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INHIBITION KINETICS OF CHLORAMPHENICOL ACETYLTRANSFERASE BY SELECTED DETERGENTS[1]

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SUMMARY Kinetic analyses indicate that the inhibitory effects of the nonionic detergents Triton X-100 and Nonidet P-40 on chloramphenicol acetyltransferase are exerted by a competitive and a non-competitive mechanism with respect to the substrates chloramphenicol and acetyl-CoA, respectively. Comparison with nonionic detergents without an aromatic moiety like that present in Triton X-100 and Nonidet P-40 suggests that the aromatic groups in these two detergents may compete with chloramphenicol for binding to the hydrophobic, active site in the chloramphenicol acetyltransferase.

The bacterial chloramphenicol acetyltransferase (CAT) gene is one of the most widely used reporter genes in molecular biology today. We reported earlier (1) the profound inhibition of CAT activity by nonionic detergents such as Triton X-100 (Triton) and Nonidet P-40 (NP-40). The inhibitory effect was substantial even at concentrations as low as 20 ppm (0.002%, w/v). Such a finding strongly argues against the use of these detergents in the preparation of cell extracts for the assay of CAT reporter enzyme activity. The use of these detergents not only can decrease the assay sensitivity by as much as 6-7 fold, but also can introduce assay variability of the same order of magnitude, which can lead to erroneous conclusions. We extended our study in this paper to the kinetic patterns of inhibition by these detergents. With respect to the substrates chloramphenicol and acetyl-CoA, a competitive and a non-competitive inhibition pattern were identified, respectively.

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MATERIALS AND METHOD

The enzyme source and the assay method were described in our previous report (1). Briefly, a crude cell extract prepared from human HeLa cells or monkey kidney CV-1 cells transfected with a CAT expression plasmid was used in most experiments. A purified preparation of CAT purchased from Sigma Chemical Company (St. Louis, MO, USA) was also used in several experiments. Acetyl-CoA was purchased from Pharmacia (Piscataway, NJ, USA). [^{14}C]Chloramphenicol (20 μCi/ml in 0.25 M Tris-HCl, pH 7.5, 54 mCi/mmol) was purchased from Amersham (Arlington Heights, IL, USA). Detergents and other chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA). The thin layer chromatographic method of Gorman et al (2) was used for CAT activity assays. For kinetic studies, the concentration of one substrate was varied while the other was held at the standard assay level. Linearity of the assay was ensured by conducting the assay below 30% conversion of the substrate [^{14}C]chloramphenicol.

RESULTS AND DISCUSSION

Figure 1 shows the effect of several commonly used nonionic (Triton, NP-40, Tween 20) and ionic detergents (sodium deoxycholate, NaDOC and sodium dodecyl sulfate, SDS) on the CAT activity assay. On first glance, the concentration of a detergent that was effective in producing 50% inhibition of the CAT activity (inhibition index, $I_{50\%}$) seemed to correlate with the critical micelle concentration (CMC) of that detergent (Table 2). Such an observation would suggest that the inhibitory effect could be related to the ability of a detergent to form micelles. However, this correlation did not hold when other detergents such as Tween 20, n-dodecyl ß-D-glucopyranoside (DG) and n-octyl ß-D- glucopyranoside (OG) were tested for their ability to inhibit CAT (Tables 1 and 2). Even though Tween-20 and DG can

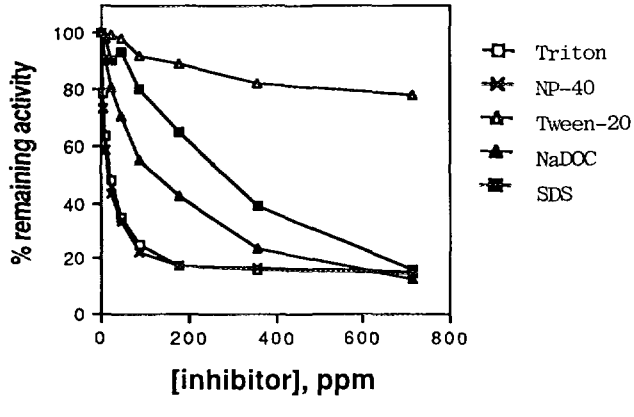

Figure 1. The inhibitory effect of selected detergents on the chloramphenicol acetyl transferase activity. Detergents are represented by symbols.

TABLE 1. EFFECT OF SELECTED NON-IONIC DETERGENTS ON THE CHLORAMPHENICOL ACETYLTRANSFERASE (CAT) ACTIVITY

Detergent	Concentration		CAT activity[1] (% of control)
	ppm	mM	
none (control)	0	0	100
Triton X-100	14	0.022	69
	43	0.069	42
	143	0.229	22
n-Dodecyl ß-D-glucopyranoside	71	0.204	147
	143	0.408	156
n-Octyl ß-D-glucopyranoside	357	1.223	159
	714	2.445	133

[1]Purified bacterial CAT enzyme purchased from Sigma Chemical Company was used in this experiment.

form micelles at lower concentrations than can NP-40 or Triton, they caused little inhibition and DG might even have stimulated the CAT activity to some extent (Table 1).

The Hill coefficient (n) shows the nature of the interaction between a CAT enzyme molecule and the inhibitor molecules. Hill plots (3) of the data in Figure 1 yield slopes approaching unity (i.e., $n \simeq 1$) (Figure 2), indicating a monomeric

TABLE 2. THE INHIBITION INDICES ($I_{50\%}$) OF SELECTED DETERGENTS AND THEIR CRITICAL MICELLE CONCENTRATIONS (CMC)

Detergent	$I_{50\%}$		CMC[1] (mM)
	ppm	mM	
Triton X-100	15	0.024	0.24
Nonidet P-40	12	0.02	0.29
Sodium deoxycholate	125	0.3	5
Sodium dodecyl sulfate	280	0.97	8.27
Tween-20	none	none	0.05
n-Dodecyl ß-D-glucopyranoside	none	none	0.19
n-Octyl ß-D-glucopyranoside	none	none	10

[1]The critical micelle concentration and molecular weight data were from Sigma Chemical Company product catalog, 1993.

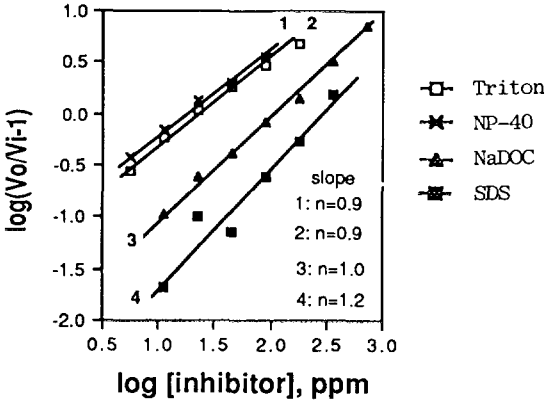

Figure 2. Hill plots of data in Figure 1. Detergents are represented by symbols. The slope (n) of each plot indicates the order of interaction between the enzyme molecule and inhibitor molecules. The slopes were close to unity, indicating monomeric interaction.

interaction between an enzyme molecule and a detergent molecule. The initial velocity and substrate concentration data were plotted according to Lineweaver and Burk (4) to examine the inhibition kinetics (Figure 3). The apparent K_m's estimated from the present data for chloramphenicol and acetyl-CoA were 5 and 55 μM, respectively. These estimates are in good agreement with the published values (Reviewed by Shaw in ref 5). From the Lineweaver-Burk plots, it is also evident that Triton, NP-40 and sodium deoxycholate inhibited CAT by a competitive mechanism with respect to the substrate chloramphenicol (Figure 3, panel A) and a non-competitive mechanism with respect to acetyl-CoA (Figure 3, panel B). The competitive inhibition kinetics indicates that an inhibitory detergent is competing for binding to the chloramphenicol binding site in the active center of the enzyme.

There are several explanations for the inhibitory effects of detergents on CAT activity. It is possible that detergents decrease the effective concentration of chloramphenicol by enhancing its solubility in the aqueous reaction environment so that less chloramphenicol is available to CAT for the transacetylation reaction (i.e., a detergent effect). However, there was no correlation between the critical micelle concentration, which reflects the micelle forming ability of a detergent, and its CAT inhibitory effect. Even with those inhibitory detergents, 50% inhibition was achieved with 10% of the critical micelle concentration. These facts do not support a detergent effect. Furthermore, aromatic dyes (triphenylmethane derivatives) such as crystal violet and ethyl violet that do not possess any detergent activity have been shown to be very potent inhibitors of CAT (6). These dyes share the same kinetic

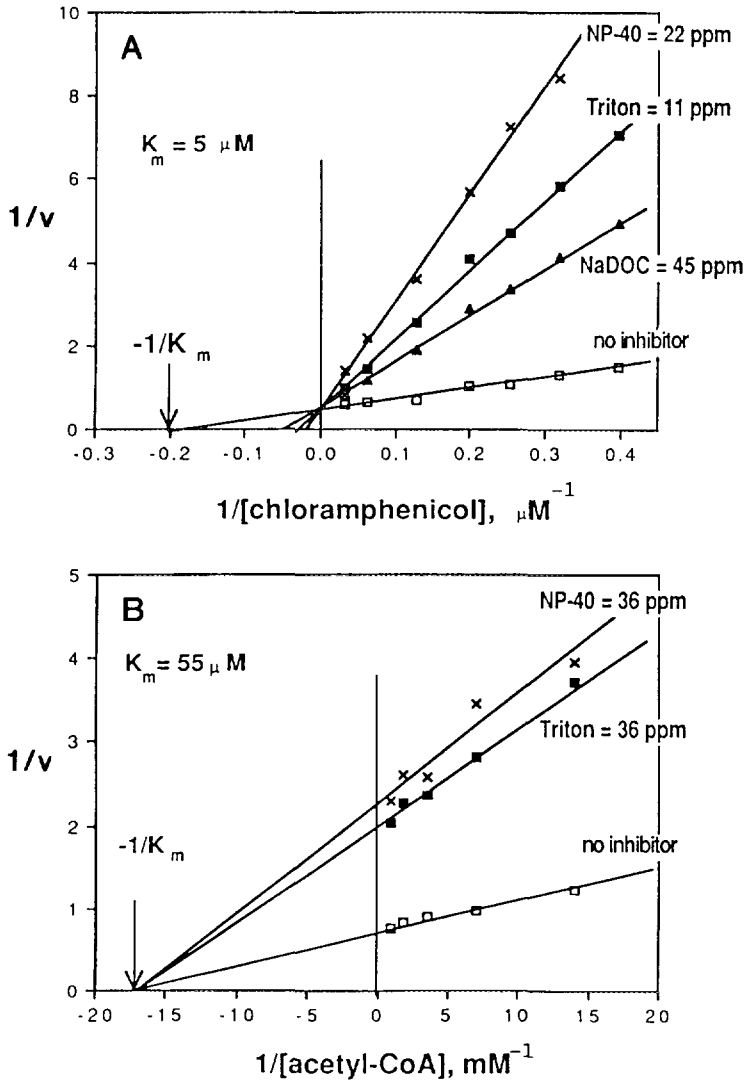

Figure 3. Lineweaver-Burk (double reciprocal) plots of initial reaction velocities vs. concentrations of chloramphenicol (Panel A) and of acetyl-CoA (Panel B).

patterns of inhibition as the detergents, that is, a competitive mechanism with respect to chloramphenicol and a non-competitive mechanism with acetyl-CoA (6). The inhibitory potencies of these structurally related dyes correlate with the number of aromatic rings in their molecules (6) and therefore their hydrophobicity. The explanation for the competitive inhibition kinetics may therefore reside in the shared structural similarity between the inhibitory detergent molecules and chloramphenicol. The inhibition was strongest by Triton and NP-40, which share a common hydrophobic octylphenol(oxy) group (Table 3). Tween-20, n-dodecyl ß-D-

TABLE 3. DETERGENTS USED IN THIS STUDY

Abbreviation	Chemical name
Triton	Octyl phenoxy polyethoxyethanol
NP-40	Octyl phenol ethylene oxide condensate
OG	n-Octyl ß-D-glucopyranoside
DG	n-Dodecyl ß-D-glucopyranoside
Tween 20	Polyoxyethylene sorbitan monolaurate
SDS	Sodium dodecyl sulfate or Sodium lauryl sulfate
NaDOC	Sodium deoxycholate

glucopyranoside and n-octyl ß-D-glucopyranoside did not inhibit CAT activity, and they do not contain an aromatic group. The binding affinity of chloramphenicol to the active site has been shown by Cullis *et al.* to be dominated by hydrophobic interaction(7). The aromatic moieties in Triton and NP-40 may therefore compete with the aromatic group of the chloramphenicol for binding to the "hydrophobic pocket" in the enzyme molecule (5) and confer the competitive inhibition.

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