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SUMMARY

To reveal previously suggested host-mediated repair of u.v.-induced lesions in dsRNA of encephalomyocarditis (EMC) virus, two sets of experiments have been carried out: (i) samples of dsRNA of EMC virus were irradiated with different doses of u.v. light and their infectivity was assayed in Krebs II cells, before and after conversion of dsRNA into ss form; (ii) samples of ssRNA of EMC virus were similarly irradiated and their infectivity was assayed before and after conversion of ssRNA into ds form. No evidence for a significant host-mediated repair of dsRNA in this virus-cell system has been obtained.

The infectivity of dsRNA of picornaviruses is known to be more resistant to u.v. irradiation than that of ss virion RNA (Bishop et al. 1967; Zavadova et al. 1968; Zavadova, 1971). Two explanations for this difference can be given: (i) the rate of photoproduct formation upon irradiation with a given u.v. source is lower in the case of dsRNA than in the case of ssRNA (Bishop et al. 1967); this explanation is in line with the results of a direct determination of photoproducts formed upon a high dose u.v. irradiation of ss and ds synthetic polynucleotides (Johns, 1967; we shall refer to this explanation as the resistance hypothesis), and (ii) host cells are able to repair some u.v.-induced lesions in ds- but not in ssRNA molecules (the repair hypothesis). The latter explanation was suggested by Zavadova (Zavadova et al. 1968; Zavadova, 1971) and was supported by the following observations.

In experiments with encephalomyocarditis (EMC) virus dsRNA, the slopes of u.v. dose-survival curves increased when the infectivity of irradiated RNA was assayed either in cells treated with caffeine, a known inhibitor of DNA repair, or in cells derived from patients with Xeroderma pigmentosum which are known to be deficient in DNA repair; on the other hand, the slopes of the survival curves obtained with irradiated ssRNA in such cells were essentially the same as in normal cells.

Repair of RNA molecules, if it exists, would have wide implications and merits further studies. We have performed experiments of two kinds using the EMC virus-specific RNA-Krebs II cell system. Preparations of dsRNA were irradiated with different doses of u.v. light and their infectivity was assayed before and after denaturation. The resistance hypothesis predicts that the conversion of irradiated dsRNA into the ss form should not affect the slope of the u.v. dose-survival curve, while under the repair hypothesis an increase in the slope is to be expected because lesions reparable in the dsRNA would become non-reparable in the ss form. The u.v. dose-survival curves have also been determined before and after the conversion of irradiated ssRNA preparations into a ds form by hybridization with complementary strands. The prediction of the resistance hypothesis is that the slope should again be unchanged, whereas the repair hypothesis demands a decrease in the slope. The experimental findings reported here are more in accord with the resistance hypothesis than with the repair hypothesis.
Fig. 1. Survival curves of virus-specific RNAs of EMC virus. (a) O—O, ssRNA; ●—●, dsRNA. Samples irradiated for 60 s received a dose of 35.7 nE/cm². (b) ●—●, dsRNA; ○—○, ssRNA derived from the irradiated dsRNA. (c) ○—○, ssRNA; □—□, ssRNA subjected to denaturation and subsequent incubation at 20 °C. (d) ○—○, ssRNA; •—•, dsRNA derived from the irradiated ssRNA by annealing with complementary strands; ●—●, dsRNA. For description of methods see text and legend to Fig. 2.

Preparations of unlabelled and ³H-labelled ss virion RNA and the ds replicative form RNA of EMC virus were obtained by extraction of the purified virus or virus-infected Krebs II cells, respectively, as described previously (Chumakov & Agol, 1976; Chumakov, 1979). The samples of RNA were dissolved at a concentration of about 1 μg/ml in a solution containing 0·1 M-NaCl, 0·01 M-tris-HCl, pH 7·3, 0·001 M-EDTA (STE) and were irradiated for different periods of time with a ‘Germicid-F’ lamp (Hungary). In some experiments, the dose of irradiation was estimated by the UMP photolysis method (Wierchowski & Shugar, 1959) and expressed in nanoEinstein/cm² (nE/cm²). In different experiments the dose rates varied approx. from 0·055 to 0·090 nE/cm²·s. The infectivity of the RNAs was assayed in DEAE-dextran-treated Krebs II cells (K. M. Chumakov, unpublished data). The specific infectivity of non-irradiated ss- and dsRNAs was 10² to 2 × 10⁶ and 10⁵ to 10⁶ p.f.u./μg, respectively.

In agreement with the data of Zavadova et al. (1968), dsRNA of EMC virus was inactivated by u.v. light four to five times more slowly than ssRNA (Fig. 1 a). Experiments in which the effect of denaturation of irradiated dsRNA on its u.v. dose-survival curve was investigated were performed in the following way. Preparations of dsRNA were dissolved in 0·1 M-LiCl, 0·01 M-tris-HCl, pH 7·3, 0·001 M-EDTA (LTE) at a concentration of 7 to
Fig. 2. Fractionation of a denatured preparation of dsRNA. A 3H-labelled non-irradiated sample of dsRNA used in the experiment shown in Fig. 1(b) was denatured and subjected to centrifugation in a 5 to 20% sucrose concentration gradient as described in the text.

10 μg/ml and were irradiated as described. At selected intervals, two samples each of 0.1 ml were withdrawn, one of which was diluted 10-fold with STE and kept frozen at −10 °C until assayed for infectivity. A 0.4 ml amount of dimethyl sulphoxide (DMSO) was added to the other sample to a final concentration of 80%. After a 3 min incubation at room temperature, which is sufficient for melting dsRNA under the conditions employed (Chumakov, 1979), 1 ml of STE containing about 50 μg of tRNA was added. The RNA was precipitated with 3 vol. of ethanol, dissolved in 0.2 ml of 2.5 × 10⁻⁴ M-EDTA, pH 7.0, and subjected to centrifugation for 210 min at 50,000 rev/min in a Beckman SW50.1 rotor in a sucrose concentration gradient prepared in 2.5 × 10⁻⁴ M-EDTA, pH 7.0. The sedimentation profile of denatured dsRNA corresponding to the non-irradiated sample from the experiment presented in Fig. 1(b) is shown in Fig. 2 (similar profiles were obtained also with denatured irradiated samples). The lighter peak A contained mainly the intact ssRNA molecules as judged by the following criteria: (i) about 90% of this material was rendered acid-soluble after digestion with pancreatic RNase in STE (5 μg/ml, 30 °C, 30 min); (ii) it sedimented predominantly at 35S when analysed in a sucrose concentration gradient prepared in STE; (iii) its specific infectivity was usually at least one order of magnitude higher than that of the original dsRNA (data not shown). Fractions corresponding to the peak A of dsRNA samples irradiated for different periods of time were frozen and assayed for infectivity simultaneously with the samples of non-denatured dsRNA. The survival curves obtained in one of these experiments are presented in Fig. 1(b). The denaturation of irradiated dsRNA resulted, somewhat unexpectedly, in a decrease in the slope of the curve rather than in an increase, as should have been the case if the repair hypothesis were correct. This decrease in the slope may be explained by a non-enzymic reversion of some u.v.-induced lesions in RNA molecules (Wierchowski & Shugar, 1959) which could occur during the treatments required for the denaturation of dsRNA and isolation of ssRNA. This explanation was supported by the fact that in control experiments the treatment of irradiated ssRNA analogous to that used for denaturation of dsRNA (except that instead of being centrifuged the samples of ssRNA were incubated at 20 °C for 210 min) resulted in
a similar decrease in the slope of the survival curve (Fig. 1c). Thus, the experiments with
denaturation of the irradiated dsRNA (Fig. 1b) provided no evidence in favour of the
repair hypothesis.

It may be argued that the non-enzymic reactivation of dsRNA exceeds that of ssRNA to
an extent sufficient to mask the effect of the enzymic repair. Therefore, we performed
another set of experiments, the results of which confirm our conclusion on the absence of
significant host cell-mediated repair of irradiated dsRNA.

In these experiments, the infectivity of ssRNA samples irradiated for different periods of
time was determined before and after conversion of the ssRNA into a ds form. This con-
version was achieved by annealing the preparations of ssRNA under study with the highly
irradiated dsRNA of EMC virus (2 min irradiation; estimated surviving infectivity about
10^{-8}). This procedure resulted in the formation of duplexes, the plus RNA strand of which
was only slightly irradiated whereas the minus strand was totally inactivated (duplexes
with both strands highly inactivated were probably also formed but their presence could
not affect the results of infectivity assays). As shown previously (Chumakov & Agol, 1976),
the inactivation of the minus RNA strand did not appreciably influence the infectivity of
dsRNA molecules and, therefore, the infectivity of annealed samples reflected solely the
extent of inactivation of the plus RNA strands of these duplexes.

A solution of EMC virus ssRNA (20 to 30 µg/ml in LTE buffer) was irradiated for
different periods of time and at each interval two samples (0.1 ml) were removed. One of
these was added to 0.1 ml of LTE and the other to 0.1 ml of LTE containing 2 to 3 µg/ml
of highly irradiated dsRNA. After addition of 0.8 ml of DMSO to each aliquot and in-
cubation at room temperature for 3 min (denaturation), the concentrations of LiCl and
DMSO were changed to 0.4 M and 60%, respectively and the incubation continued at 30 °C
for an additional 30 min (annealing) (Chumakov, 1979). The RNA was precipitated with
ethanol in the presence of carrier tRNA, dissolved in 0.01 M-tris-HCl, 0.0001 M-EDTA,
pH 7.3, heated at 50 °C for 2 min to disrupt aggregates (Roy & Bishop, 1970) and after
addition of NaCl to a final concentration of 0.1 M, the samples annealed with dsRNA were
treated with pancreatic RNase (0.01 µg/ml, 30 min, room temperature) to inactivate
ssRNA species; the samples annealed in the absence of dsRNA were treated in the same
way, but without addition of the RNase. The infectivity of the two sets of ssRNA samples
was assayed simultaneously. As a control, a set of dsRNA samples irradiated for different
periods of time was assayed in each experiment of this type. Prior to determination of
infectivity, these samples were treated with 0.4 M-LiCl and 60% DMSO for 30 min at
30 °C, precipitated with ethanol, dissolved and heated as described above; then NaCl was
added to 0.1 M. The survival curves obtained in one of these experiments are shown in
Fig. 1(d). It is seen that the conversion of irradiated ssRNA into a ds form resulted only in a
minor, if any, decrease in the slope.

Thus both approaches used in the present study failed to reveal any significant repair of
u.v.-irradiated dsRNA of EMC virus in Krebs II cells. In addition, no apparent effect of
treating the cells with caffeine on the survival curve of irradiated dsRNA was observed in
this system (data not shown). It can be argued that host cells used here differed from those
used by Zavadova et al. (1968) with respect to the efficiency of dsRNA repair. Such a
possibility could certainly not be excluded, but it should be noted that the difference in u.v.
sensitivities of ds- and ssRNAs in our experiments was very close to that observed by
Zavadova et al. (1968).

Another point also merits consideration. The value of e^{-1D} (the dose which resulted in
e-fold inactivation of RNA, a reference point accepted in quantitative studies on u.v.
inactivation) for ssRNA obtained in the present study (~ 1.5 nE/cm²) lies very close to that
calculated from the data of Bishop et al. (1967) for ssRNA of poliovirus (~ 1.4 nE/cm²).
However, the dsRNA of EMC virus in our study has been found to be much more resistant to u.v. treatment ($e^{-1}D \sim 7.8 \text{nE/cm}^2$) than the dsRNA of poliovirus in the work of Bishop et al. (1967) ($\sim 2.4 \text{nE/cm}^2$). It remains to be established whether this difference in u.v. sensitivity of dsRNA of the two viruses reflects some inherent peculiarities of their structure or is due to a difference in the experimental techniques used.

It should be noted that our experiments were designed to reveal only that kind of repair which affects dsRNA but not ssRNA molecules. Any other types of host-mediated repair mechanisms, independent of the strandedness of RNA, could not be detected in the present study.

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REFERENCES


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