Lawrence Berkeley National Laboratory
Recent Work

Title
DETERGENT EFFECTS ON A REVERSE TRANSCRIPTASE ACTIVITY AND ON INHIBITION BY RIFAMYCIN DERIVATIVES

Permalink
https://escholarship.org/uc/item/3tq2j2js

Author
Thompson, Frances M.

Publication Date
1972-08-10

eScholarship.org Powered by the California Digital Library
University of California

Submitted to Science

LBL-919
Preprint

DETERGENT EFFECTS ON A REVERSE TRANSCRIPTASE ACTIVITY AND ON INHIBITION BY RIFAMYCIN DERIVATIVES

Frances M. Thompson, Louis J. Libertini, Urs R. Joss
and Melvin Calvin

RECEIVED
LAWRENCE
RADIATION LABORATORY

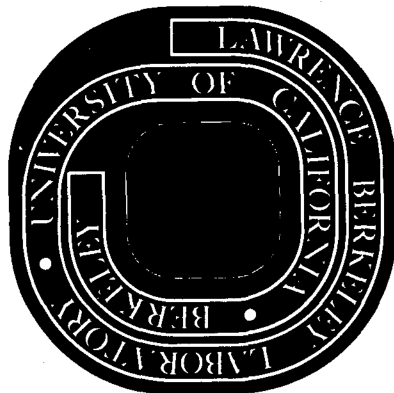
LIBRARY AND
DOCUMENTS SECTION

August 10, 1972

AEC Contract No. W-7405-eng-48

For Reference

Not to be taken from this room



LBL-919

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

0 0 0 0 3 8 0 2 0 9 7

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

Abstract. A reverse transcriptase activity, extracted from virally transformed cells, is activated by very low concentrations of non-ionic 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 (2,3,4). This testing of derivatives has been done in the presence of non-ionic detergents using whole virus particles as the source of reverse transcriptase activity.

In the study reported here, one previously tested and two new rifamycin derivatives (5) are compared for their inhibitory effect on an RNA-instructed DNA polymerase (RIDP) from transformed tissue culture cells (6) as a function of non-ionic detergent concentrations. We were able to show that non-ionic 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.

RIDP activity as used in this report is defined by the assay given in the caption to Figure 1. The activity was extracted (7) from UCl-B tissue culture cells transformed by Moloney leukemia virus (8), but could not be detected in un-infected, non-transformed cells. The extraction procedure normally included a step which solubilized the RIDP activity by using a non-ionic detergent.

The non-ionic 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 mM an increasing amount of RIDP activity was lost. The results are summarized in Figure 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 four-fold increase in the protein concentration of the assay (BSA added) or by the presence of 0-0.4% DMSO 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 mM by dilution or dialysis. However, the remaining activity still exhibited activation characteristics very similar to those shown in Figure 1 for fully active RIDP (7).

In addition to using highly diluted aliquots of the very active RIDP extracts obtained with detergent solubilization, the minimal activity obtained without detergent solubilization was assayed for detergent activation and 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 a solubilizing agent 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 present in the RIDP assay was also found to significantly alter the extent of RIDP inhibition obtained from a given amount of a rifamycin derivative. The results for three derivatives are summarized in Figure 2. A Triton X-100 concentration of 0.005% 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 seen in Figure 3 suggested an extraction of the rifamycin derivatives into detergent micelles. Strong evidence for this extraction was provided by co-chromatography of one derivative, DMB, with Triton X-100 on Sephadex gel filtration columns. More than 80% of the DMB applied to a Sephadex G-50 column in 0.5% Triton X-100 was eluted at the exclusion volume with the detergent micelles while less than 0.1% was eluted at the exclusion volume when detergent was omitted from the DMB solution.

The results presented here 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%. These same limitations on appropriate detergent concentrations would apply to Nonidet P-40 (Shell Chemicals), a commonly used detergent very 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%-0.023%. 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 since the activation by the three different detergents occurs at comparable molar concentrations which are approximately an order of magnitude below those of micelle formation by Triton DN-65.

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

Frances M. Thompson

Louis J. Libertini

Urs R. Joss

Melvin Calvin

Laboratory of Chemical Biodynamics

University of California

Berkeley, California 94720

References and Notes

1. W. Wehrli and M. Staehelin, Bacteriol. Rev. 35, 290 (1971).
2. C. Gurgo, R. K. Ray, L. Thiry and M. Green, Nature New Biol. 229, 111 (1971).
3. S. Tang, F. Herrera, R. G. Smith, M. Reitz, G. Lancini, R. Ting and R. Gallo, J. Nat. Cancer Inst., in press.
4. M. Green, J. Bragdon and A. Rankin, Proc. Nat. Acad. Sci. USA 69, 1294 (1972).
5. A. Tischler, U. R. Joss and M. Calvin, submitted to J. Org. Chem.
6. Both transformed and non-transformed cells were kindly supplied by Dr. A. J. Hackett, Cell Culture Laboratory (PH 43-63-13, Special Virus-Cancer Program) Naval Biomedical Research Laboratory, University of California, Berkeley.
7. F. M. Thompson, L. J. Libertini, U. R. Joss and M. Calvin, submitted to Biochemistry.
8. A. J. Hackett and S. S. Sylvester, Nature, in press.
9. J. Kelly and H. L. Greenwald, J. Phys. Chem. 62, 1097 (1958).
10. This work was supported, in part, by the U.S. Atomic Energy Commission. Support is gratefully acknowledged to the Elsa U. Pardee Foundation for Cancer Research (F.M.T. and U.R.J.) and The Damon Runyon Fund for Cancer Research (L.J.L.).

0 0 0 0 3 8 0 2 0 0 8

Figure Legends

Figure 1. Effect of non-ionic detergents on RIDP activity. Assays were done in 100 μl which is 82-94 mM Tris-HCl (pH = 7.8), 100 mM KCl, 0.2 mM dithiothreitol, 0.02 mM ^{3}H-dTTP (1 C/mmole), 10 μg/ml poly-rA:oligo-dT, 0.1 mM $MnCl_2$, and 2-4% glycerol. The detergents were added to the assays to yield the concentrations indicated in the figure. Assays were started by the addition of a chosen amount of enzyme extract and were incubated for 30 min at 37°C. All points on each curve were determined from at least duplicate assays. Maximal activity for each detergent was determined by averaging the activities in assays with detergent concentrations ≥0.06 mM.

(○) The detergent added in the assay was Triton X-100, an octyl phenoxypolyethoxyethanol (Rohm and Haas) with an average of 9-10 polyoxyethylene residues; M.W.∿ 650. The RIDP used was solubilized and stored in 0.125% Triton X-100 and diluted to 0.0032% for addition to the assays; protein = 0.45 μg per assay and maximal activity = 200 pmol/hr/μg; dimethylsulfoxide (DMSO) = 0.24-0.40%.

(●) The detergent added in the assay was Triton DN-65 (Rohm and Haas), a non-ionic detergent prepared by reacting one mole of a mixture of n-octyl and n-decyl alcohols with approximately seven moles of ethylene oxide and approximately two moles of propylene oxide; M.W.∿ 570. The RIDP used was solubilized and stored in 0.1% Triton DN-65 and diluted to 0.0025% for addition to the assays; protein = 0.38 μg per assay and maximal activity = 250 pmol/hr/μg; DMSO = 0.25-0.30%.

(x) The detergent added in the assay was Triton X-1017, prepared by chromatographing Triton X-100 according to the method of Kelly (9) and pooling molecules containing 10 to 17 polyoxyethylene residues (yield = 17.6%); M.W.∿ 790. The RIDP used was solubilized and stored in 0.1% Triton X-1017 and diluted to 0.0025% for addition to the assays; protein = 0.50 μg per assay and maximal activity = 266 pmol/hr/μg.

(▲) The detergent added in the assay was Brij-35 (Sigma), a polyoxyethylene-23-lauryl ether; M.W.∿ 1200. The RIDP used was solubilized and stored in 0.1% Triton DN-65 and diluted to 0.0025% for addition to the assay. The 0.0088 mM contributed by the Triton DN-65 added with the enzyme was included in the detergent concentration given in the figure. Protein = 0.38 μg per assay and maximal activity = 132 pmol/hr/μg.

(△) Polyethylene glycol-400 (Fisher) was added in the assay instead of a detergent; M.W.∿ 400. The RIDP used was solubilized and stored in 0.1% Triton DN-65 and diluted to 0.0025% for addition to the assay. The 0.0088 mM contributed by the Triton DN-65 added with the enzyme was included in the detergent concentration given in the figure. Protein = 0.38 μg per assay and maximal activity (determined from the maximal activity with the Triton DN-65) = 132 pmol/hr/μg.

Figure 2. RIDP inhibition by three rifamycin derivatives as a function of Triton X-100 concentration. Assays were done as described in Figure 1 with the additions indicated below. Each assay contained 0.45 μg protein of the same RIDP extract (solubilized by 0.1% Triton X-100). Control activity (170-190 pmol/hr/μg) was taken as the average of at least 4 points done in duplicate at concentrations ≥0.005% on a Triton X-100 activation curve which was run at the same time as the curve shown in the figure.

(○) 0.25% DMSO and 25 μg/ml (0.026 mM) 2',6'-dimethyl-N(4')-benzyl-N(4')-[desmethyl]rifampicin (DMB).

(□) 0.40% DMSO and 40 μg/ml (0.026 mM) di-rifaldehyde azine (rifamazine).

(△) 0.30% DMSO and 7.5 μg/ml (0.0079 mM) rifaldehyde (N-amino-azacyclopentadecane)-hydrazone (rifazacyclo-16).

Figure 3. RIDP inhibition by rifazacyclo-16 and micelle formation as a function of three detergents.

RIDP assays, done as described in Figure 1, included 0.3% DMSO and 7.5 μg/ml rifazacyclo-16. Control activity was determined by averaging 18 assays which were ≥0.06 mM in the appropriate detergent and omitted only the rifazacyclo-16.

(—△) Triton DN-65 was added in the assays which contained 0.38 μg protein of an RIDP extracted with 0.1% Triton DN-65. Control activity = 226 pmol/hr/μg.

(—o—) Triton X-100 was added in the assays which contained 0.45 μg protein of an RIDP extracted with 0.1% Triton X-100. Control activity = 186 pmol/hr/μg.

(—□—) Brij-35 was added in the assays which contained 0.38 μg protein of an RIDP extracted with 0.1% Triton DN-65. Control activity = 132 pmol/hr/μg. The 0.0088 mM contributed by the Triton DN-65 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 mμ with excitation at 320 mμ in detergent solutions which were 100 mM Tris-HCl (pH = 7.8), 100 mM KCL, 4% glycerol, 0.3% DMSO and 1×10^{-3} mM TNS. [TNS strongly fluoresces in the non-aqueous environment of detergent micelles while giving only a weak fluorescence in the buffer (7)].

(——) Triton DN-65 added

(– –) Triton X-100 added

(—-—) Brij-35 added

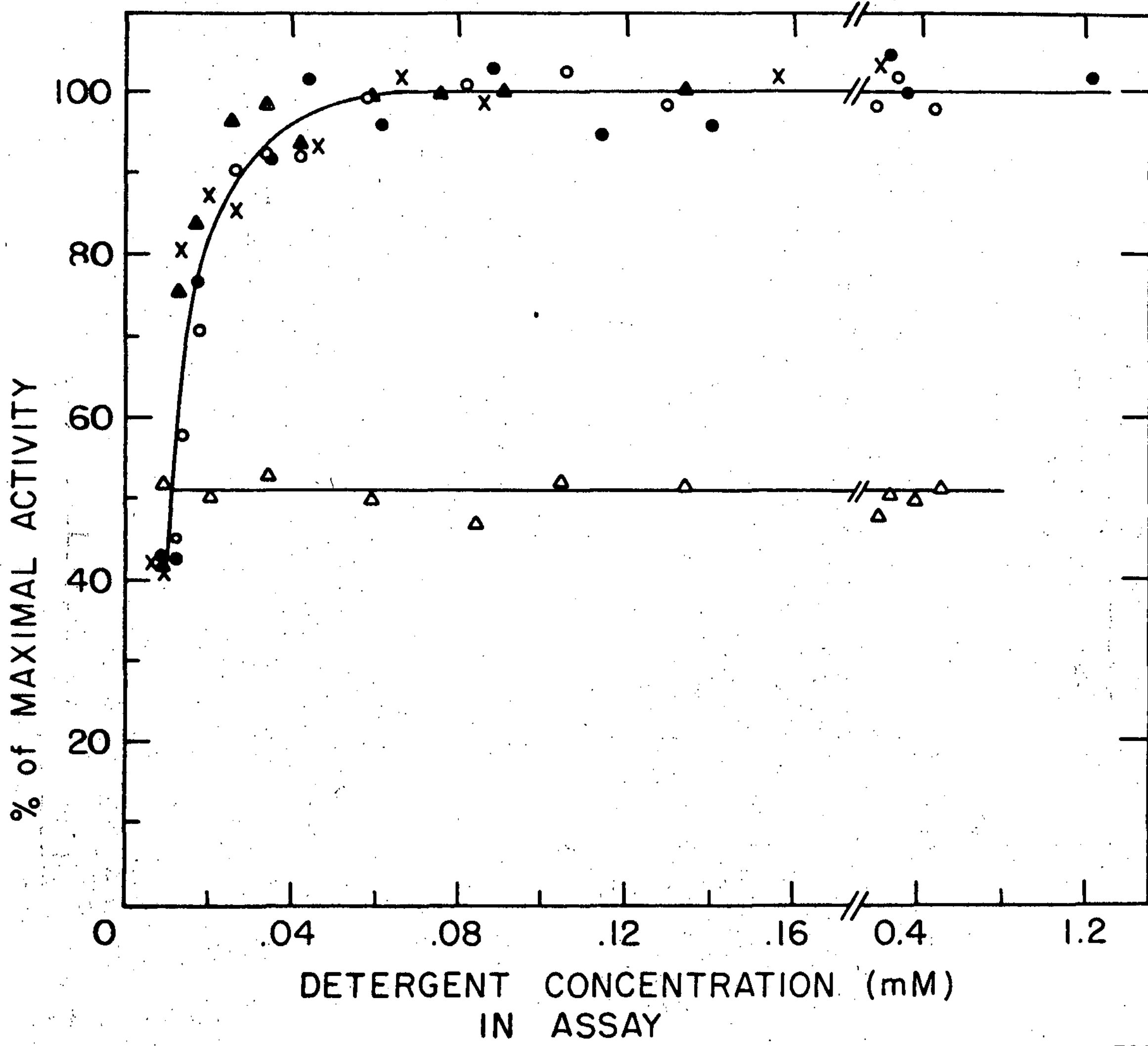


Fig. 1

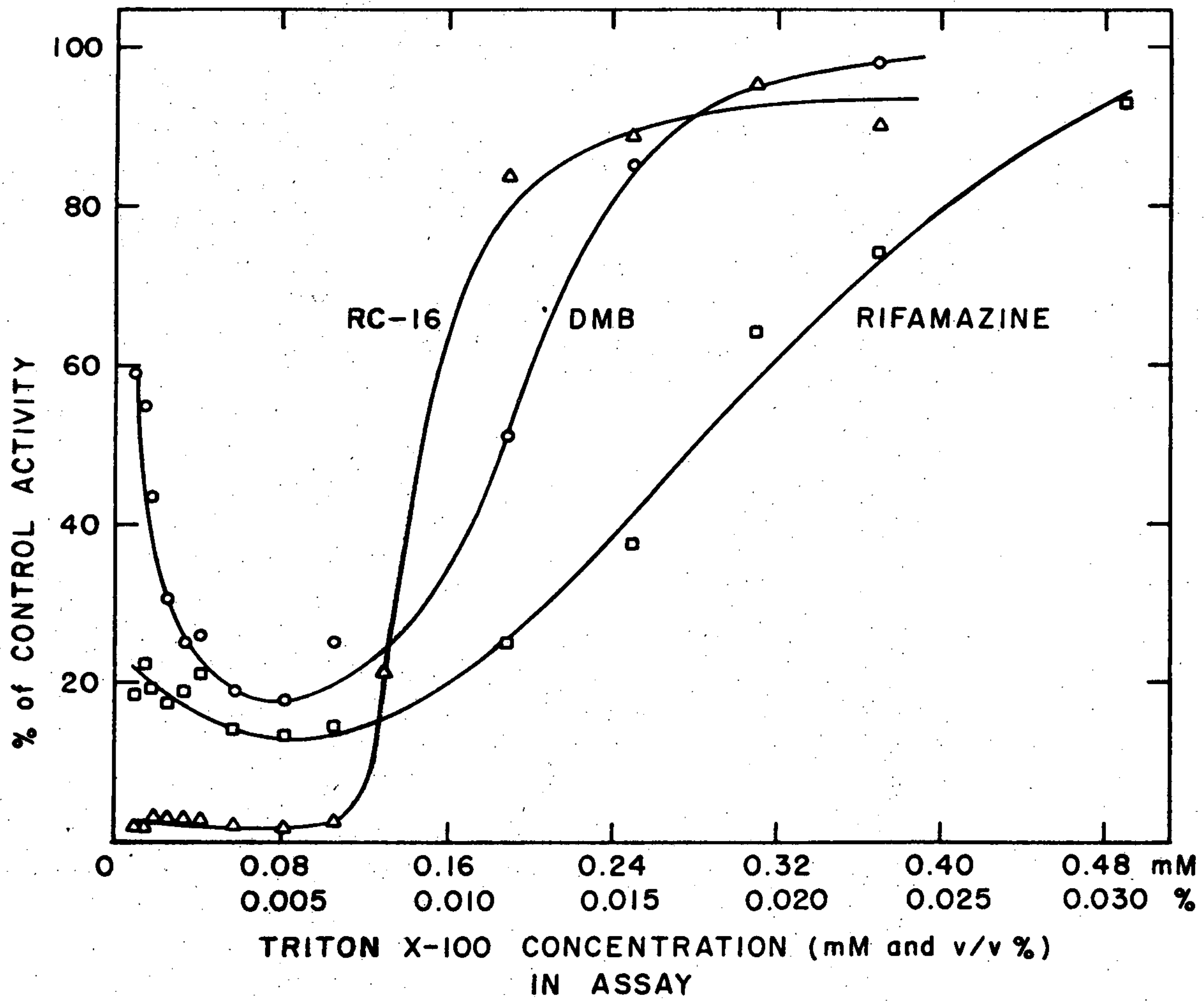


Fig. 2

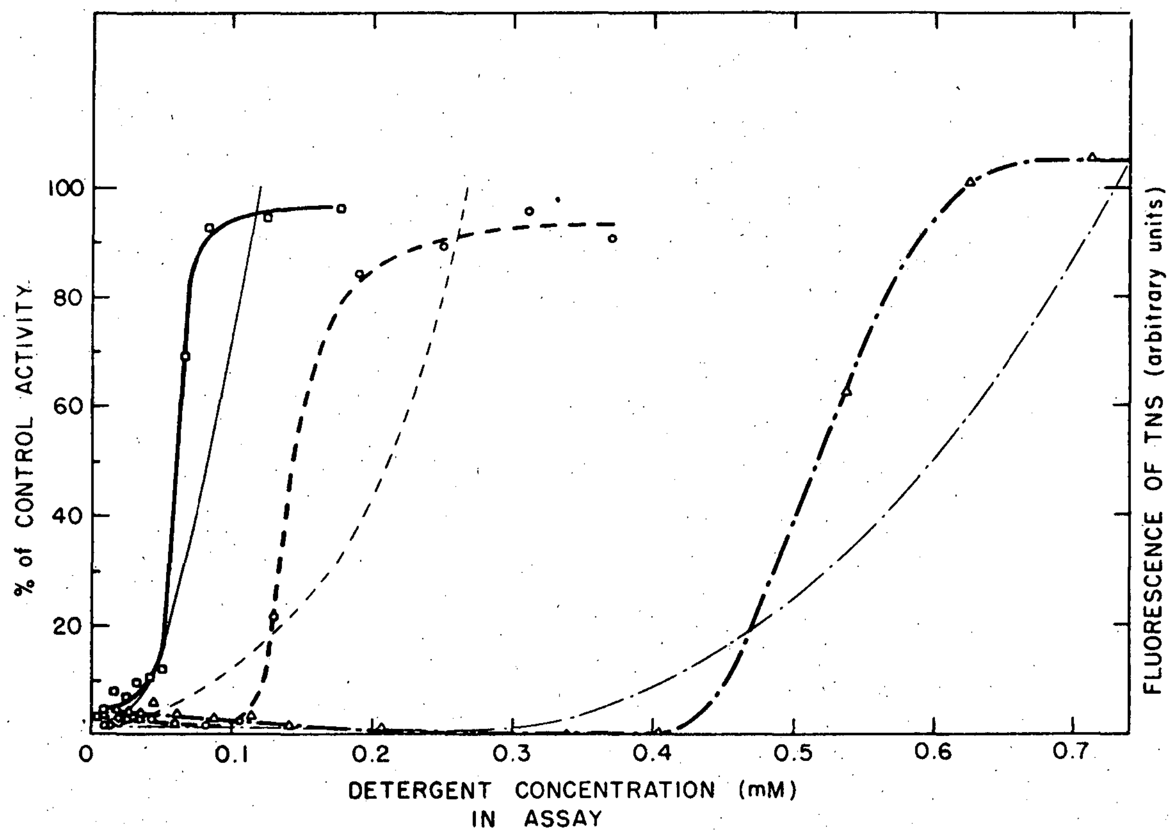


Fig. 3

XBL 728-4713

LEGAL NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

TECHNICAL INFORMATION DIVISION
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720