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Enveloped Virus Inactivation by Fatty Acid Derivatives

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The enveloped bacteriophage ϕ6 has been shown to be a valuable model system for the preliminary screening of compounds that might be expected to inactivate herpes simplex virus and other enveloped mammalian viruses. A variety of fatty acid derivatives that form fluid micelles in aqueous media have been found to be potent inactivators of ϕ6. The chemical nature of the polar head group, the length of the alkyl chain(s), and the extent and geometry of unsaturation are all important parameters in determining the antiviral effectiveness of this class of compounds.

Recent studies have shown that some nonionic amphiphilic molecules are potent inactivators of a variety of lipid-containing viruses (2, 3, 5, 6). As a step toward the possible future development and use of amphiphiles as antiviral agents in vivo, we are investigating the structural parameters that are important for the inactivation of viruses by these molecules. The enveloped bacteriophage ϕ6 has been shown in several studies (5, 6) to have a similar sensitivity to these types of agents as does herpes simplex virus. Thus, ϕ6 can be used as a model system for the preliminary screening of a range of amphiphilic molecules that have various polar head groups and alkyl chains. In the accompanying paper in this volume (4), we report that long-chain unsaturated alcohols and monoglycerides are extremely potent inactivators of both ϕ6 and herpes simplex virus type 2. We have performed a more comprehensive survey of the inactivation of ϕ6 by fatty acid derivatives, and the results of that survey are reported in this note.

Bacteriophage ϕ6 (1, 8) was exposed, in buffered aqueous medium, to various concentrations of fatty acids and fatty acid derivatives at 25°C. Bacteriophage ϕ6 was added to NBY medium (8) at 25°C to give approximately 10^7 plaque-forming units per ml. The fatty acid derivatives were solubilized in ethanol at 100 times the desired final concentration and added to the virus suspension. After 30 min of incubation, samples were diluted and assayed for surviving plaque-forming units (3, 8). All compounds (best grade available) were purchased from the Sigma Chemical Co., St. Louis, Mo., and used without further purification. Chromatographic checks of some of the compounds revealed no detectable amounts of other products. The data in Table 1, which shows the concentration of a given amphiphile required to reduce ϕ6 survival to 1% within 30 min, indicate that a wide variety of amphiphiles exhibit potent effects against ϕ6. The most potent compounds are the unsaturated alcohols, which are considered in more detail in the accompanying paper (4). Some of the acetates and methyl esters are also very active, as are all three dimethylamides tested. Saturated compounds of alkyl chain lengths of 12 or 14 carbons are more effective than similar compounds having longer or shorter chains. *cis*-Unsaturated compounds of alkyl chain lengths of 16 or 18 carbons are more active than the corresponding saturated or *trans*-unsaturated compounds. None of these compounds at the concentrations used in this study affects the growth rate of the host bacterium, *Pseudomonas phaseolicola*.

The single alkyl chain compounds that are active against ϕ6 form micellar aggregates in the aqueous medium, and our general hypothesis is that "fluid micelles" having a somewhat polar surface interact with ϕ6 to inactivate the virion. The only compound having two alkyl chains that inactivated ϕ6 in our survey was dicaprylin, the diglyceride having two 8-carbon saturated alkyl chains. Because diacyl compounds form vesicular aggregates as opposed to the micellar aggregates formed by monoacyl compounds, the activity of dicaprylin is surprising, especially because none of the other (longer chain) diglycerides tested or any phospholipids tested (data not presented) is active against ϕ6. The relatively short alkyl chains of dicaprylin perhaps result in vesicles having significantly different properties from those composed of molecules having longer alkyl chains.

TABLE 1. *Inactivation of bacteriophage φ6 by fatty acid derivatives*

Derivative	Alkyl chain concn (μg/ml) that reduces φ6 survival to 1%[a]												
	8:0	10:0	12:0	14:0	14:1 (Δ9 *cis*)	16:0	16:1 (Δ9 *cis*)	18:0	18:1 (Δ9 *cis*)	18:1 (Δ9 *trans*)	18:2 (Δ9, 12 *cis*)	18:3 (Δ9, 12, 15)	18:3 (Δ6, 9, 12)	20:4 (Δ5, 8, 11, 14)
Acid	NA	NA	NA	NA	50	NA	10	NA	3	50	10	20	4	5
Alcohol	NA	10	3	1	1	NA	1	NA	2	0.5	4	4	0.5	2
Acetate	—	20	3	10	4	50	20	NA	10	NA	20	—	—	NA
Methyl ester	—	20	3	4	4	50	3	NA	3	NA	2	20	NA	20
Ethyl ester	20	20	20	20	20	NA	20	NA	NA	NA	—	NA	50	NA
Dimethylamide	—	—	10	4	—	—	—	—	3	—	—	—	—	—
Monoacylglyceride	NA	—	50	NA	—	50	7	NA	3	—	1	—	—	—
Diacylglyceride	20	—	—	—	—	NA	—	NA	NA	—	—	—	—	—
Triacylglyceride	NA	NA	NA	—	—	NA	NA	NA	NA	NA	NA	NA	—	—

[a] NA, Not active, φ6 survival >5% following treatment at 50 μg/ml; —, not tested due to lack of commercial availability of compound.

φ6 was exposed to dicaprylin at 3 μg/ml for 15 min at various temperatures as indicated in Table 2 in NBY medium. In some instances, other acylglyceride was added to the virus suspension 1 min before addition of dicaprylin. The resultant data presented in Table 2 show that, at low concentrations, dicaprylin is a much better inactivator of φ6 at 35°C than at lower temperatures, suggesting that the dicaprylin aggregates must be "fluid" to be very active against φ6.

Also presented in Table 2 are data on the effect of the presence of other, non-inactivating, acylglycerides on the inactivation of φ6 by dicaprylin. Tricaprylin provides only a small amount of protection, but the unsaturated 18-carbon diglyceride diolein almost completely protects φ6 from inactivation by dicaprylin. These results on the interference with virus inactivation are qualitatively similar to those obtained for the inactivation of φ6 by long-chain unsaturated alcohols and monoglycerides, as described in the accompanying paper (4).

In the case of the interference by diolein with the virus inactivating activity of dicaprylin, we are dealing with two compounds that each form vesicular aggregates in the aqueous medium (7). We have correspondingly detected some examples of interference with virus inactivation in the case of monoacyl inactivating and interfering compounds, each of which probably forms micellar aggregates (7). For example, elaidyl acetate completely protects φ6 from inactivation by myristoleyl acetate when the mass ratio of interferer to inactivator is 10 to 1 (data not shown). Our present hypothesis concerning these interference effects is that mixed vesicular or micellar aggregates form rapidly in the aqueous medium, even though the compounds are added separately. There are, however, other possibilities, and future experiments should elucidate the mechanisms of both inactivation and interference.

We have reported in this note that many amphiphilic molecules that form fluid micelles with a somewhat polar surface in aqueous me-

TABLE 2. *Effects of temperature and presence of other acylglycerides on inactivation of φ6 by dicaprylin (3 μg/ml)*

Other acylglyceride present	Temp (°C)	% Inactivation
None	20	21
	25	41
	30	59
	35	99
Diolein (50 μg/ml)	35	10
Tricaprylin (50 μg/ml)	35	85

dium are fairly potent inactivators of the enveloped bacteriophage ϕ6. Other studies (4, 5, 6) have shown that most compounds which inactivate ϕ6 are also active against herpes simplex virus and at least a few other enveloped mammalian viruses. It thus appears that the work on some of the agents used in this study should be extended to include herpes simplex and perhaps other lipid-containing viruses.

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