High-performance capillary electrophoretic analysis of chloramphenicol acetyl transferase activity

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ABSTRACT

This study highlights the potential utility of high-performance capillary electrophoresis (HPCE) for monitoring enzyme activity. Free-zone capillary electrophoresis is used to rapidly and reproducibly analyze the activity of the bacterial enzyme chloramphenicol acetyl transferase (CAT) which converts the substrates acetyl coenzyme A (CoA) and chloramphenicol to acetyl chloramphenicol and CoA. The results of this study indicate that HPCE may be an excellent tool for studying enzyme activities since it has several advantages over standard single parameters assays, most notably, the ability to monitor both loss of substrate and appearance of products simultaneously. Conditions have been identified for optimal separation of the substrate (chloramphenicol) from the products (acetylated derivatives). This presents a unique potential of HPCE for the analysis of enzymatic reactions that may be applied to areas of analytical research presently utilizing enzymatic reactions. One such analytical method is the CAT assay used for analysis of gene promoter activity. In this study, HPCE is shown to yield similar quantitative results with nonradiolabelled substrate in a fraction of the time. HPCE has several advantages over standard techniques including speed of analysis, no need for radiolabelled substrate, small sample volumes, high sensitivity/resolution and excellent quantitative capabilities.

INTRODUCTION

High-performance capillary electrophoresis (HPCE) is a versatile, relatively new analytical technique that has combined the quantitative precision and instrumental control of high-performance liquid chromatography (HPLC) with the resolving power of electrophoresis. HPCE differs from other analytical separation techniques, such as HPLC, in that it is capable of unprecedented separation efficiency, *i.e.* has the potential for generating several hundred thousand theoretical plates [1]. HPCE is also capable of automated microscale electrophoretic separation of a number of samples in a reproducible manner within a relatively short period of time. Although there are some limitations with the

present technology, the resolving power, speed, quantitative ability, reproducibility and sensitivity at the femtomole level has made HPCE a valuable technique in the biomedical sciences (reviewed in refs. 2–4). HPCE has advanced tremendously since its conception almost a decade ago. Much of the early efforts were focussed on defining conditions for the separation of smaller molecules, a few of which include anticancer drugs [5], organic compounds [6,7] and metal ions [8]. More recent research efforts have applied the resolving power and sensitivity of HPCE to the analysis of macromolecular components of the cell such as peptides [9,10] (for review see ref. 4), proteins [2] and oligonucleotides [11–15] (for review see ref. 3).

There has been little in the literature regarding the use of HPCE for monitoring enzymatic reactions of either clinical or basic science interest. In this study we use, as a model system, the bacterial enzyme chloramphenicol acetyl transferase (CAT) to dem-

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onstrate the utility of HPCE for monitoring enzyme activity. CAT is an enzyme synthesized in several strains of chloramphenicol-resistant bacteria [16]. The properties of the enzyme vary according to the bacterial strain from which it is purified and no CAT is representative of all the enzymes. Typically, the native enzymes have a molecular mass of 80 000, a quaternary structure of four identical subunits of 20 000 and isoelectric points ranging from 4.0–5.4 [17]. As indicated by its name, CAT catalyzes the conversion of the substrates chloramphenicol and acetyl coenzyme A (acetyl-S-CoA), to the products 3-acetyl-chloramphenicol and coenzyme A (HS-CoA) as described by eqn. 1. The acetyl group of the 3-acetylchloramphenicol congener can apparently undergo a non-enzymatic, pH-dependent migration to form the 1-acetylchloramphenicol product (eqn. 2) which then can be further acetylated enzymatically (eqn. 3).

chloramphenicol + acetyl-S-CoA →

$\qquad$ 3-acetyl chloramphenicol + HS-CoA $\qquad$ (1)

3-acetyl chloramphenicol $\rightleftharpoons$

$\qquad$ 1-acetyl chloramphenicol $\qquad$ (2)

1-acetyl chloramphenicol + acetyl-S-CoA →

$\qquad$ 1,3-acetyl chloramphenicol + HS-Co A $\qquad$ (3)
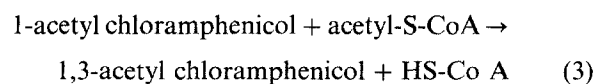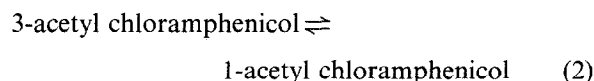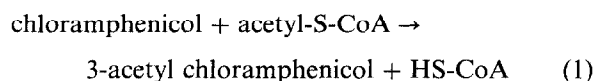
One of the classic extensions of this particular enzyme activity is its use in the molecular biology technique that specifically exploits the enzyme-catalyzed acetylation of chloramphenicol. The CAT assay is used to characterize the transcriptional activity of a eukaryotic promoter and of regulatory sequences in the 5′ flanking domains [17] found upstream of actively transcribed genes [18]. Ideally, promoter activity is measured by the amount of gene product produced in response to a regulator of the specific promoter. However, not all gene products are convenient to assay. Therefore, to circumvent this problem, the coding sequence of the bacterial CAT gene is connected to a promoter and/or 5′ flanking domain in question. Cells are transfected with an expression vector containing the CAT gene downstream of the specific promoter sequence to be evaluated and the cells are treated with an activator. If the sequence has promoter activity, transcription of the CAT gene ensues and, in the presence of the

cellular translational machinery, the mRNA is translated to yield an active CAT enzyme. Because there is not a eukaryotic counterpart of the CAT gene, the enzyme activity can be directly and quantitatively measured in the extract of the cell. The level of CAT enzyme activity corresponds to the amount of CAT synthesized, which in turn reveals the level of activity of the promoter.

In this study, we show that enzyme-dependent loss of reactants and formation of products can be monitored accurately and reproducibly with HPCE. The use of borate as a buffer is necessary for this separation since its apparent complexation with chloramphenicol results in the separation of this substrate from the acetylated products. The ability to monitor CAT activity with HPCE is not only of interest from an enzymological perspective, but is discussed in terms of the potential extrapolation of these findings to improve upon a present day molecular biological technique, the CAT assay.

EXPERIMENTAL

Chemicals and reagents

Chloramphenicol was obtained from Boehringer Mannheim, the acetyl coenzyme A from Pharmacia, the diacetyl chloramphenicol, CAT (isolated from *E. coli*), coenzyme A, sodium tetraborate, dithiothreitol (DTT), glycerol and boric acid from Sigma and the Tris–Cl from ICN Chemicals. All solutions were made with Milli-Q purified distilled water. [^{14}C]Chloramphenicol was purchased from New England Nuclear (NEN).

Incubation conditions for CAT activity

CAT was stored as a stock solution in 50% glycerol, 10 mM Tris–HCl, 0.5 mM DTT, pH 7.5 at a stock concentration of 1 Unit/μl. Acetyl coenzyme A and chloramphenicol were maintained at stock concentrations of 4 mM (in water) and 11 mM (in 95% ethanol) respectively. All components were stored at -20°C, and kept on ice until added to the incubation mixture. For CAT activity, the appropriate amount of enzyme was added to Tris buffer (20 mM Tris–HCl pH 7.5) containing acetyl CoA and chloramphenicol, and incubated in a water bath equilibrated at either 27, 37, 44 or 55°C for varied times. The reaction was terminated by boiling the sample for 2 min. For stability studies at 27°C, the

sample was simply allowed to stand in a Beckman minivial within a capped 4-ml sample tube in the P/ACE 2050 unit.

CAT enzymatic assay

[¹⁴C]Chloramphenicol thin-layer chromatographic (TLC) analysis. The CAT assays using TLC were performed following the procedure of Gorman *et al.* [17] with slight modifications. The 150-μl reaction mixture contained 20 mM Tris–Cl, pH 7.5, 0.513 mM [¹⁴C]chloramphenicol (NEN 1.3 mCi/mmol, 0.1 μCi), 0.533 mM acetyl coenzyme A (Pharmacia, Sweden) and 1 U CAT enzyme. The enzymatic reaction was performed at 27°C for different time periods at which point the reactions were stopped by boiling the sample. The [¹⁴C]chloramphenicol and its acetylated derivatives were extracted with 500 μl of ethyl acetate. The organic phase was removed and dried down on a speed vac. The residue was dissolved in 30 μl of ethyl acetate and spotted on a silica gel TLC plate. The TLC plate was placed in a tank containing chloroform–methanol (90:10) for ascending chromatography. Following the chromatography the TLC plate was autoradiographed.

HPCE analysis. The CAT assay mixtures were prepared for HPCE by filtration through a 0.22-μm syringe filter. The fused-silica capillary (47 or 57 cm × 50 μm I.D.; uncoated; 7 cm from the detector to the outlet) was equilibrated with the running buffer consisting of 100 mM borate/tetraborate buffer at pH of 8.3 (a minimum 15-min rinse or preferably overnight equilibration). Each method involved a 1-min rinse with running buffer, injection of sample, separation, 1-min rinse with 0.1 M NaOH and finally, a 2-min rinse with running buffer. Unless otherwise noted, the sample was pressure injected for 1–5 s (*ca.* 1.3 nl/s) into the capillary and separation carried out on a Beckman P/ACE Model 2050 at 25 kV, 32 μA, 25°C. Unless otherwise noted, detection was at wavelength $\lambda = 200$ nm. Data were collected and peak migration time and area were analyzed using Beckman System Gold Version 6.0 software.

Effect of DTT on coenzyme stability

Coenzyme A (0.5 mg/ml in 100 mM borate buffer, pH 8.3) was incubated with or without 20 mM DTT at 27°C. The samples were kept at 4°C until incubation at 27°C and analysis initiated by HPCE at time 0 and 7 h.

RESULTS

Fig. 1 shows the chemical structures of the substrates chloramphenicol and acetyl coenzyme A in their fully protonated form. Fig. 2 shows an electropherogram of standard acetyl coenzyme A, chloramphenicol, diacetyl chloramphenicol and coenzyme A. A net negative charge on coenzyme A at a pH of 8.3 accounts for the clear separation from chloramphenicol which is uncharged. Separation of the acetylated chloramphenicol from the chloramphenicol is interesting since both are neutrally charged under these conditions. Acetylated chlor-
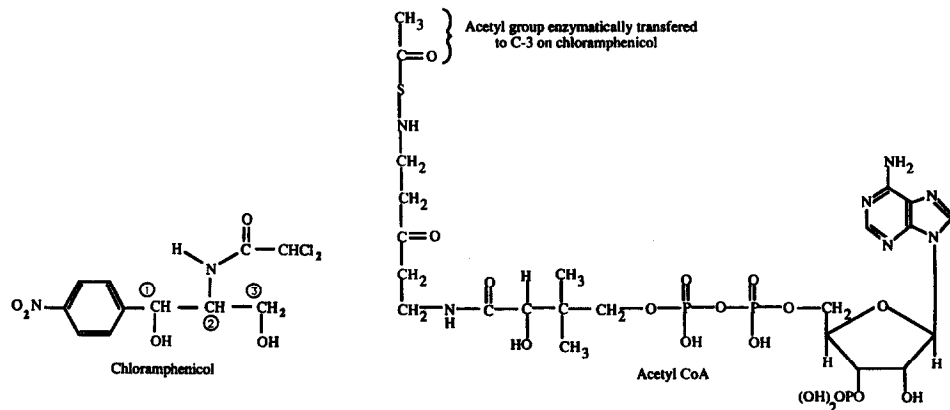

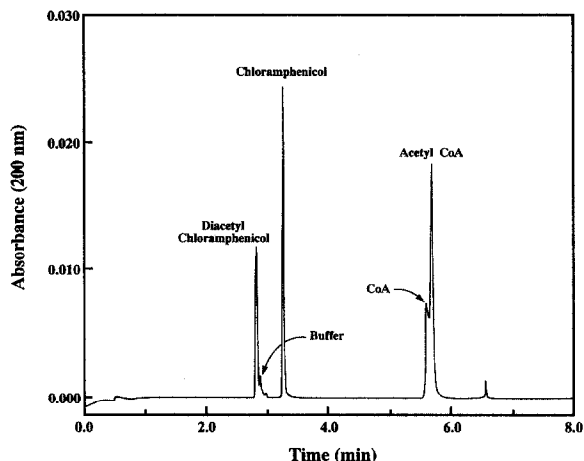Fig. 1. Chemical structures of chloramphenicol and acetyl coenzyme A.

Fig. 2. HPCE separation of CAT enzyme substrates and products. Purified acetyl coenzyme A, coenzyme A, chloramphenicol and diacetylated chloramphenicol (all at a concentration of *ca.* 500 mg/ml in 20 m*M* Tris buffer pH 7.5) were injected by pressure for 1 s (equivalent to 1.3 nl or 650 pg) into a 57 cm × 50 μm capillary and separated at 25 kV.

amphenicol migrates, as expected, with endoosmotic flow (EOF; the bulk flow of buffer towards the cathode; neutral molecules migrate with this) but surprisingly, chloramphenicol elutes later than EOF, indicating that other variables are playing a role in the separation. Borate appears to be a variable since separation in phosphate buffer at the same pH did not lead to the resolution of chloramphenicol and the acetylated derivatives which both comigrated with EOF (data not shown). Acetyl coenzyme A and coenzyme A were found to be barely separable under these experimental conditions (coenzyme A, 5.7 min; acetyl coenzyme A, 5.8 min in the 57-cm capillary) probably as result of the thiol group of CoA not being ionized at this pH. Purified monoacetyl chloramphenicol was not commercially available as a pure standard and therefore the identity of the acetylated chloramphenicol peak was determined by a process of elimination. Using purified standards, the minimum detectable on-column mass of both the diacetylated and unacetylated forms of chloramphenicol was *ca.* 1 pg and optimal at $\lambda = 200$ nm. Detection sensitivity for these types of compounds at $\lambda = 200$ nm was approximately 2–3 times greater than at $\lambda = 214$ nm (data not shown). Addition of CAT to 25 m*M*

Tris–Cl buffer, pH 7.5 containing a 500 μ*M* concentration of both chloramphenicol and acetyl coenzyme A led to a measurable loss of substrate and appearance of products. Fig. 3 illustrates the enzyme-dependent (1 Unit) loss of chloramphenicol and acetyl coenzyme A and the corresponding appearance of peaks which represent acetylated forms of chloramphenicol and coenzyme A as described by the equations in the Introduction. While a minor amount of diacetyl chloramphenicol may be produced (typically <1%), there was no indication that it was separable under these conditions from the major product, 3-acetyl-chloramphenicol. When an enzymatic reaction mix is spiked with diacetylchloramphenicol, the acetylated chloramphenicol peak is increased with no other observable peaks (data not shown). Determination of the peak area was made for calculation of the percent conversion of chloramphenicol to acetylated chloramphenicol as a function of time at this temperature and is shown in Fig. 4A. A linear rate of formation of product occurs within 30 min and thus represents the useful reaction time for kinetic studies. Conversion was complete at 2 h at 27°C even though approximately 15% of the substrate was still available. The leveling off may have been due either to the temperature-dependent inactivation of CAT or to a product feedback inhibition. The quantitative results obtained with HPCE are similar to those obtained by the more laborious TLC method using ^{14}C-labelled substrate (Fig. 4A and B). The data shown in Fig. 4A were obtained by linear scanning of the autoradiogram obtained from TLC (Fig. 4B). The slight difference in the saturation level with the two assay methods may be due to inaccuracy of linear densitometry scanning of the autoradiogram. The temperature-dependency of the CAT activity is illustrated in Fig. 5. As expected, higher temperatures resulted in higher enzyme activity with optimal production of monoacetyl chloramphenicol in 1 h occuring at 44°C. A temperature of 55°C appears to be less productive, perhaps due to a more rapid inactivation of the enzyme. As expected, the CAT-catalyzed conversion was found to be dependent upon the concentration of both the reactants and the enzyme with maximal production of acetylated chloramphenicol and coenzyme A occurring at a 500 μM concentration of both reactants with 1 Unit of CAT (data not shown). Increasing the substrate
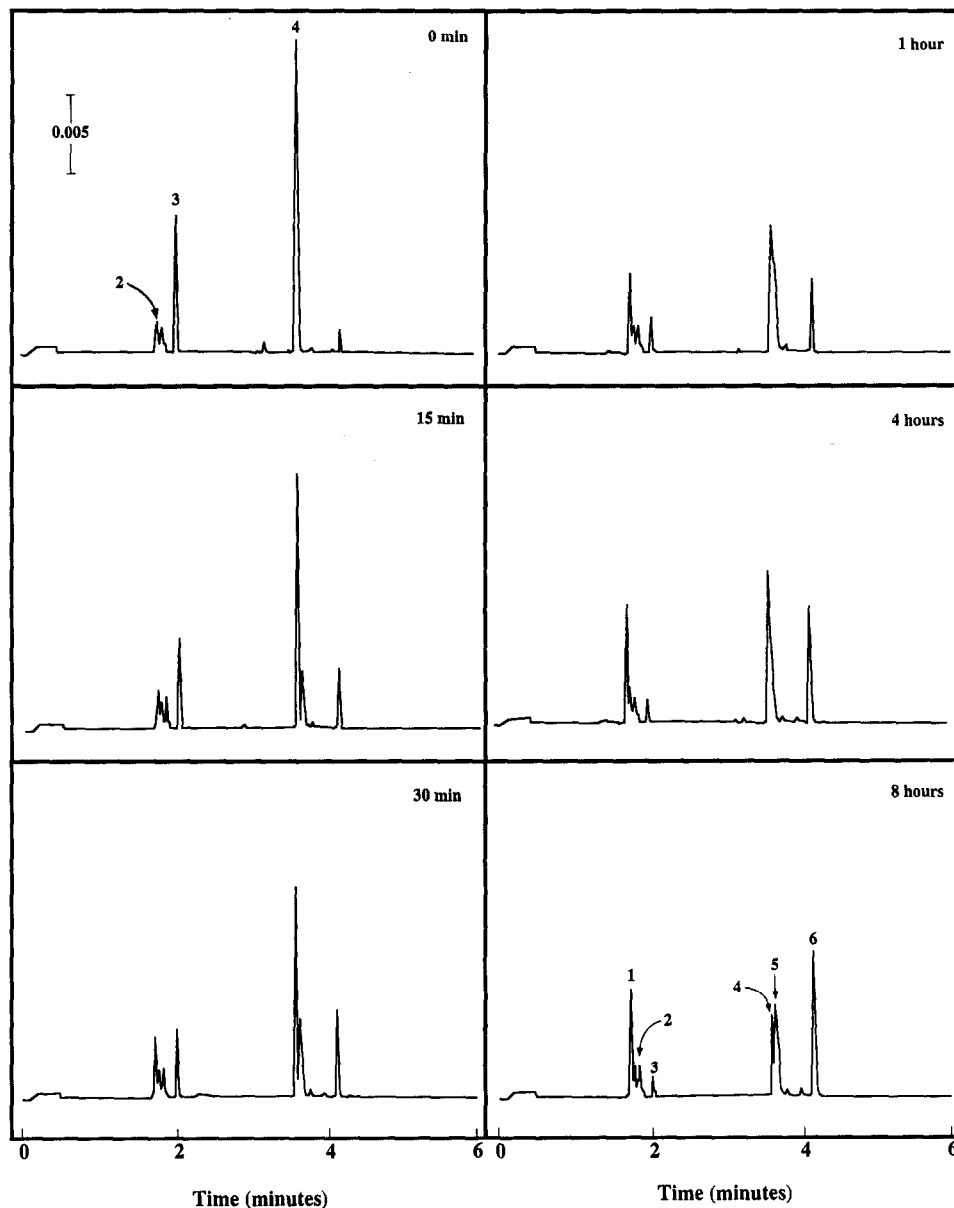
Fig. 3. HPCE analysis of the CAT-catalyzed acetylation of chloramphenicol. Chloramphenicol (500 μM), acetyl coenzyme A (500 μM) incubated in 20 mM Tris–Cl at 27°C for 0, 15, 30, 60, 240 and 480 min in the presence of 1 Unit of CAT. The reaction volume for each sample was 150 μl. The resultant mixtures were diluted 1:1 with tetraborate buffer pH 8.3 and pressure injected for 5 s into a 47 cm × 50 μm capillary and separated at 25 kV. Identified peaks: 1 = acetylated chloramphenicol; 2 = Tris–HCl; 3 = chloramphenicol; 4 = acetyl CoA; 5 = CoA; 6 = product of CoA.

beyond this concentration did not enhance production of the acetylated chloramphenicol.

Loss of substrate (chloramphenicol and acetyl coenzyme A) was due solely to the activity of the

enzyme and not to a non-enzymatic degradation since exposure to 23°C for 36 h or 100°C for 2 min had a negligible effect on the amount of each of the substrates or the shape of the peaks (data not
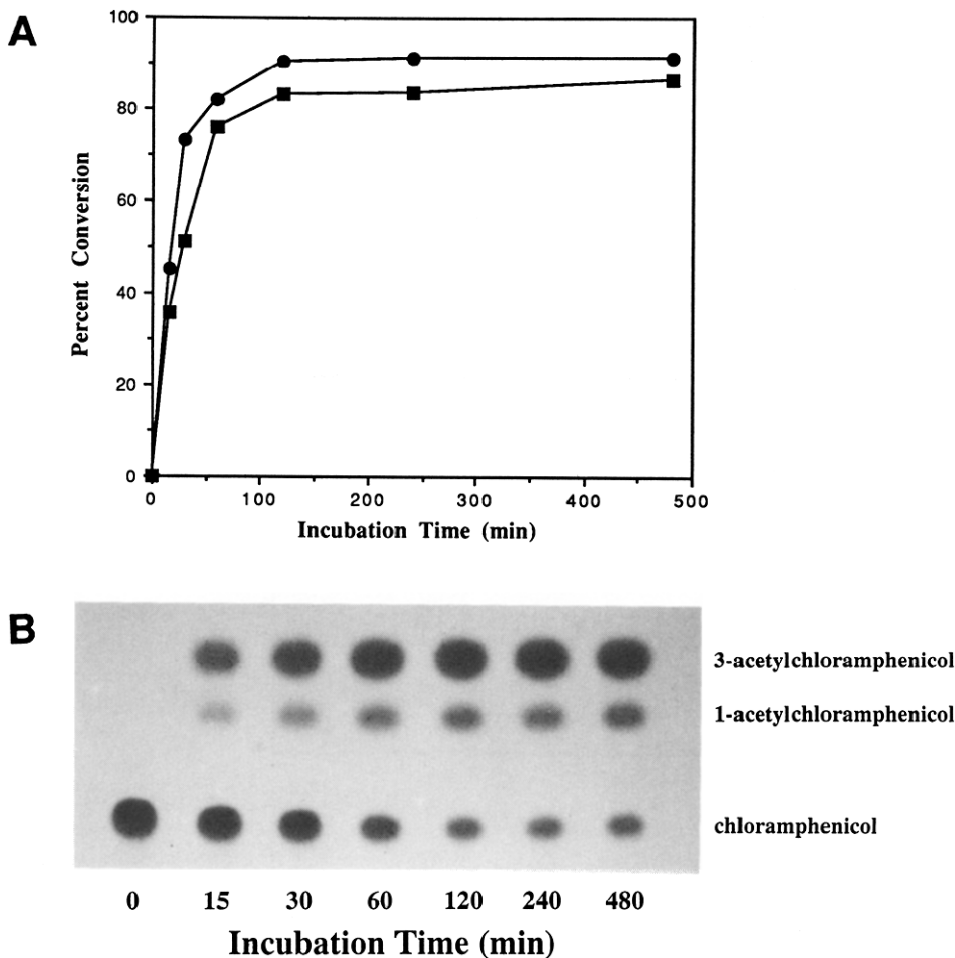
A

B

Fig. 4. CAT-catalyzed production of acetylated chloramphenicol. (A) A comparison of the percent conversion of chloramphenicol to acetyl chloramphenicol by 1 Unit of CAT at 27°C using HPCE analysis (■) and the standard TLC method (●). Percent conversion was determined by the peak area for the HPCE analysis and linear scanning densitometry of the autoradiogram for the TLC method. (B) Autoradiograph of TLC separation of the [^{14}C]acetylchloramphenicol from the [^{14}C]chloramphenicol substrate.

shown). This was not the case for the reaction product coenzyme A which eluted as a relatively broad peak and appeared to undergo a temperature-dependent, non-enzymatic degradation at temperatures greater that 4°C. At 27°C, the time-dependent increase in magnitude of the peak at *ca.* 6.0 min clearly results from the loss of coenzyme A (Fig. 6). The identity of this peak has not been determined unequivocally, but the possibility that it represents ADP, pantothenic acid or β-mercaptoethylamine (subcomponents of coenzyme A) has been eliminated since these standards did not co-migrate with this peak (data not shown). Interestingly, the presence of DTT appeared to prevent the loss of coenzyme A. Analysis of the samples at time 0 and 7 h of incubation at 27°C showed that the presence of DTT prevented the formation of the peak previously observed, indicating that this is most likely an oxidized form of coenzyme A (Fig. 7A). The absence of DTT led to the total conversion of coenzyme A to the oxidized product over the course of 7 h (Fig. 7B). The remarkably unstable nature of coenzyme A under these experimental conditions is highlighted in graphic form in Fig. 8 which shows the non-enzymatic loss at 27°C in comparison with the other CAT reaction components. In contrast to coenzyme
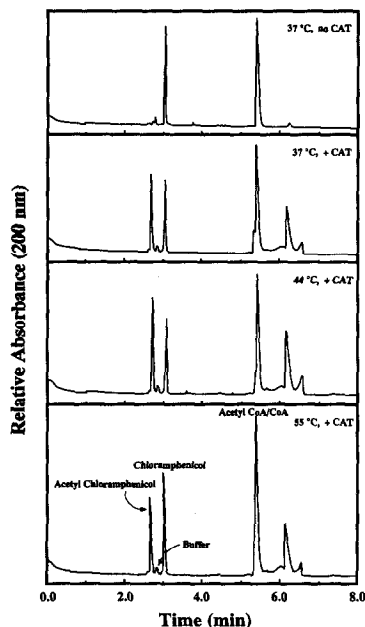
Fig. 5. Temperature-dependence of the CAT-catalyzed conversion of substrate to product. Chloramphenicol (500 μM), acetyl coenzyme A (500 μM) incubated in 20 mM Tris–Cl at 37, 44 and 55°C for 1 h. One unit of CAT was added to the indicated reactions in a total volume of 150 μl. Samples were pressure injected (3 s) into a 57 cm × 50 μm capillary and separated at 25 kV.

A, chloramphenicol, acetyl chloramphenicol and acetyl coenzyme A were very stable under similar conditions since no loss of these components was observed over the course of 8 h. The slight increase in their concentration over the course of the experiment is most likely due to a concentrating effect result from evaporation of solvent at 27°C. Despite the use of rubber caps on the sample vials, a loss of approximately 5% of sample volume per hour has been routinely observed (data not shown). This is a significant loss with microvials the capacity of which is approximately 25 μl.

DISCUSSION

In this study, we have demonstrated that both the substrates and products of the CAT enzymatic reaction can be separated rapidly by HPCE with a borate buffer system at pH 8.3. Loss of substrates (acetyl coenzyme A and chloramphenicol) and the

formation of products (acetylated chloramphenicol and coenzyme A) could be clearly observed in a manner dependent upon time and temperature, as well as reactant and enzyme concentration. The observed separation of chloramphenicol from its acetylated derivative is curious in light of the fact that they both are neutrally charged under these experimental conditions and hence would be expected to migrate with EOF. The fact that chloramphenicol elutes later than the EOF leads to two possible hypotheses. The first is based on the potential for chloramphenicol to interact with the capillary wall. It seems unlikely, based on the structure of chloramphenicol, that this molecule would be ionized at a pH of 8.3. Thus, it is possible that the presence of intact hydroxyl groups make chloramphenicol amenable to interaction (polar) with the capillary wall (SiO_3) which would ultimately retard its migration. In contrast, acetylated chloramphenicol is less polar (especially the diacetylated derivative which has both hydroxyl groups masked), interacting with the wall to a substantially lesser degree and would thus migrate with EOF. The second hypothesis exploits the potential of chloramphenicol to interact with borate. Since borate is known to complex with vicinal hydroxyls, it is possible that it forms a complex with the two free hydroxyl groups of chloramphenicol, imparting a net negative charge to the molecule at pH 8.3. This complexation is not possible with either the mono- or diacetylated chloramphenicol and hence, this molecule comigrates with the endoosmotic flow. This latter possibility appears to be the case. Separation of idential samples in phosphate and borate buffer at the same pH showed that chloramphenicol and the acetylated derivatives were not resolved in phosphate buffer but instead, comigrate with EOF (data not shown). These results suggest that, as shown by Lui et al. [19] with sugars, borate may be forming specific complexes with chloramphenicol but not acetylated chloramphenicol.

With respect to the monitoring of enzyme activity, HPCE has advantages over standard colorimetric or radiometric analysis which monitor changes in a single parameter. This includes not only the time required for analysis of a single sample, but also the fact that conditions can be found in which all components (substrates and products) can be monitored simultaneously. For the case of CAT, activity
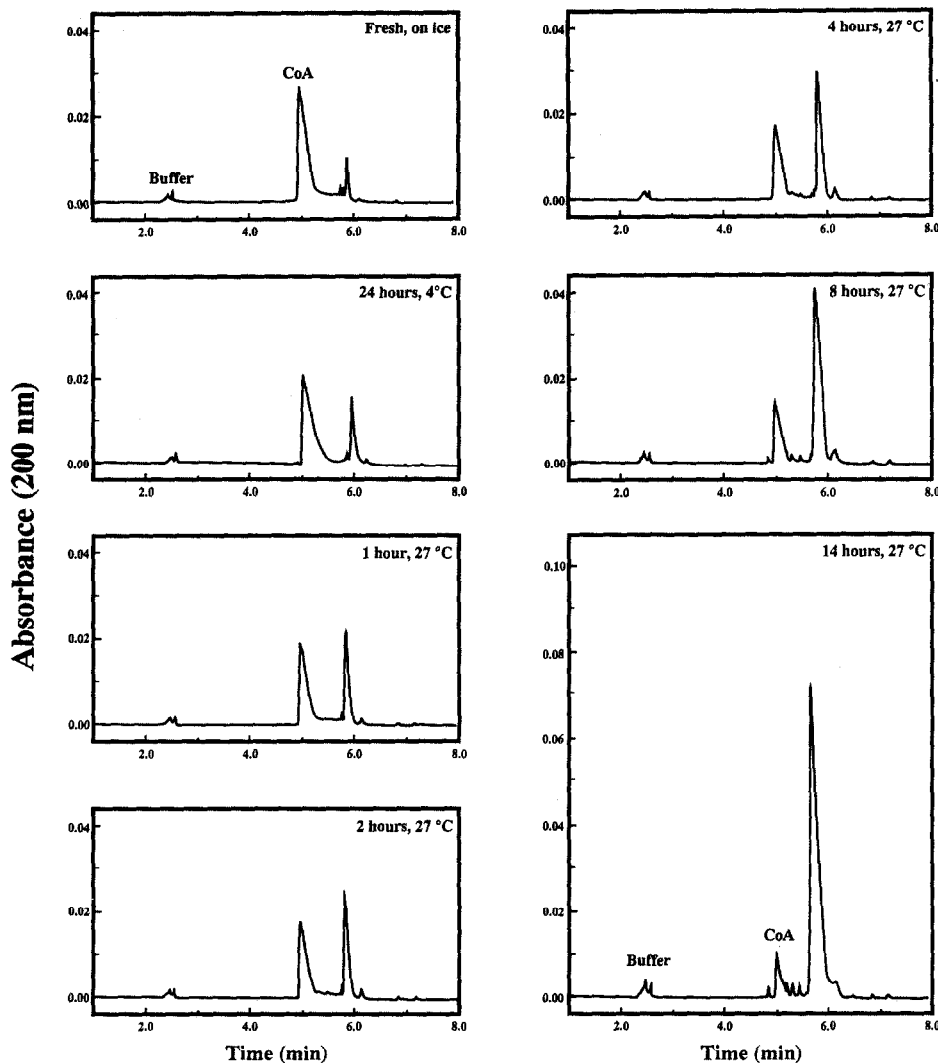
Fig. 6. HPCE monitoring of the non-enzymatic loss of coenzyme A. Coenzyme A (500 μg/ml) was incubated on ice or at 27°C in 20 mM Tris–Cl and sampled at specific intervals. Sample injection was by pressure for 1 s into a 57 cm × 50 μm capillary and separation at 25 kV.

can be followed as a function of both chloramphenicol loss and/or acetylchloramphenicol formation. Additionally, the speed of HPCE analysis allows for protocol modification to be tested rapidly. For example, a 10-min analysis determined that incubation in boiling water (*i.e.* boiling) had little or no effect on substrate stability and could thus serve to terminate enzyme activity. HPCE analysis also demonstrated that, in contast to reports on the

stability of acetyl coenzyme A in cell extracts [20], this component was apparently very stable under our experimental conditions while the product coenzyme A was found to be very unstable. The identity of the peak resulting from prolonged exposure of coenzyme A to 27°C was not any of the obvious possibilities such as subcomponents of coenzyme A. Through the use of the reducing agent DTT, it became apparent that coenzyme A was
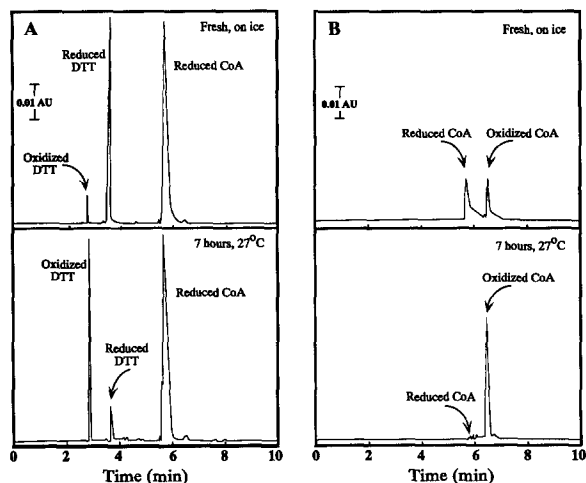
Fig. 7. Dithiothreitol stabilization of coenzyme A. Coenzyme A (0.5 mg/ml in borate buffer, pH 8.3) in the presence (A) and absence (B) of 20 mM DTT at 27°C at time 0 and 7 h. The reaction volume for each sample was 50 μl. An aliquot was sampled by pressure injection for 3 s into a 57 cm × 50 μm capillary and separated at 25 kV.

Fig. 8. HPCE analysis of the stability of CAT substrates and products in the absence of enzyme. Acetyl coenzyme A (1 mg/ml), coenzyme A (500 μg/ml), diacetyl chloramphenicol (500 μg/ml) and chloramphenicol (500 μg/ml) were incubated individually at 27°C in 20 mM Tris–HCl and sampled at specific intervals (1-s injection; 57 cm × 50 μm capillary; 25 kV). Percent maximum concentration was obtained through peak areas and plotted vs. the incubation time.

undergoing oxidation, possibly forming a dimer through a disulphide bond. The ability to detect the rapid loss of any reaction component is relevant to the development of an assay for enzyme activity. In the case of CAT, the estimation of enzyme activity, as measured by the appearance of coenzyme A, would be largely underestimated as a result of rapid oxidation of this molecule in a temperature-dependent, non-enzymatic manner.

Perhaps one of the more obvious extensions of the results of this study is the potential extrapolation to the molecular biology technique that has exploited the enzyme-catalyzed acetylation of chloramphenicol. With the standard CAT assay the aim is to measure the amount of enzyme (and hence the activity of the promoter) by the addition of [14C]chloramphenicol and acetyl coenzyme A to an extract of the cells containing (or lacking) the enzyme. Active enzyme catalyzes the acetylation of 14C-labeled chloramphenicol using acetyl coenzyme A as the acetyl group donor. Labelled products (both mono- and diacetylated chloramphenicol) are separated from the substrate by TLC and results are qualitatively assessed by autoradiography. Quantitative assessment of product formation is determined by liquid scintillation counting after spots have been
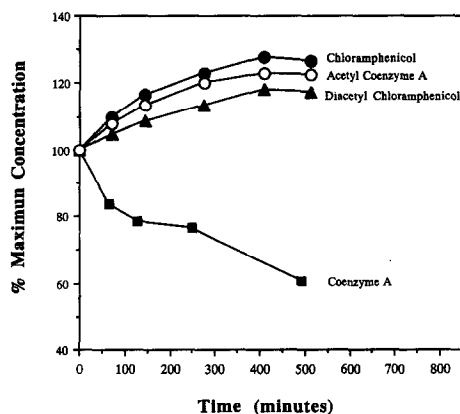
scraped from the thin-layer plate or by scanning densitometry of the autoradiogram. Another method involves a two-phase extraction of the 14C-labelled product into liquid scintillation cocktail using [14C]acetyl coenzyme A and unlabelled chloramphenicol [21].

In the study described here, concentrations of unlabelled chloramphenicol and acetylcoenzyme A similar to those used in a standard CAT assay were used. The use of a Tris buffer concentration (20 mM) lower than that typically used in a standard CAT assay (167–570 mM; cf. refs. 20 and 22) reduced the size of the buffer peak which migrates close to acetyl chloramphenicol without affecting enzymatic activity. Parallel experiments using 14C-labelled chloramphenicol as substrate, followed by analysis with the standard TLC method and HPCE analysis, show that similar quantitative data are obtained. This clearly indicates that CAT-induced acetylation of chloramphenicol can be rapidly monitored with HPCE and may be a useful tool for the evaluation of the transcriptional promoting activity of specific sequences of DNA. This approach presents several potential advantages over the TLC method presently used (Table I). First, HPCE significantly decreases the amount of time needed to obtain qualita-

TABLE I

COMPARISON OF HPCE AND TLC ANALYSIS OF CAT ENZYME ACTIVITY

	HPCE	TLC
Speed for analysis of single sample	6 min	Up to 24 h
Radioactivity required	No	Yes
Sample size (μl)	10	150
Quantitation: number of steps	1	3
method	Peak area analysis	Scintillation counting
Cost considerations	—	^{14}C-Substrate purchase
	—	Radioactivity disposal
Automation	Yes	No
Simultaneous analysis of multiple samples	No	Yes

tive and quantitative data. Typical times for obtaining quantitative data with the TLC method may be as long as 24 h when including ethylacetate extraction, chromatography, autoradiography and scintillation counting. The data presented here indicate that, with HPCE, the equivalent quantitative data may be obtained in less than 10 min per sample. Moreover, CAT activity by any assay is measured by the presence of both the mono- and diacetylated forms of chloramphenicol. HPCE separation under the conditions described in this study results in the apparent comigration of all acetylated forms and, hence, peak area is a true quantitative representation of activity. In addition to eliminating the biohazard of working with radioactivity, all of the advantages of using a non-isotopic assay follow. The cost of the assay is dramatically reduced by eliminating the cost of purchasing [^{14}C]chloramphenicol or [^{14}C]acetyl coenzyme A and disposing of the radioactive products. Finally, a single HPCE analysis uses a smaller reaction mixture volume (as little as 10 μl) to make nanoliter volume injections. This is substantially less than the 150 μl typically required for TLC or liquid scintillation cocktail extraction and, hence, reduces the amount of precious cell extract needed.

CONCLUSIONS

These data highlight new potential uses for HPCE. First, the possibility that the rapid, reproducible nature of HPCE analysis for the evaluation of enzyme activities is identified with the particular

advantage that both loss of substrate as well as the increase in product(s) can be monitored. Finally, the potential exists for HPCE to circumvent the tedious methodology presently used to characterize the transcriptional activity of eukaryotic promoters using the CAT assay.

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