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## Detergent Effects on a Reverse Transcriptase Activity and on Inhibition by Rifamycin Derivatives


Abstract. *A reverse transcriptase activity, extracted from virus-transformed cells, is activated by very low concentrations of nonionic detergents. These same detergents also significantly reduce the effectiveness of certain rifamycin derivatives as inhibitors of the polymerase activity when the detergents are present at micelle-forming concentrations.*


The bacterial DNA-dependent RNA polymerase is specifically inhibited by certain rifamycin derivatives (*1*). Such specificity for a particular polymerase has encouraged attempts to test many rifamycin derivatives for specific inhibition of the viral reverse transcriptase (*2*). This testing of derivatives has been done in the presence of nonionic detergents with whole virus particles as the source of reverse transcriptase activity.

In our study, one previously tested and two new rifamycin derivatives (*3*) are compared for their inhibitory effect on an RNA-instructed DNA polymerase (RIDP) from transformed tissue culture cells (*4*) as a function of nonionic detergent concentrations. We were able to show that nonionic detergents are important activators of the RIDP. However, at detergent concentrations significantly greater than those required to activate the RIDP, the rifamycin derivatives lose inhibitory effectiveness. This loss of effectiveness could be correlated to the formation of detergent micelles.

As used in this report RIDP activity is defined by the assay given in the caption to Fig. 1. The activity was extracted (*5*) from UC1-B tissue culture cells transformed by Moloney leukemia virus (*6*), but could not be detected in uninfected, nontransformed cells. The extraction procedure normally included sonication of washed cells, precipitation at 50 percent saturated ammonium sulfate, resuspension, and solubilization of the RIPD activity with a nonionic detergent.

The nonionic detergent concentration in the RIDP assay strongly influenced the RIDP activity. As the concentration of detergent in the assay was reduced below approximately 0.05 m*M*, an increasing amount of RIDP activity was lost (Fig. 1). The detergent requirement for full activity could not be satisfied by polyethylene glycol. This activation by detergents was not altered by as much as a fourfold increase in the protein concentration of the assay [bovine serum albumin (BSA) added] or

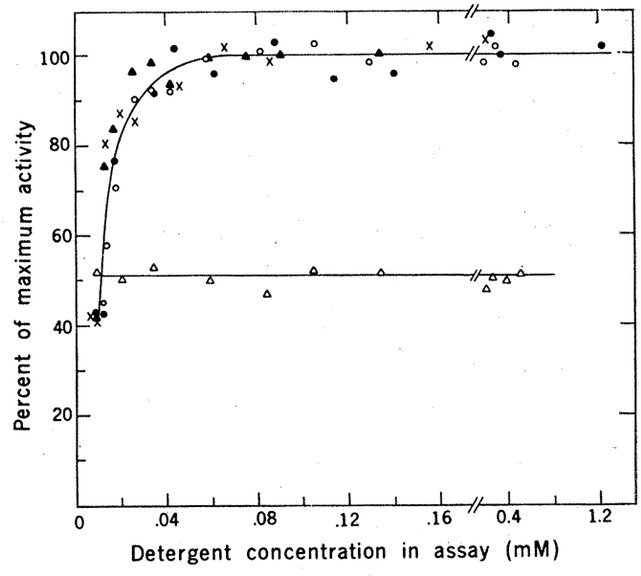



Fig. 1. Effect of nonionic detergents on RIDP activity. Assays were done in 100-$\mu$l volumes which consisted of 82 to 94 m*M* tris-HCl (*p*H 7.8), 100 m*M* KCl, 0.2 m*M* dithiothreitol, 0.02 m*M* [$^3$H]deoxythymidine triphosphate (1 c/mmole), 10 $\mu$g of polyriboadenylate·oligodeoxythymidylate per milliliter, 0.1 m*M* $MnCl_2$, and 2 to 4 percent glycerol. The detergents were added to the assays to yield the concentrations indicated. Assays were started by the addition of a chosen amount of enzyme extract and were incubated for 30 minutes at 37°C. All points on each curve were determined from at least duplicate assays. The maximum activity for each detergent was determined by averaging the activities in assays with detergent concentrations $\geq$ 0.06 m*M*. (○) The detergent added in the assay was Triton X-100, an octylphenoxypolyethoxyethanol (Rohm and Haas) with an average of nine to ten polyoxyethylene residues and a molecular weight of ~ 650. The RIDP was solubilized and stored in 0.125 percent Triton X-100 and diluted to 0.0032 percent for addition to the assays; the protein concentration was 0.45 $\mu$g per assay, the maximum activity was 200 pmole hour$^{-1}$ $\mu$g$^{-1}$, and the dimethyl sulfoxide concentration was 0.24 to 0.40 percent. (●) The detergent added in the assay was Triton DN-65 (Rohm and Haas), a nonionic detergent prepared by reacting 1 mole of a mixture of *n*-octyl and *n*-decyl alcohols with approximately 7 moles of ethylene oxide and approximately 2 moles of propylene oxide; the molecular weight was ~ 570. The RIDP was solubilized and stored in 0.1 percent Triton DN-65 and diluted to 0.0025 percent for addition to the assays; the protein content was 0.38 $\mu$g per assay, the maximum activity was 250 pmole hour$^{-1}$ $\mu$g$^{-1}$, and the dimethyl sulfoxide concentration was 0.25 to 0.30 percent. (×) The detergent added in the assay was Triton X-1017, prepared by chromatographing Triton X-100 according to the method of Kelly and Greenwald (*7*) and pooling molecules containing 10 to 17 polyoxyethylene residues (yield, 17.6 percent); the molecular weight was ~ 790. The RIDP was solubilized and stored in 0.1 percent Triton X-1017 and diluted to 0.0025 percent for addition to the assays; the protein content was 0.50 $\mu$g per assay, and the maximum activity was 266 pmole hour$^{-1}$ $\mu$g$^{-1}$. (▲) The detergent added in the assay was Brij-35 (Sigma), a polyoxyethylene-23 lauryl ether with a molecular weight of ~ 1200. The RIDP was solubilized and stored in 0.1 percent Triton DN-65 and diluted to 0.0025 percent for addition to the assay. The concentration of Triton DN-65 (0.0088 m*M*) added with the enzyme was included in the detergent concentration given in the figure. The protein concentration was 0.38 $\mu$g per assay and the maximum activity was 132 pmole hour$^{-1}$ $\mu$g$^{-1}$. (△) Instead of a detergent, polyethylene glycol-400 [molecular weight ~ 400 (Fisher)] was added in the assay. The RIDP was solubilized and stored in 0.1 percent Triton DN-65 and diluted to 0.0025 percent for addition to the assay. The concentration contributed by the Triton DN-65 (0.0088 m*M*) added with the enzyme was included in the detergent concentration indicated. The protein was 0.38 $\mu$g per assay and the maximum activity (determined from the maximum activity with the Triton DN-65) was 132 pmole hour$^{-1}$ $\mu$g$^{-1}$.

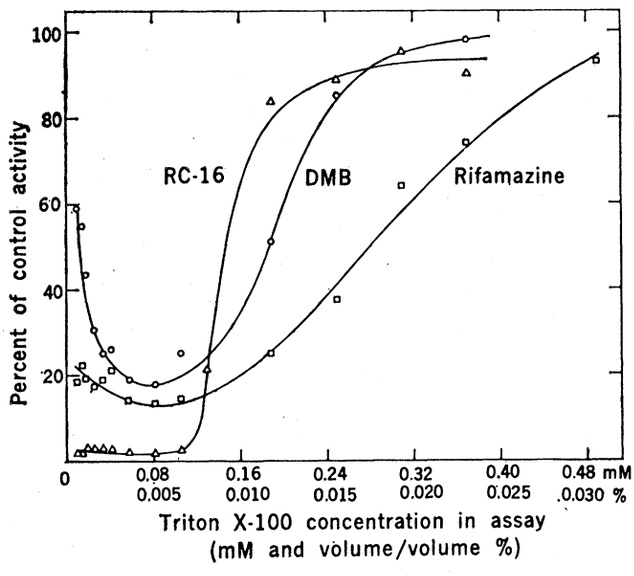
by the presence of 0 to 0.4 percent dimethyl sulfoxide in the assay.

The RIDP activity was also found to undergo an irreversible inactivation when the concentration of the detergents in the extracts was reduced below 0.04 m*M* by dilution or dialysis. However, the remaining activity still exhibited activation characteristics very similar to those shown in Fig. 1 for fully active RIDP (*5*).

In addition to the use of highly diluted samples of the very active RIDP extracts obtained with detergent solublization, the minimum activity obtained without detergent solubilization was assayed for detergent activation and was found to have characteristics identical to those of the solubilized RIDP. The above result suggests that the detergents are activators of the RIDP activity in addition to being solubilizing agents, and that the activation by detergents is an intrinsic property of the enzyme rather than an artifact introduced by detergent solubilization. The fact that the purified Triton X-100 (Triton X-1017) activated as well as commercial Triton X-100, Triton DN-65, and Brij-35 makes unlikely the possibility that the activation is caused by a minor component or contaminant in the three crude detergents. The activation by the detergents and not by polyethylene glycol suggests that the enzyme has an important, accessible hydrophobic region.

The detergent concentration in the RIDP assay also significantly altered the extent of RIDP inhibition obtained from a given amount of a rifamycin derivative. The results for three derivatives are summarized in Fig. 2. A Triton X-100 concentration of 0.005 percent allowed maximum inhibition by all three derivatives, even though their sensitivity to the detergent concentration was very different. Both lower and higher concentrations of Triton X-100 caused a reduction in the RIDP inhibition by the rifamycin derivatives. The reduced ability of the derivatives to inhibit the RIDP at low detergent concentrations may be an artifact caused by the incomplete enzyme activation at these concentrations. It is interesting, however, that the extent of reduced RIDP inhibition at low detergent concentrations seems to be dependent on the rifamycin derivative causing the inhibition.

The reduced ability of the rifamycin derivatives to inhibit the RIDP activity at high detergent concentrations was found to correlate with the formation of detergent micelles. Figure 3 shows the relief of RIDP inhibition by a rifamycin derivative and the micelle formation as a function of the concentration of three detergents. The correlation between relief of RIDP inhibition and micelle formation shown in Fig. 3 suggested an extraction of the rifamycin derivatives into detergent micelles. Evidence for this extraction was provided by co-chromatography of one derivative, DMB (see Fig. 3 legend), and Triton X-100 on Sephadex gel filtration columns. More than 80 percent of the DMB applied to a Sephadex G-50 column in 0.5 percent Triton



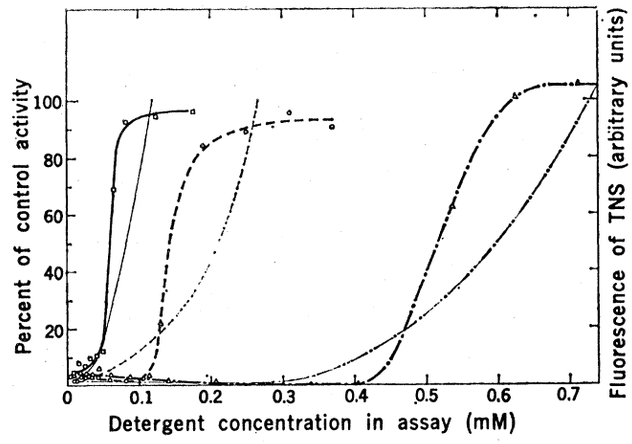




Fig. 2 (left). Inhibition of RIDP by three rifamycin derivatives as a function of Triton X-100 concentration. Assays were done as described in Fig. 1 with the additions indicated below. Each assay contained 0.45 $\mu$g of protein of the same RIDP extract (solubilized by 0.1 percent Triton X-100). Control activity (170 to 190 pmole hour$^{-1}$ $\mu$g$^{-1}$) was taken as the average of at least four points done in duplicate at concentrations $\geq$ 0.005 percent on a Triton X-100 activation curve which was run at the same time as the curve shown. (○) Dimethyl sulfoxide (0.25 percent) and [25 $\mu$g/ml (0.027 m*M*)] 2′,6′-dimethyl-*N*$^{1'}$-benzyl-[*N*$^{1'}$ desmethyl]rifampicin (DMB). (□) Dimethyl sulfoxide (0.40 percent) and dirifaldehyde azine (rifamazine)[40 g/ml (0.027 m*M*)]. (△) Dimethyl sulfoxide (0.30 percent) and rifaldehyde (*N*-aminoazacyclohexadecane) hydrazone (rifacyclo-16) [7.5 $\mu$g/ml (0.0079 m*M*)]. Fig. 3 (right). Inhibition of RIDP by rifazacyclo-16 and micelle formation as a function of three detergents. Assays of RIDP, as described in Fig. 1, included dimethyl sulfoxide (0.3 percent) and rifazacyclo-16 (7.5 $\mu$g/ml). Control activity was determined by averaging 18 assays which were $\geq$ 0.06 m*M* in the appropriate detergent and omitted only the rifazacyclo-16. (△) Triton DN-65 was added in the assays which contained 0.38 $\mu$g of protein of an RIDP extracted with 0.1 percent Triton DN-65. Control activity was 226 pmole hour$^{-1}$ $\mu$g$^{-1}$. (○) Triton X-100 was added in the assays which contained 0.45 $\mu$g of protein of an RIDP extracted with 0.1 percent Triton X-100. The control activity was 186 pmole hour$^{-1}$ $\mu$g$^{-1}$. (□) Brij-35 was added in the assays which contained 0.38 $\mu$g of protein of an RIDP extracted with 0.1 percent Triton DN-65. The control activity was 132 pmole hour$^{-1}$ $\mu$g$^{-1}$. The concentration of the Triton DN-65 (0.0088 m*M*) added with the enzyme was not included in the detergent concentration indicated in the figure. Detergent micelle formation was measured as the fluorescence of 2-*p*-toluidinylnaphthalene-6-sulfonate (TNS) at 440 nm with excitation at 320 nm in detergent solutions which were 100 m*M* tris-HCl (*p*H 7.8), 100m*M* KCl, 4 percent glycerol, 0.3 percent dimethyl sulfoxide and 1 $\times$ 10$^{-3}$ m*M* TNS. [TNS strongly fluoresces in the nonaqueous environment of detergent micelles while giving only a weak fluorescence in the buffer (*5*).] (—•—) Triton DN-65 added; (— —) Triton X-100 added; (—) Brij-35 added.

X-100 was eluted at the exclusion volume with the detergent micelles, while less than 0.1 percent was eluted at the exclusion volume when detergent was omitted from the DMB solution.

Our results indicate that the detergent concentrations to be used in assays which test for the inhibition of RIDP by rifamycin derivatives should lie between the concentration required for full RIDP activation and the one which gives micelle formation. The range of Triton X-100 concentrations which meets these requirements is very narrow, from 0.004 to 0.006 percent. These same limitations on appropriate detergent concentrations would apply to Nonidet P-40 (Shell Chemicals), a commonly used detergent similar to Triton X-100. However, Triton DN-65 is not subject to these restrictive limitations. Even though Triton DN-65 has approximately the same efficiency in solubilizing the RIDP in the extraction procedure as does Triton X-100 and activates as well as Triton X-100, it has a concentration range between full activation of the RIDP and micelle formation which is much wider, 0.004 to 0.023 percent. In addition to indicating the unusual appropriateness of Triton DN-65 for RIDP studies involving rifamycin derivatives, the wide range of Triton DN-65 concentrations between full RIDP activation and micelle formation has further implications in terms of the activation phenomenon. The mechanism of action must be a molecular one because the activation by the three different detergents occurs at comparable molar concentrations that are approximately an order of magnitude below those of micelle formation by Triton DN-65.

In summary, we have found that the nonionic detergent concentration is a significant variable in RIDP assays. The detergents not only are activators of the RIDP activity but also form micelles that interfere with RIDP inhibition by rifamycin derivatives.

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## Electrical Responses of Insect Central Neurons: Augmentation by Nerve Section or Colchicine

Abstract. *Intracellular recording from the somata of central motor neurons in the cockroach* Periplaneta americana *normally shows little or no electrical response evoked by soma depolarization or by antidromic stimulation. Within 4 days after either cutting the axon or administration of colchicine, large action potentials can regularly be recorded from cell bodies of metathoracic motor neurons. Each experimental procedure evokes formation of a dense, perinuclear ribonucleic acid ring in the soma of neurons showing augmented electrical responses.*

Cellular communication may occur either by way of specific chemical substances or by electrical events. Because of the specialized electrical aspect of communication in the nervous system and the high level of metabolic activity, neurons provide an exceptional opportunity to examine the relation between biochemical and bioelectrical activity. The reaction of a nerve cell body to injury of its axon (axon reaction) is known to involve major changes in ribonucleic acid (RNA) and protein metabolism. In neurons of vertebrates, these metabolic changes are correlated with alterations in the organization of various cellular components, such as the disappearance of large basophilic Nissl aggregates of rough endoplasmic reticulum, enlargement and vesiculation of the Golgi apparatus, and an increase in nucleolar size (*1*). The above cytological changes associated with the axon reaction are correlated with an increased electrical excitability in cat motor neurons (*2*).

Central neurons of some insects also show marked morphological changes in response to axon injury. The most obvious component is a dense perinuclear ring of RNA that reaches its maximum

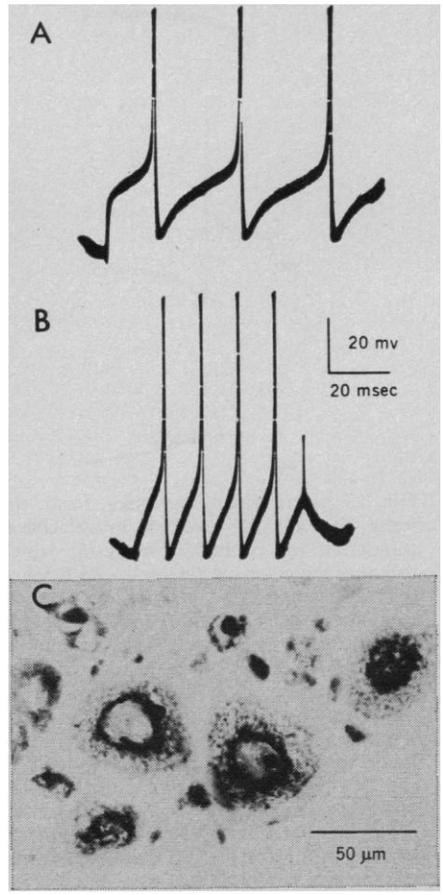


Fig. 1. Intracellular recording of responses in the soma of cell 28 evoked by depolarization of the soma membrane. (A) Six days after cutting the axon of cell 28 in nerve 5. The depolarizing pulse applied across the soma membrane is maintained for the duration of the record. (B) Four days after treatment with 5 percent colchicine. The depolarizing pulse is maintained during the burst of activity and its termination is indicated by the small vertical deflection at the extreme right. Note the large overshooting action potentials present in both records. Normally little or no response to intracellular depolarization can be recorded from the soma of this cell. (C) Section stained with Pyronine-malachite green showing motor neuron cell bodies in the metathoracic ganglion 4 days after treatment with 5 percent colchicine. The dense perinuclear basophilic ring in the two large central cells is similar to the RNA ring seen after nerve section (*3*).