A novel substrate for assays of gene expression using chloramphenicol acetyltransferase

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We describe the use of 1-deoxychloramphenicol (1-deoxyCm) as an alternative to chloramphenicol (Cm) for the assay of chloramphenicol acetyltransferase (CAT). 1-deoxyCm yields a single acetylated product, in contrast to the three which can result using Cm as substrate, thereby simplifying the quantitative analysis of CAT activity for gene expression studies.

Of the many reporter genes used to monitor the effects of *cis*- and *trans*-acting transcriptional elements *in vitro* and *in vivo*, CAT has probably found the widest acceptance. The method of Gorman *et al.* (1), and variations thereof, make use of [¹⁴C]-labelled Cm and acetyl-CoA, usually in conjunction with separation of the substrate and its acetylated products by thin-layer chromatography (TLC). One of the limitations of such assays for quantitative studies arises from the chemistry of the CAT reaction; namely, the non-enzymic rearrangement of the reaction product 3-acetylCm to 1-acetylCm followed by a second cycle of acetylation to form a third radioactive product, 1,3-diacetylCm. As 1-acetylCm is acetylated relatively slowly by CAT but is bound to the enzyme with an affinity which approaches that of Cm itself (2), it follows that assays which measure yield of total acetylated Cm or 3-acetylCm alone cannot be truly quantitative.

Recent studies, with the type III variant of CAT have shown that 1-deoxyCm (1-hydroxyl replaced by a proton) is a good substrate (2, 3). A steady-state kinetic analysis of type I CAT (specified by the CAT genes of Tn9, pBR325, and all cassettes in common use for gene expression studies) yielded the comparative data for 1-deoxyCm and Cm which are shown in Table I.

The results of a TLC experiment using 10⁻³ to 10⁻⁷ units of type I CAT (Specific Activity = 155 units mg⁻¹) are shown in Figure 1. Reactions were carried out for 60 min. at 25°C in 0.1 mL of 50 mM Tris-HCl (pH 7.5) containing 100 mM NaCl, 0.1 mM EDTA, 0.4 mM acetyl-CoA and 0.1 mM Cm or 1-deoxyCm, and terminated by the addition of 1 mL of ethyl acetate. The specific radioactivity of [¹⁴C]-Cm and 1-deoxyCm was 54 mCi mmol⁻¹ and 0.25 µCi (46% of total) from each assay was analysed by TLC. Production of 3-acetylCm and 3-acetyl,1-deoxyCm was observed down to 10⁻⁶ units of CAT (6.4 pg) indicating similar sensitivity of assays using the alternative substrates.

The use of radioactive 1-deoxyCm instead of Cm for CAT assays obviates the need to take account of multiple products and should simplify measurement of enzyme activity by direct

scintillation counting of sections of TLC plates or by densitometric analysis of autoradiographs.

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Figure 1. Autoradiograph of TLC analysis of CAT reactions using equivalent concentrations of Cm (Tracks 1–6) and 1-deoxyCm (Tracks 7–12). Assays contained the following quantities of type I CAT: none (Tracks 1, 7), 10⁻³ units (2, 8), 10⁻⁴ units (3, 9), 10⁻⁵ units (4, 10), 10⁻⁶ units (5, 11), and 10⁻⁷ units (6, 12). Resolved species are 1,3-diacetylCm (A), 3-acetylCm (B), 1-acetylCm (C), Cm (D), 3-acetyl,1-deoxyCm (E), and 1-deoxyCm (F).

Table I. Kinetic parameters for type I CAT

Substrate	kcat	Km (acyl acceptor)	Km (acyl donor)	
			acetyl-CoA	butyryl-CoA
Cm	97 s ⁻¹	11 µM	55 µM	
	140 s ⁻¹	26 µM		156 µM
1-deoxyCm	38 s ⁻¹	36 µM	55 µM	
	60 s ⁻¹	75 µM		89 µM

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