ($sB' + sB''$), rejoined breaks ($rB' + rB''$) and the sum of the both.

RESULTS

The yield of chromosome breaks of various types is recorded in Table 1. A dose of 70 R produced chromosome breaks of a mean frequency of 271.1 per 100 cells which consist of all types of the aberrations and include gaps also. When the chemical

Table 1. Frequencies of various chromosome aberrations in combination treatments of nucleosides or nucleotides with 70 R of X-rays (per 100 cells)

Treatments	Total number of cells	Simple breaks				Rejoined breaks				Total number of breaks	Probability falling outside the range t	Gaps
		sB'	sB''	Total	Probability falling outside the range t	rB'	rB''	Total	Probability falling outside the range t			
X-rays	360	44.7	79.7	124.4		61.1	1.9	63.0		187.5		84.2
dTTP+X-rays	200	47.0	71.0	118.0	$P > 0.5$	54.5	2.0	56.5	$P > 0.5$	174.5	$P > 0.5$	75.5
(dATP+dGTP+dCTP)+X-rays	200	27.5	74.5	102.0	$P > 0.5$	61.0	1.5	62.5	$P > 0.5$	164.5	$P > 0.5$	89.5
(dATP+dGTP+dCTP+dTTP)+X-rays	200	27.0	53.5	80.5	$0.01 < P < 0.02$	47.0	1.0	48.0	$0.2 < P < 0.3$	128.5	$0.02 < P < 0.05$	69.0
(dATP+dGTP+dCTP+UTP)+X-rays	200	22.0	61.0	83.0	$0.01 < P < 0.02$	42.5	1.5	44.0	$0.05 < P < 0.1$	127.0	$0.02 < P < 0.05$	68.0
(ATP+GTP+CTP+dTTP)+X-rays	200	39.0	51.5	90.5	$0.02 < P < 0.05$	58.5	1.0	59.5	$P > 0.5$	150.0	$0.1 < P < 0.2$	70.0
(ATP+GTP+CTP+UTP)+X-rays	200	29.5	63.0	92.5	$P = 0.05$	60.0	2.5	62.5	$P > 0.5$	155.0	$0.1 < P < 0.2$	74.0
(AR+GR+CR+TdR)+X-rays	200	36.5	56.5	93.0	$0.05 < P < 0.1$	54.5	0.5	55.0	$0.4 < P < 0.5$	148.8	$0.1 < P < 0.2$	77.0

sB' =chromatid breaks; sB'' =isolocus chromatid breaks; rB' = rejoined chromatid breaks; rB'' =rejoined isolocus chromatid breaks.

treatments with nucleosides or nucleotides were combined with irradiation, in any of the combination treatments there is a trend toward the decrease in frequency with almost all of the types of breaks. With rejoined breaks, however, the statistical analysis did not prove the significant difference from the value of the X-ray control in all the combination cases, though in the both cases of (dATP+dGTP+dCTP+dTTP)+X-rays and (dATP+dGTP+dCTP+UTP)+X-rays the extent of the decrease is high. On the contrary, with regard to simple breaks a significant difference was shown in the three cases of (dATP+dGTP+dCTP+dTTP)+, (dATP+dGTP+dCTP+UTP)+, and (ATP+GTP+CTP+dTTP)+X-rays treatments. The value of P, the probability of falling

outside the range t , is $0.01 < P < 0.02$ in both of the first and the second cases and it is $0.02 < P < 0.05$ in the last case. Even in the cases of (ATP+GTP+CTP+UTP)+ and (AR+ GR+CR+TdR)+X-rays treatments, the frequency of simple breaks was reduced to an appreciable extent and P is nearly 0.05 in the former but in the latter it is between 0.05 and 0.1.

In our previous study a considerable decrease in the frequency of total detectable breaks was observed only in the case of simultaneous supply of AR, GR, CR and TdR, whereas the omission of any one, two or three, as a rule, did not affect the frequency (Iwabuchi *et al.* 1966). However, in the same nucleoside test, *i.e.* (AR+GR+CR+TdR)+X-rays, of the present series of the experiments, the sum of simple and rejoined breaks did not differ significantly from that observed in the X-ray control, although a considerable extent of decrease was shown in this combination experiment. The different results between these two series may be due to the different amounts of these precursors which became available for rejoining within the cells in problem.

The frequency of gaps also decreased in some cases of the combination of four nucleotides. However, as mentioned earlier, some proportion of the aberrations scored as gaps might be true chromatid breaks. Then decisive argument with gaps must be avoided.

DISCUSSION

In the present experiments we examined the modifying effects of ribonucleo- and deoxyribonucleotides on the frequency of X-ray-induced chromosome aberrations. A significant decrease in aberration yield was shown in the experiments with dATP+dGTP+dCTP+dTTP mixture and with dATP+dGTP+dCTP+UTP mixture. Some extent of the decrease was observed in the three cases of the combination of four ribosyl compounds (ATP, GTP, CTP, UTP) and the combinations of three ribosyl and one deoxyribosyl compounds (ATP, GTP, CTP, dTTP) and (AR, GR, CR, TdR). When dTTP or the mixture of three deoxyribosyl compounds (dATP, dGTP, dCTP) was supplied there was no remarkable difference from the X-ray control.

It is generally accepted that the majority of the initial breaks produced by X-rays during interphase is restored, the part of the rest is involved in exchange formation and the other portion remained open and appears as simple breakage at mitosis (cf. Lea 1947). The acceleration of the restitution process would cause the decrease in frequency of the total detectable breaks and the prevention of this process would result in the increase of the frequency. Kihlman (1963) observed that the pretreatment with TdR does not influence the sensitivity of *Vicia* chromosomes. In the present experiment, the supply of dTTP did not result in any appreciable difference from the X-ray control with the frequency of breaks. Therefore significant decrease of the total breaks, sum of simple and rejoined breaks, in both cases of (dATP+dGTP+dCTP+dTTP)+ and (dATP+dGTP+dCTP+UTP)+X-ray treatments seems to be the reflection of the accel-

eration of restitution process. When interpreting the results obtained here in the light of the general evidence on DNA synthesis, it will be probably suggested that DNA replication is involved in the rejoining of X-ray-induced breaks, at least in the restitution process to restore the original chromosome continuity, though the problem with the rejoining to form exchanges still remained obscure.

Substitution of UTP for dTTP in the combination of all four deoxyribonucleotides gives the same effect in reducing the yield of breaks as that observed in (dATP+dGTP+dCTP+dTTP)+X-ray treatment. Since uridyate is a common precursor of thymidylate, the conversion of UTP to dTTP might proceed faster than the conversion process of other three ribosyl compounds to corresponding deoxyribonucleoside triphosphates.

A significant difference in the frequency of breaks was not observed between (ATP+GTP+CTP+UTP)+X-ray treatment and the X-ray control. This probably indicates that RNA synthesis does not involve in the process of rejoining, at least with great extent.

SUMMARY

The combination experiments of X-irradiation with some mixture solutions of various nucleotides were undertaken with the chromosomes of the ovular tissue cells of *Trillium kamtschaticum*.

Thymidine triphosphate did neither reduce nor increase significantly both frequencies of simple and rejoined breaks produced by X-rays. An equimolar mixture solution of three deoxyribonucleotides of dATP, dGTP and dCTP did not also result in any significant difference in the frequency of breaks from the X-ray control.

When the four components commonly found in DNA or RNA were supplied as the form of nucleoside or nucleotide, there was a tendency toward the decrease of the frequency of breaks. The significant decrease in the frequency of simple breaks from that of the X-ray control was recognized in the three cases of (dATP+dGTP+dCTP+dTTP)+, (dATP+dGTP+dCTP+UTP)+, and (ATP+GTP+CTP+dTTP)+X-ray treatments.

These results suggest the involvement of DNA synthesis in the process of reunion of X-ray-induced breaks, at least in restitution to restore the original continuity of chromosomes.

ACKNOWLEDGEMENT

The authors are grateful to Dr. Hajime Matsuura for his helpful suggestions in the work and to Professor Ichitaro Harada for his critical reading of the manuscript. We are further indebted to Mr. Takashi Sone for his kind help with X-irradiation treatments.

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