Structural and Mechanistic Studies of Galactoside Acetyltransferase, the Escherichia coli LacA Gene Product*

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Escherichia coli galactoside acetyltransferase (GAT) is a member of a large family of acetyltransferases that O-acetylate dissimilar substrates but share limited sequence homology. Steady-state kinetic analysis of overexpressed GAT demonstrated that it accepted a range of substrates, including glucosides and lactosides which were acetylated at rates comparable to galactosides. GAT was shown to be a trimeric acetyltransferase by cross-linking with dimethyl suberimidate. Fluorometric analysis of coenzyme A binding showed that there is a fluorescence quench associated with acetyl-CoA binding whereas CoA has no effect. This difference was exploited to measure dissociation rates for both CoA and acetyl-CoA by stopped-flow fluorometry. The rate of dissociation of CoA (2500 s⁻¹) is at least 170-fold faster than k₅ₐₚ for any substrate tested. The fluorescence response to acetyl-CoA binding is entirely due to Trp-139 since treatment of GAT by [¹⁴C]iodoacetamide resulted in complete inactivation of the enzyme and the incorporation of label into histidyl and cysteynil residues to approximately equal extents. Following replacement of His-115 by alanine, label was incorporated solely into cysteynil residues. Furthermore, the substitution results in an 1800-fold decrease in k₅ₐₚ suggesting that His-115 has an important catalytic role in GAT.

Galactoside acetyltransferase (EC 2.3.1.18; GAT) transfers an acetyl group from acetyl-CoA to the 6-hydroxyl of certain galactopyranosides (1). GAT is encoded by the lacA gene of the lac operon of Escherichia coli, and consequently lactopyranosides induce expression of lacA coordinately with the lacZ and lacY genes which encode β-galactosidase and lactase permease, respectively (2). The latter two enzymes have clearly defined roles in the catabolism of lactose whereas GAT does not; its function in microbial metabolism remains ill-defined since lacA plus minus strains of E. coli are equally viable under laboratory conditions (3). Andrews and Lin (4) proposed that the lacA gene product might have a role in detoxification, by facilitating the elimination of non-metabolizable lactopyranosides from the cell since acetylated lactopyranosides are not taken up by cells (3).

E. coli GAT has been studied extensively by Zabin and co-workers (5–8). Both the DNA sequence of the lacA gene (5) and the amino acid sequence of GAT (6) have been determined independently. Although GAT was reported to be dimeric (7), the subsequent determination of the exact subunit molecular mass (22,700 Da (6)) from sequencing together with a value of 65,300 Da from ultracentrifugation studies for the native molecular mass (8) suggests that GAT might be a trimer. Steady-state kinetic studies with IPTG as acetyl acceptor indicated that GAT catalyzed acetyl transfer by a compulsory order ternary complex mechanism with acetyl-CoA as the leading substrate (9).

GAT is a member of a large family of acetyltransferase enzymes that share regions of amino acid sequence homology. Downie (10) reported extensive homology throughout the amino acid sequence of GAT and of the nodL gene product of Rhizobium leguminosarum, a lipooligosaccharide acetyltransferase. Although the precise nature of the substrate for lipooligosaccharide acetyltransferase is unclear, like GAT it acetylates the 6-hydroxyl group of a galactopyranoside ring (11). More limited areas of homology have been found between the C-terminal regions of the amino acid sequences of GAT, serine acetyltransferases, and a class of enzymes that may conveniently be described as xenobiocic acetyltransferases (10, 12). Since enzymes of the xenobiocic acetyltransferase class acetylate antibiotics such as chloramphenicol as well as virginnamid-like compounds (12–14), the evident dissimilarity of the acetyl acceptors for this class of acetyltransferases suggests that the region of homology in their amino acid sequences could represent a structural feature required for binding coenzyme A.

GAT shows no primary structure homology to a particularly well-defined example of an acetyltransferase enzyme, type III chloramphenicol acetyltransferase (CATIII), for which a three-dimensional structure is available (15, 16). CATIII is a homotrimer (3 × 25 kDa) with a kinetic mechanism that involves the formation of a ternary complex via a random order of addition of substrates (17). The catalytic mechanism of CATIII has been studied in some detail. The N² of the imidazole of His-195, conserved in all members of the CAT family, acts as a general base, abstracting a proton from the hydroxyl group of chloramphenicol, thereby promoting nucleophilic attack by the oxygen at the carbonyl of the thioester of acetyl-CoA. The mechanism proceeds via a tetrahedral intermediate wherein the resulting oxanyion is stabilized by hydrogen bonding to the hydroxyl of Ser-148, another conserved residue. Substitution of Ser-148 and His-195 by Ala in CATIII results in 53-fold and 9 × 10⁵-fold decreases in k₅ₐₚ respectively (18, 19). Another class of acetyltransferases that may be distinct from either the CAT or the GAT family includes choline acetyltransferase and carnitine palmitoyltransferase, both of which have also been reported to have catalytic histidyl residues (20, 21).
In the present study on GAT we show that GAT is indeed a trimeric acetyltransferase, report the results of steady-state and pre-steady-state kinetic studies of the enzyme, and examine the fluorescence response of GAT to coenzyme binding. We also present evidence from site-directed mutagenesis and chemical modification studies that suggests that GAT contains a catalytically important histidyl residue.

EXPERIMENTAL PROCEDURES

Materials

Cibacron Blue-Sepharose was prepared as described by Lowe and Pearson (22) and CoA-Sepharose was prepared by covalently linking CoASH to epoxy-activated Sepharose 2B (Pharmacia Biotech, Inc.) as described by the manufacturers’ instructions.

Overexpression of GAT and Site-directed Mutagenesis

The lacA gene was amplified from pgm8 (5) by the polymerase chain reaction using the primers P1 (5'-CGGATCCAGAGGATATGTTTAATGAACATGCCAATGACC) and P2 (5'-CCTCTAGA(GTTGAAGCG-GAGGCCAAGCG-CAGCG) in 67 mM Tris-HCl, pH 8.3, 16 mM (NH4)2SO4, 6.7 mM MgCl2, 10 mM 2-mercaptoethanol, 6.7 mM EDTA, 1.5 mM GATP, 1.5 mM dCTP, 1.5 mM dTTP, bovine serum albumin (0.17 mg/ml), and 2.5 units of Taq polymerase. The resulting polymerase chain reaction fragment was digested with BamHI and XbaI, cloned in BamHI/XbaI digested M13 mp18, and the sequence of the insert was determined. The lacA gene was excised by digesting with Smal and PstI and cloned in Smal/PstI-digested pkK223-3 yielding pkK223-GAT.

Oligonucleotide-directed mismatch mutagenesis was performed using the deoxyuridine selection protocol with the dut ung E. coli strain RZ1032 (23). The oligonucleotides (mismatches underlined) used were: 5'-GGGTGACAGGGGCCTCGAAGCS (His-115 -→ Ala), 5'-GACCTC- CGATA(T/A)AGACGTTATTG (Trp-139 Ala), and 5'-GAACCATGCCAATGACC (5'.

Fluorescence Spectra

The fluorescence spectrum of each protein was obtained using an SLM 4000Os fluorometer with a thermostated cuvette block by methods similar to those employed for CATs (27). Tryptophan fluorescence was selected by excitation at 297 nm from a 200-watt Hg-Xe lamp. The emission slit width was 4 nm. A typical sample contained 2 μM protein (as GAT monomers) in TSE buffer, pH 7.5, equilibrated to 25 °C. Emission spectra were corrected for photomultiplier sensitivity.

Fluorometric Titrations

Manual titrations of GAT (0.7 μM in monomers) in TSE buffer, pH 7.5, were performed as described by Ellis et al. (28). Data were plotted as fluorescence intensity versus [acetyl-CoA] and were fitted to a hyperbolic function using Graft (29) to calculate dissociation constants.

Stopped-flow Fluorometry

A SF 1.7MV stopped-flow spectrometer (Applied Photochemistry, Leatherhead, United Kingdom), with a dead time of 1.6 ms, was used for the measurement of the dissociation rate constants of CoA and acetyl-CoA (as described in Ref. 28). The light source was a 150-watt xenon lamp, 297 nm was selected as the excitation wavelength and a 1-mm slit width used. Fluorescence was observed through a 335-nm cut-on filter. The temperature of the drive syringes and observation chamber was maintained by water circulation. Stock solutions of ligands and enzyme were diluted to the desired concentrations with degassed TSE buffer, pH 7.5, immediately prior to loading into the drive syringes. Typically, 10 traces were collected to be signal-averaged for subsequent nonlinear regression analysis. Dissociation rate constants were found to be independent of the enzyme substrate ratio.

Chemical Modification of GAT with Iodoacetamide

Time courses of chemical modification were monitored at 25 °C in TSE buffer, pH 7.5. Stock solutions of iodoacetamide were made up in TSE buffer and the pH was adjusted to 7.5. The reagent (final concentrations 10, 20, 30, 40, and 50 mM) was added to GAT at zero time, and at appropriate time intervals, samples were withdrawn, diluted in TSE buffer, and assayed for GAT activity.

[14C]iodoacetamide was diluted with unlabeled reagent to give a final specific activity of 368 MBq/mmol. Wild-type GAT was incubated in TSE buffer containing 50 mM [14C]iodoacetamide. Samples were withdrawn at intervals and the extent of covalent incorporation was monitored by filter binding as described by Keenathous et al. (30). In preparative analyses, wild-type GAT and the H115A variant (100 nmol) were each treated with 50 mM [14C]iodoacetamide. After 4 h the reaction was quenched by adding 2-mercaptoethanol (final concentration 0.1 mM) and a sample of the treated protein in each case was counted to establish the stoichiometry of modification. The remainder was subjected to acid hydrolysis, and the products were resolved by thin layer electrophoresis (Polygram SiIG silica) (30). Following staining with fluorescent camine, the plate was cut into strips and analyzed by scintillation counting to identify modified amino acids.

RESULTS

Overproduction and Purification of GAT—The E. coli lacA gene was previously cloned on a 3.2-kilobase fragment in pgm8 (5). The lacA open reading frame was amplified from pgm8 using primers P1 and P2, producing a copy of the lacA gene with an ATG start codon, a good RBS (from the cat toxin gene (31)) and BamHI and XbaI sites 5' and 3', respectively, to the coding sequence. The sequence of the amplified gene was verified, then the modified lacA gene was cloned in pkK223-3. Induction of expression with IPTG resulted in levels of GAT activity elevated approximately 40-fold over the level found in E. coli JM101 carrying pgm8. GAT was purified as described above using a modification of the method of Zabin and Fowler (24).

Subunit Structure—Since a comparison of the subunit Mr of 22,700 derived from the amino acid sequence of GAT (6) and 8,0, containing 0.1 M NaCl and 0.4 M dithiothreitol. The cross-linking reaction was quenched by the addition of Tris-HCl, pH 7.5, 2-mercaptoethanol and SDS to give final concentrations of 50 mM, 40 mM, and 0.3% (w/v), respectively. The cross-linked protein was analyzed by SDS-polyacrylamide gel electrophoresis using a continuous phosphate buffer system (26).
the native $M_c$ of 65,300 determined by ultracentrifugation (8) suggested that GAT might be trimeric, this possibility was examined by cross-linking GAT with dimethyl suberimidate. Only three species were observed (results not shown) after cross-linking and their $M_c$ values corresponded to those of the monomer, dimer, and trimer of GAT, confirming the prediction that GAT is trimeric.

Kinetic Properties of GAT—A preliminary analysis showed that kinetic parameters for overproduced GAT with IPTG as acetyl acceptor were similar to those reported by Musso and Zabin (9) who also demonstrated that $p$-nitrophenol sugars were substrates for GAT but did not report kinetic parameters. In fact, $p$-nitrophenol-$\beta$-D-pyranogalactoside (PNP$_{\beta}$Gal) is a reasonable substrate for GAT with a $K_m$ value an order of magnitude lower than that for IPTG. Consequently, the kinetic parameters for a range of $p$-nitrophenol sugars have been examined, as well as the effects of the alternative acyl donors propionyl-CoA and butyryl-CoA (Table I).

Generally, the major effect of altering either the acetyl donor or the acceptor is expressed in changes in $k_{cat}$. Altering the structure of the acetyl acceptor has little effect on the $K_m$ value for either substrate, with the exception of the $K_m$ for acetyl-CoA with PNP$_{\beta}$Gal as acetyl acceptor. However, the changes in $k_{cat}$ suggest that PNP$_{\beta}$Gal is the best substrate, followed by ONP$_{\beta}$Gal, whereas PNP$_{\beta}$Gal, PNP$_{\beta}$Glc, and PNP$_{\beta}$Lac are all relatively poor substrates. Moreover, the presence of the nitro group on the phenyl ring makes little difference since phenyl-$\beta$-D-glucopyranoside is $\beta$-d-glucopyranoside $\alpha$-acetylated approximately 20-fold more slowly than PNP$_{\beta}$Gal. GAT appears to prefer sugars without N-acyetyl groups. The major effect of increasing the chain length of the acetyl donor is a decrease in the value of $K_{cat}$. Although propionyl-CoA is almost as effective as acetyl-CoA as an acetyl donor ($K_{cat}$ decreased only 2.5-fold), butyryl-CoA is a very poor substrate; $K_{cat}$ is decreased 270-fold and $K_m$ is increased 2-fold compared to acetyl-CoA.

Musso and Zabin (9) proposed that GAT follows an ordered sequential mechanism with acetyl-CoA as the leading substrate. If such a mechanism applies, it should be possible to extract $K_m$ values for acetyl-CoA from the results of steady-state kinetic analysis, and show that such values for $K_m$ do not depend on the structure of the acetyl acceptor. The data for acetyl-CoA, shown in Table I, confirms that the nature of the acetyl acceptor has little effect on the kinetically derived $K_m$ values, as expected for the proposed mechanism.

Fluorescence Responses to the Binding of Acetyl-CoA and CoA—The association of acetyl-CoA with GAT provokes a quench of intrinsic protein fluorescence of approximately 29%, as illustrated in Fig. 1. The use of 297 nm as the excitation wavelength avoids problems arising through inner filter effects from acetyl-CoA since the CoA moiety does not absorb significantly above 285 nm. As such, the observed quench is a genuine intrinsic fluorescence response of the protein to the association of the ligand and shows that GAT binds acetyl-CoA in the absence of an acetyl acceptor.

The results observed with unesterified CoA as the ligand are somewhat unexpected in that, despite the marked quench of fluorescence intensity on binding acetyl-CoA, GAT shows virtually no fluorescence response on association with CoA (Fig. 1). The fluorescence quench induced by acetyl-CoA binding allowed the direct determination of the dissociation constant for the GAT-acetyl-CoA binary complex by fluorometric titration. The $K_d$ value of 20.8 $\mu$M obtained by this technique is significantly lower than the values estimated from steady-state kinetics. Binding of the substrate analogue, ethyl-CoA, also results in a fluorescence quench of the same magnitude as that produced by acetyl-CoA, however, the $K_d$ for ethyl-CoA is approximately 300 $\mu$M.

Stopped-flow Fluorometry—The binding of acetyl-CoA to GAT at 25 °C was found to proceed too rapidly to be followed by stopped-flow fluorometry, making the measurement of the association rate constant impossible. However, the marked difference in the fluorescence responses of GAT to the binding of CoA and acetyl-CoA provided the opportunity to monitor coenzyme dissociation using a displacement reaction.

A sample of 4 $\mu$M GAT was preincubated with 150 $\mu$M acetyl-CoA (syringe concentrations) and mixed with 400 $\mu$M CoA in the stopped-flow apparatus. The observed increase in fluorescence (due to the displacement of acetyl-CoA) was small (2%) and, when fitted to a single exponential expression, occurred at a rate in excess of 600 s$^{-1}$ at 25 °C. In the reciprocal experiment, the dissociation rate constant for CoA was found to be too fast to measure at 25 °C in that no fluorescence change was observed.

In view of this, together with the uncertainty inherent in monitoring small amplitude changes at very fast rates for acetyl-CoA dissociation, the procedure was repeated at a number of lower temperatures. On plotting these data as $\ln(k_{on})$ versus temperature (results not shown), the linear fit was extrapolated to estimate the dissociation rate at 25 °C. For acetyl-CoA this rate was found to be approximately 720 s$^{-1}$, and a much faster rate of the order of 2500 s$^{-1}$ for CoA.

From fluorometric titrations, the $K_s$ of acetyl-CoA was found to be 20.8 $\mu$M and, knowing $k_{cat} = 720$ s$^{-1}$, $K_s$ may be calculated to be $3.5 \times 10^7$ M$^{-1}$s$^{-1}$. Clearly, neither the association of

| Substrate pair        | $k_{cat}$ | Acetyl acceptor | Acetyl donor | Acetyl donor |
|-----------------------|----------|----------------|--------------|--------------|
|                       | s$^{-1}$ | $K_m$ (M)       | $K_m$ (M)    | $K_d$ (M)    |
| BtCoA/PNP$_{\beta}$Gal| 0.054   | 25.2           | 221          | 81           |
| PrCoA/PNP$_{\beta}$Gal| 5.8     | 45.6           | 98           | 49           |
| AcCoA/phenyl-$\beta$Gal| 13.0   | 63.4           | 59           | 66           |
| AcCoA/PNP$_{\beta}$Gal| 14.7   | 63.4           | 104          | 50           |
| AcCoA/ONP$_{\beta}$Gal| 8.9    | 67.9           | 82           | 62           |
| AcCoA/PNP$_{\beta}$Glc| 1.42   | 57.9           | 75           | 50           |
| AcCoA/PNP$_{\beta}$Lac| 1.84   | 40.2           | 65           | 39           |
| AcCoA/PNP$_{\beta}$Lac| 1.84   | 60.2           | 266          | 40           |
acetyl-CoA nor the dissociation of CoA during product release are rate-limiting in the transacetylation reactions studied here.

Replacement of Trp-139—The primary structure of GAT contains only two tryptophanyl residues, Trp-63 and Trp-139. Furthermore, only Trp-139 lies in the region of general homology found in this class of acetyltransferases that is presumed to be important for CoA binding. Consequently, Trp-139 was replaced by phenylalanine and tyrosine in site-directed mutagenesis experiments. Preliminary investigation indicated that the activities of W139F and W139Y GAT were very similar (data not shown). Consequently, only W139F GAT was characterized since the Trp → Phe substitution allows fewer opportunities for novel interactions in the substituted enzyme. Compared to wild-type GAT, the $K_m$ values for acetyl-CoA and PNPβGal for W139F are unchanged; $k_{cat}$ is decreased approximately 10-fold as a consequence of the substitution (Table II).

The emission spectra in Fig. 1 demonstrate the loss of intensity (~46%) due to the replacement of Trp-139 by phenylalanine. It is clear that the two tryptophan residues within each GAT monomer contribute equally to the observed fluorescence of the wild-type native trimer. The simple additive nature of these fluorescence contributions indicates that homogeneous radiation-less transfer between them does not have a significant effect on their fluorescence properties when excited at 297 nm, near the red edge of the absorption spectrum for tryptophan.

The position of the maximum emission wavelength varies slightly between the GAT variants, reflecting subtle differences in the nature of the local environment of each tryptophan (Fig. 1). The spectrum of W139F GAT, retaining only Trp-63, shows a red shift in the emission peak ($\lambda_{max} = 341$ nm), compared to the spectrum of wild-type GAT ($\lambda_{max} = 337$ nm); indicative of either a relatively hydrophobic local environment for Trp-139 or greater solvent accessibility for Trp-63.

W139F GAT retains little or no fluorescence response to acetyl-CoA (or CoA) association (Fig. 1). Hence, it is almost certain that Trp-139 is entirely responsible for the tryptophan-related fluorescence response of wild-type GAT to the binding of acetyl-CoA.

Replacement of His-115 and Ser-162—Comparison of the sequences of the nodA acetyltransferase (LAT) and GAT revealed that only one histidine and one serine residue are conserved in the sequence alignment, namely, His-115 and Ser-162. Each residue is each replaced by alanine in order to determine the likelihood that these residues might play a similar role in GAT as His-195 and Ser-148 do in CAT$_{III}$. The kinetic properties of the substituted enzymes, H115A and S162A GAT, are shown in Table II. S162A GAT is very similar to wild-type enzyme, suggesting that Ser-162 is not catalytically important. In contrast, the His-115 → Ala substitution leads to a dramatic decrease in catalytic activity. Consequently, H115A GAT was overexpressed in E. coli X901LacA46, a strain with the cat$_{II}$ gene inserted into the lacA reading frame to ensure that no GAT was produced from expression of the chromosomal lacA gene. When compared to wild-type GAT, the H115A variant showed an 1800-fold decrease in $k_{cat}$, whereas the $K_m$ value for PNPβGal was decreased 5-fold and the $K_m$ for acetyl-CoA was unchanged. Dissociation constants for acetyl-CoA, measured by fluorometric titration for H115A GAT and S162A GAT are 20.1 and 21.6 $\mu$M, respectively, suggesting that acetyl-CoA binding is not affected by either substitution.

Chemical Modification of GAT with Iodoacetamide—Wild-type GAT is irreversibly inhibited by iodoacetamide. The pseudo-first order rate of inactivation ($k_{i}$) was determined at several different concentrations of reagent (results not shown). Since $k_{i}$ is directly proportional to the iodoacetamide concentration, inhibition is a simple bimolecular process (rate constant $= 0.017$ $M^{-1}s^{-1}$). Protection against inhibition is observed in the presence of the non-hydrolyzable substrate analogue ethyl-CoA or PNPβGal (Fig. 2a) with ethyl-CoA providing better protection. However, the degree of protection afforded by PNPβGal may be limited by the relatively low concentration used compared to the presumably high binding constant for this substrate.

The stoichiometry of inactivation was examined by treatment of GAT with [14C]iodoacetamide (Fig. 2b). Clearly, incorporation of label does not directly reflect inhibition of GAT activity suggesting that iodoacetamide modifies more than one residue in GAT. In fact, after 4 h, approximately 2 nmol of [14C]label were incorporated per nanomole of GAT monomers. In order to determine the type of residue modified by the reagent, [14C]iodoacetamide treatment of both wild-type and H115A GAT was followed by acid hydrolysis and the resulting labeled amino acids were separated by thin-layer electrophoresis (30). In each case the stoichiometry of inactivation was similar, approximately 2 nmol of label incorporated per nmol of enzyme monomer. However, the pattern of residues modified in the wild-type and substituted enzymes were different (Fig. 2c). In wild-type GAT, carboxymethylcysteine and 3-carboxymethylhistidine were found in approximately equal amounts (37.8 and 49.3% of the total counts recovered, respectively) indicating that iodoacetamide modifies both cysteinyl and histidyl residues in the wild-type protein. In contrast, in H115A GAT carboxymethylcysteine is the major product (84.8% of total counts recovered), suggesting that His-115 is the histidyl residue modified by iodoacetamide in wild-type GAT.

**DISCUSSION**

Cross-linking GAT with dimethyl suberimidate has confirmed the suggestion from earlier M, determinations that GAT is a trimeric acetyltransferase. There is also evidence that a xenobiotic acetyltransferase enzyme that acetylates chloramphenicol in Agrobacterium tumefaciens is a trimer.3 Other members of the acetyltransferase family to which GAT belongs may also prove to be trimeric.

It is clear from steady-state kinetic analysis that GAT has a broad substrate specificity since it will acetylate galactosides, glucosides, and lactosides. Such a broad specificity supports the proposal (4) that GAT may act as a detoxifying enzyme, acetylating non-metabolizable sugars to prevent their re-entry into the cell.

The kinetic results support the mechanism postulated by Musso and Zabin (9). Certainly, acetyl-CoA binds to the free

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2. E. coli X901LacA46 was prepared by, and was a kind gift from Dr. I. A. Murray, Dept. of Pharmaceutical Chemistry, UCSF School of Pharmacy, San Francisco, CA.

3. I. A. Murray and W. V. Shaw, unpublished results.
enzyme and the association rate constant for acetyl-CoA and the dissociation rate constant for CoA are much greater than \( k_{cat} \). The rate-determining step is therefore likely to be either the loss of acetylated acceptor from the binary complex or the interconversion of the substrate and the product ternary complexes.

The fluorescence response to acetyl-CoA binding has been shown to be entirely due to Trp-139. Moreover, this tryptophanyl residue is in the C-terminal portion of GAT that is homologous to other acetyltransferase sequences. In fact, Trp-139 is found in other acetyltransferases including the nodL product (LAT) as well as enzymes of the xenobiotic acetyltransferase class, but not in serine acetyltransferase where it is replaced by a methionyl residue. Since the \( K_m \) value for acetyl-CoA for W139F GAT is similar to that of wild-type, it is unlikely that acetyl-CoA binding has been impaired by this substitution. The intrinsic fluorescence of GAT is quenched by acetyl-CoA but not CoA suggesting that Trp-139 may be close to the binding site of the acetyl group of the thioester and consequently to the active site of GAT. The fact that the Trp-139 \( \rightarrow \) Phe substitution results in a substantial decrease in \( k_{cat} \) supports this hypothesis, but the observed differentiation between acetyl-CoA and CoA may be due to another and more subtle effect. In the better characterized example of CATIII, the intrinsic fluorescence of Trp-152 is enhanced by association of acetyl-CoA but not CoA (27), although Trp-152 is in fact 7 Å from the thiol of CoA in the structure of the CATIII-CoA binary complex and would be expected, from structural considerations alone, to respond equally well to CoA or acetyl-CoA binding.

It is clear that Ser-162 appears to have no catalytic function despite its conservation throughout most of the acetyltransferase family. In contrast, His-115 seems certain to be important for catalysis, although it is conserved only in the alignment between GAT and the nodL product. The 1800-fold decrease in \( k_{cat} \) in H115A GAT is a modest one compared to the \( 9 \times 10^5 \)-fold decrease in \( k_{cat} \) observed for CATIII upon replacement of His-195 by Ala (19). Although it is possible that the function of His-115 may not be strictly analogous to that of His-195 in CATIII, nonetheless it is clearly of critical importance to the catalytic activity of GAT.

Chemical modification with iodoacetamide results in inactivation of GAT. The modification of histidyl and cysteinyl residues alike in wild-type GAT, but only of cysteinyl residues in H115A GAT, suggests that iodoacetamide modifies His-115 and one or both of the two cysteinyl residues of GAT. The fact that the stoichiometry of inactivation of H115A GAT is 2:1 suggests that both of the cysteinyl residues of GAT are fully modified in this variant, whereas in wild-type GAT either only one cysteine is modified or both cysteinyl residues are partially modified. It is unlikely that the cysteinyl residues of GAT have a catalytic function since, unlike His-115, neither are conserved in the sequence alignment between GAT and the nodL product (LAT).

Our interest in extending the work of others on the lacA gene

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**Figure 2.** a, inactivation of GAT with iodoacetamide. GAT was treated with iodoacetamide as described under Experimental Procedures. b, stoichiometry of iodoacetamide inactivation. GAT was treated with \([^{14}C]\)iodoacetamide as described under Experimental Procedures. At intervals, samples were removed and the fraction of the initial activity remaining and the extent of incorporation of \(^{14}C\) label into the protein were determined. c, high voltage electrophoresis of acid hydrolysates of wild-type and H115A GAT modified by \([^{14}C]\)iodoacetamide. Hydrolysates were subjected to electrophoresis at pH 6.5 on a silica plate. The plate was stained with fluorescamine, the tracks were cut into strips, and the radioactivity was located by liquid scintillation counting of the strips. The positions of carboxymethylated amino acid standard compounds after electrophoresis are indicated: A, 1-carboxymethylhistidine; B, 3-carboxymethylhistidine; C, 1,3-dicarboxymethylhistidine; and D, carboxymethylcysteine.
product has arisen in the main from a desire to rationalize structure-function relationships for a large and growing apparent “superfamily” of microbial acetyltransferases that may, in time, be separated into subgroups by various criteria. Although in some respects the prototype of the superfamily, CAT shows no sequence homologies with the enzymes mentioned here and with GAT in particular. What does stand out thus far is the likelihood that GAT is, like CAT, a trimeric enzyme with a similar kinetic mechanism and a critical histidyl residue. Studies in progress on the nodL product (LAT), on serine acetyltransferase, and on members of the curious “xenobiotic acetyltransferase” class of heterogeneous acetyltransferases should help to clarify the co-evolution of structure and function within the proposed superfamily.

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REFERENCES
1. Zabin, I., Kepes, A., and Monod, J. (1959) Biochem. Biophys. Res. Commun. 1, 289–292
2. Zabin, I., Kepes, A., and Monod, J. (1962) J. Biol. Chem. 237, 253–257
3. Wilson, T. H., and Kashket, E. R. (1969) Biochim. Biophys. Acta 173, 501–508
4. Andrews, K. J., and Lin, E. C. C. (1976) J. Biol. Chem. 251, 510–513
5. Hediger, M. A., Johnson, D. P., Nierlich, D. P., and Zabin, I. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 6414–6418
6. Fowler, A. V., Hediger, M. A., Musso, R. E., and Zabin, I. (1985) Biochimie (Paris) 67, 101–108
7. Brown, J. L., Brown, D. M., and Zabin, I. (1967) J. Biol. Chem. 242, 4254–4258
8. Zabin, I. (1963) J. Biol. Chem. 238, 3300–3306
9. Musso, R. E., and Zabin, I. (1973) Biochemistry 12, 553–557
10. Downie, J. A. (1989) Mol. Microbiol. 3, 1649–1651
11. Bloemberg, G. V., Thomas-Oates, J. E., Lugtenberg, B. J. J., and Spank, H. P. (1994) Mol. Microbiol. 11, 793–804
12. Parent, R., and Roy, P. H. (1992) J. Bacteriol. 174, 2891–2897
13. Tennigkeit, J., and Matzura, H. (1991) Gene (Amst.) 98, 113–116
14. Allignet, J., Londe, V., Simenel, C., Delepierre, M., and Solh, N. E. (1993) Gene (Amst.) 130, 91–98
15. Leslie, A. G. W., Moody, P. C. E., and Shaw, W. V. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 4133–4137
16. Leslie, A. G. W. (1990) J. Mol. Biol. 213, 167–186
17. Kleanthous, C., and Shaw, W. V. (1984) Biochem. J. 223, 211–220
18. Lewendon, A., Murray, I. A., Shaw, W. V., Gibbs, M. R., and Leslie, A. G. W. (1990) Biochemistry 29, 2075–2080
19. Lewendon, A., Murray, I. A., Shaw, W. V., Gibbs, M. R., and Leslie, A. G. W. (1994) Biochemistry 33, 1944–1950
20. Carlini, L. A., and Hersh, L. B. (1993) J. Neurochem. 61, 247–253
21. Brown, N. F., Anderson, R. C., Caplan, S. L., Foster, D. W., and McGarry, J. D. (1994) J. Biol. Chem. 269, 19157–19162
22. Lowe, C. R., and Pearson, J. C. (1984) Methods Enzymol. 104, 97–113
23. Künkel, T. A., Roberts, J. D., and Zakour, R. A. (1987) Methods Enzymol. 154, 367–382
24. Zabin, I., and Fowler, A. V. (1984) Anal. Biochem. 136, 493–496
25. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
26. Weber, K., Pringle, J. R., and Osborn, M. (1972) Methods Enzymol. 26, 3–27
27. Ellis, J., Murray, I. A., and Shaw, W. V. (1991) Biochemistry 30, 10799–10805
28. Ellis, J., Bagshaw, C. R., and Shaw, W. V. (1991) Biochemistry 30, 10806–10813
29. Leatherbarrow, R. J. (1992) Grafit Version 3.0, Erithacus Software Ltd., Staines, UK
30. Kleanthous, C., Cuill, P. M., and Shaw, W. V. (1985) Biochemistry 24, 5307–5313
31. Murray, I. A., Hawkins, A. R., Keyte, J. W., and Shaw, W. V. (1988) Biochem. J. 252, 173–179