Kinetic Analysis of the Catalytic Mechanism of Serotonin N-Acetyltransferase (EC 2.3.1.87)*

(Received for publication, September 17, 1997, and in revised form, October 20, 1997)

Jacqueline De Angelis‡, Jonathan Gastel§, David C. Klein§, and Philip A. Cole‡

From the ‡Laboratory of Bioorganic Chemistry, The Rockefeller University, New York, New York 10021 and the §Section on Neuroendocrinology, Laboratory of Developmental Neurobiology, NICHD, National Institutes of Health, Bethesda, Maryland 20892

Serotonin N-acetyltransferase (arylalkylamine N-acetyltransferase, AANAT, EC 2.3.1.87) is the penultimate enzyme in melatonin biosynthesis. This enzyme is of special biological interest because large changes in its activity drive the large night/day rhythm in circulating melatonin in vertebrates. In this study the kinetic mechanism of AANAT action was studied using bacterially expressed glutathione S-transferase (GST)-AANAT fusion protein. The enzymologic behavior of GST-AANAT and cleaved AANAT was essentially identical. Two-substrate kinetic analysis generated an intersecting line pattern characteristic of a ternary complex mechanism. The dead end inhibitor analog desulfo-CoA was competitive versus acetyl-CoA and noncompetitive versus tryptamine. Tryptophol was not an alternative substrate but was a dead end competitive inhibitor versus tryptamine and an uncompetitive inhibitor versus acetyl-CoA, indicative of an ordered binding mechanism requiring binding of acetyl-CoA first. N-Acetyltryptamine, a reaction product, was a noncompetitive inhibitor versus tryptamine and uncompetitive with respect to acetyl-CoA. Taken together these results support an ordered BiBi ternary complex (sequential) kinetic mechanism for AANAT and provide a framework for inhibitor design.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Laboratory of Bioorganic Chemistry, Rockefeller University, 1230 York Ave., New York, NY 10021. Tel.: 212-327-7241; Fax: 212-327-7243; E-mail: cole@rockvax.rockefeller.edu.

‡ The abbreviations used are: AANAT, serotonin N-acetyltransferase or arylalkylamine N-acetyltransferase; GST, glutathione S-transferase; GST-AANAT, glutathione S-transferase-arylalkylamine N-acetyltransferase fusion protein; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); Kᵢ, Kᵣ, intercept; Kₛ, Kₛ, slope.

‡ E. Koonin (National Institutes of Health), personal communication.
MATERIALS AND METHODS

**Chemicals**

The following were purchased: acetyl-CoA (Pharmacia Biotech Inc.); tryptamine-HCl, desulfo-CoA, glutathione-agarose, DTNB (Sigma); sodium phosphate, dithiothreitol, guanidinium-HCl, EDTA (Fisher Scientific); tryptophol (Aldrich); and [14C]acetyl-CoA (60 Ci/mol) (NEN Life Science Products). N-Acetyltryptamine was synthesized by reacting tryptamine-HCl (500 mg, 2.5 mmol) with acetic anhydride (260 mg, 2.5 mmol) in the presence of excess triethylamine (1.75 ml). After vigorous stirring at room temperature for 50 min, the mixture was partitioned between ethyl acetate (80 ml) and water (70 ml). The organic phase was washed with saturated aqueous NaHCO3 (50 ml), 0.1 M HCl (50 ml), and saturated aqueous NaCl (30 ml). The organic phase was dried over Na2SO4 (anhydrous), and the resultant was concentrated in vacuo to afford N-acetyltryptamine as off-white crystals (88% yield). Purity (>95%) was established by TLC and 1H NMR.

**Expression and Purification**

The entire open reading frame of the DNA encoding sheep AANAT in the plasmid vector pET15b (5) was excised with XhoI and religated in-frame to pGEX-4T-1 and transformed into *Escherichia coli* strain BL21(DE3)LyS8. A frozen stock of this strain (10 µl) was used to inoculate 25 ml of Luria Broth containing ampicillin (100 µg/ml) and chloramphenicol (34 µg/ml) in a culture flask and grown overnight at 37 °C in a floor shaker until the absorption at 595 nm was equal to 0.5–0.6. The flasks were cooled to room temperature by standing at 4 °C for 20 min and then treated with isopropyl-1-thio-β-D-galactopyranoside (to a final concentration of 0.2 mM). The cultures were maintained at 24 °C for an additional 5 h. The cells were pelleted by centrifugation (4 °C, 5,000 x g, 10 min) and the cell paste (5.2 g) snap frozen with liquid N2 and stored at −80 °C. The cell paste was resuspended in 30 ml of lysis buffer (1 × phosphate-buffered saline, 10 mM dithiothreitol, 10% glycerol, 10 mM EDTA, pH 6.9), and the suspension was lysed by passage through a French pressure cell at 12,000 p.s.i. Insoluble protein and cell debris were removed by centrifugation (4 °C, 27,000 x g, 30 min followed by 4 °C, 100,000 x g, 120 min), and the supernatant (25 ml) was snap frozen with liquid N2 and stored at −80 °C. The thawed solution was then incubated with Nutator (Fisher Scientific) mixing with 2 ml of glutathione-agarose (100 mg of dried resin, swollen with 20 ml of H2O, then pre-equilibrated in lysis buffer by washing twice with 10 ml, pelleting in a 50-ml centrifuge tube at 2,000 × g in a swinging bucket centrifuge for 5 min) at room temperature for 30 min. The mixture was centrifuged at 2,000 × g for 5 min and the supernatant carefully pipetted away. The pelleted resin was resuspended and washed twice with lysis buffer + 263 mM NaCl (10 ml each) at 4 °C. Subsequently, the glutathione-agarose resin was incubated at room temperature for 30 min on a Nutator with 20 ml of lysis buffer + 113 mM NaCl + 50 mM glutathione (whose pH was adjusted to 7 with 8 N NaOH). The resin was pelleted, and the supernatant (20 ml) was recovered and dialyzed (2 × 500 ml) at 4 °C against storage buffer (43.5 mM sodium phosphate, 1.4 mM potassium phosphate, 337 mM NaCl, 2.7 mM KCl, 5 mM dithiothreitol, 1 mM EDTA, 10% glycerol, pH 6.9). The protein concentration postdialysis was 0.5 mg/ml (total yield 10 mg) as determined by Bradford assay referenced to bovine serum albumin standard. Purity was approximately 90% as determined by 10% SDS-polyacrylamide gel electrophoresis (Coomasie staining). The protein was stored (−80 °C) at 0.5 mg/ml concentration or after Centricon (Amicon Inc., Beverly, MA) ultrafiltration, at 4.2 mg/ml, and maintained stable enzyme activity for at least 4 months.

The GST-AANAT fusion protein was cleaved with thrombin and then purified according to the manufacturer’s instructions (Pharmacia) to produce GST-free AANAT and protein concentration determined by Bradford assay.

**Enzyme Assays**

AANAT activity was measured primarily using a newly developed spectrophotometric assay. An established radiochemical assay was also used with minor modifications (12).

**DTNB Product Detection Assay**—This assay is based on the detection of CoASH generated during acetyl transfer by reaction with the thiol reagent DTNB (19). This assay was typically performed using a buffer containing 0.05 mM sodium phosphate, pH 6.8, 500 mM NaCl, 2 mM EDTA, 0.05 mg/ml bovine serum albumin, variable acetyl-CoA (0.1–3 mM), and variable tryptamine (0.05–1 mM) at 30 °C in 0.3 ml in 1.5-ml microcentrifuge tubes. Reactions were initiated with enzyme (3 µl, 5–30 mM final concentration) that had been preincubated (10–100-fold) in 50 mM sodium phosphate, 500 mM NaCl, 2 mM EDTA, and 0.05 mg/ml bovine serum albumin in the absence of reducing agent immediately before use and maintained on ice during the assay. The reactions were quenched for 0–3 min with 0.6 ml of a buffer containing guanidinium-HCl (3.2 mM), sodium phosphate (0.1 mM), pH 6.8. These mixtures were treated with 0.1 ml of DTNB (2 mM, 0.1 mM sodium phosphate, pH 6.8, 10 mM EDTA), vortexed, and allowed to stand for 5 min before absorbance readings were performed at 412 nm (thiophenolate-quantified assuming ε = 13.7 × 10³ M⁻¹ cm⁻¹) (19). Background absorbances (with all components added including enzyme) were measured and subtracted from the total absorbance. A background correction was made for each acetyl-CoA concentration because acetyl-CoA had a small contaminant of free thiol (1–2% presumably free CoASH). The rate of conversion of acetyl-CoA to CoASH in the absence of amine was negligible over the course of the assay. Activity was linear with time for at least 3 min at high (2 mM) and low (0.1 mM) acetyl-CoA. Velocity measurements were made under initial conditions where reaction of the limiting substrate did not exceed 10%.

**Radiochemical Assay**—A modification of an established radiochemical assay (12) was used in which the concentration of tryptamine (1 mM) is incubated with [14C]acetyl-CoA (1 mM; specific activity = 1.24 Ci/mmol) and N-[14C]acetyltryptamine was measured.

**Comparative Analysis of Assays**—The apparent specific activities...
measured with both methods were essentially identical (~20% difference). All assays were performed at least twice with duplicate measurements typically within 10%. Absorbance drift was minimal with fresh solutions (presumably because of slow air oxidation) over the 20–30 min necessary for assay completion.

### Kinetic Analysis

**K_i(app) Measurements**—Measurement of K_i(app), for acetyl-CoA employed an acetyl-CoA concentration range of 0.1–2 mM (0.4 K_i – 8 K_i) at fixed and near saturating tryptamine (1 mM). Measurement of K_i(app) for tryptamine and serotonin employed a substrate concentration range of 0.05–1 mM (0.3 K_i – 6 K_i) at fixed and near saturating acetyl-CoA (2 mM). Data were fitted to the equation

\[
v = V_{\text{m}} \cdot S/(K_a + S) \tag{Eq. 1}
\]

using a nonlinear least squares approach (Macintosh computer program Kaleidograph™, Reading, PA), and the kinetic constants ± S.E. errors are reported in Table I.

**Two-substrate Kinetic Measurements**—Two-substrate kinetic analysis was performed with substrate concentrations shown in Fig. 3, and the data were fitted to the sequential (ternary complex) mechanism equation (Equation 2) using the computer program KinetAsyst II™ (Insil-Kinetics, State College, PA) based on the algorithms of Cleland (20),

\[
v = V_{\text{m}} \cdot S/(K_a + K_b + K_a \cdot S + B + K_{\text{cat}} + A + A + B) \tag{Eq. 2}
\]

using a nonlinear least squares approach. Kinetic constants ± S.E. are shown in Table II. K_m = K_m of acetyl-CoA in this work, K_m = dissociation constant for acetyl-CoA (dissociation constant to free enzyme where acetyl-CoA binds prior to tryptamine), K_m = K_m of tryptamine. Fitting to a ping-pong mechanism gave a significantly larger (5-fold) sum of squares of the residuals.

**Kinetic Measurements with Inhibitors**—Competitive inhibition kinetic analysis was done by fitting all of the data points to the linear competitive inhibition equation of KinetAsyst II™ based on the algorithms of Cleland (20),

\[
v = V_{\text{m}} \cdot S/K_a (1 + I/K_a) \tag{Eq. 3}
\]

using a nonlinear least squares approach. The fixed substrate was assumed to be saturating. Kinetic constants ± S.E. are shown in Table III.

Noncompetitive inhibition kinetic analysis was done by fitting all of the data points to the linear noncompetitive inhibition equation of KinetAsyst II™ based on the algorithms of Cleland (20),

\[
v = V_{\text{m}} \cdot S/[K_a (1 + I/K_a) + S/(1 + I/K_a)] \tag{Eq. 4}
\]

using a nonlinear least squares approach. Kinetic constants ± S.E. are shown in Table III.

Uncompetitive inhibition kinetic analysis was done by fitting all of the data points to the linear uncompetitive inhibition equation of KinetAsyst II™ based on the algorithms of Cleland (20),

\[
v = V_{\text{m}} \cdot S/[K_a (1 + S/I/K_a)] \tag{Eq. 5}
\]

using a nonlinear least squares approach. Kinetic constants ± S.E. are shown in Table III.

The abbreviations are K_m = K_m, intercept and K_m = K_m, slope based on double-reciprocal plot analysis according to the nomenclature of Cleland (20). The data for individual experiments with each inhibitor versus a varied substrate were fit to all three inhibitor models. Choice of kinetic fit was based on a combination of visual inspection and comparison of S.E. values and residuals for all three inhibition types applied to the data sets (20). In the cases where uncompetitive inhibition was assigned, there were no significant improvements in the standard errors or sum of squares of the residuals (less than 2-fold) by including the extra inhibitory constant K_i. In the cases where competitive models were assigned, there were no significant improvements in the S.E. or the sum of squares of the residuals (less than 2-fold) by including the extra inhibitory constant K_i. The lines drawn through the data points in the figures are derived from the fitted equations above.

### RESULTS

**Enzyme Production**—Expression of the sheep GST-AANAT fusion plasmid in E. coli resulted in the production of active soluble GST-AANAT fusion protein (>5 mg/liter of culture). Purification using glutathione affinity chromatography afforded nearly homogeneously pure recombinant protein with the predicted molecular mass (approximately 50 kDa) as determined by SDS-polyacrylamide gel electrophoresis (see Fig. 2).

GST-free AANAT was obtained by thrombin cleavage of GST-AANAT (Fig. 2). The resulting product had kinetic characteristics essentially identical to those of GST-AANAT (Table I). GST-AANAT was used for further kinetic analysis because it was found to be more stable and easier to work with. The k_{cat} of 25 s^{-1} for recombinant AANAT is similar to the reported k_{cat} (80 s^{-1}) for a pure N-acetyl-CoA-dependent acetyltransferase (22). Furthermore, it was unlikely that the enzyme contained a large fraction of inactive material because preparations obtained using a variety of purification protocols had essentially identical turnover numbers.

**Assay Development**—Using the DTNB assay, GST-AANAT reactions display linear activity versus time for at least 3 min in the absence of reducing agents, and enzyme activity is linear with respect to enzyme concentration up to 500 nM. After background subtraction, interference from trace reducing agents from the enzyme preparations or the acetyl-CoA was shown to be inconsequential. There is insignificant CoASH formation in the absence of amine substrate in the enzyme range employed. As little as 1 nmol of product formation (3 μM) is detected reliably in a 0.3-ml reaction.

K_m values for tryptamine and acetyl-CoA obtained with the DTNB assay showed good agreement with published values obtained with native sheep pineal AANAT3 (3); specific activity values were approximately 1,000-fold higher for recombinant protein (3). These values are also in a convenient range to perform kinetic mechanism studies as outlined below. The k_{cat(app)} and K_m(app) generated with serotonin were nearly indistinguishable from those obtained with tryptamine (Table I).

**Two-substrate Kinetics**—K_m values were obtained for tryptamine using a range of acetyl-CoA concentrations. A double-reciprocal analysis of these data formed an intersecting line pattern (Fig. 3) that is characteristic of a ternary complex (sequential) mechanism. In contrast, a ping-pong mechanism is typically characterized by a parallel line pattern (21).

---

3 When assayed at similar ionic strength (I ~ 0.1), the K_i(app) values for acetyl-CoA (0.1 mM) and tryptamine (0.1 mM) for GST-AANAT are nearly identical to the published data for native AANAT (see Ref. 3).

---
Table I

| Inhibitor       | Varied substrate | Constant substrate | Inhibitor pattern | $K_i$ (mM) | $K_m$ (mM) |
|-----------------|------------------|--------------------|-------------------|------------|------------|
| Desulfo-CoA     | Acetyl-CoA       | Tryptamine (1 mM)  | Competitive       | 1.02 ± 0.20 | 1.49 ± 0.23 |
| Tryptophol      | Tryptamine       | Acetyl-CoA (1 mM)  | Noncompetitive    | 0.12 ± 0.45 | 0.680 ± 0.078 |
| N-Acetyltryptamine | Tryptamine       | Acetyl-CoA (0.2 mM) | Competitive       | 0.160 ± 0.12 | 0.916 ± 1.160 |

**Table III**

Inhibitor data with GST-AANAT

See "Materials and Methods" and Figs. 5–7 for further details. Values are shown ± S.E.

| Inhibitor       | Varied substrate | Constant substrate | Inhibitor pattern | $K_i$ (mM) | $K_m$ (mM) |
|-----------------|------------------|--------------------|-------------------|------------|------------|
| Desulfo-CoA     | Acetyl-CoA       | Tryptamine (1 mM)  | Competitive       | 1.02 ± 0.20 | 1.49 ± 0.23 |
| Tryptophol      | Tryptamine       | Acetyl-CoA (1 mM)  |Noncompetitive     | 0.12 ± 0.45 | 0.680 ± 0.078 |
| N-Acetyltryptamine | Tryptamine       | Acetyl-CoA (0.2 mM) | Competitive       | 0.160 ± 0.12 | 0.916 ± 1.160 |

**DISCUSSION**

DTNB-based AANAT Assay—Previously published kinetic assays for AANAT have relied on radioactive incorporation of acetyl ($^3$H or $^{14}$C) into the acetylated product (3, 12, 23) or high pressure liquid chromatographic analysis (24, 25). These assays were developed for high sensitivity as required for detection of enzyme activity in small biological samples. However, they require complex extraction procedures and are not prac-
tical for routine analysis of large numbers of samples as required for detailed mechanistic studies or inhibitor screens. The less complicated DTNB assay described in this report meets this requirement.

The potential difficulty with this approach was that reducing agents such as dithiothreitol and β-mercaptoethanol react with DTNB (Ellman's reagent). Indeed, GST-AANAT is somewhat unstable in the absence of reducing agents, losing greater than 50% activity within 2 h at 4°C (data not shown). However, the enzyme was stable within the short incubation periods used in these studies (less than 3 min). It was shown unequivocally that CoASH generation was coupled tightly to N-acetyltryptamine formation, and the activity was linear with time and enzyme concentration. An attractive feature of the DTNB detection assay is that it allows any potential amine substrate to be tested easily, providing it does not react with DTNB. Of note, a continuous spectrophotometric assay with DTNB was not possible because DTNB inhibited GST-AANAT activity.

**Kinetic Mechanism of GST-AANAT**—A generally useful approach to kinetic analysis of two-substrate enzymes is one in which both substrates are varied within the same experiment. It is well accepted that a double-reciprocal plot that results in intersecting lines suggests a ternary complex mechanism and that a parallel line pattern is characteristic of a ping-pong mechanism (21). Previous experiments using this approach to analyze crude preparations on AANAT from rat and bird pineal glands suggest that different mechanisms were involved (23, 26). This difference is inconsistent with the high homology among vertebrate AANATs, especially within the putative binding domains and the putative catalytic site (4, 10). The reported differences in the apparent mechanism of catalysis may reflect contaminants in the partially purified preparations as the assays appeared to be performed under similar conditions of pH and ionic strength. The potential problem with contaminants is avoided by the use of purified expressed GST-AANAT as described in this report.

The most important advance in this study was the evidence that a ternary complex mechanism is involved, as indicated by the clear intersecting line pattern. It should be noted that although this analytical approach has correctly predicted the mechanistic behavior of the best characterized acetyltransferases (15, 27, 28) it is not impossible that a covalent enzyme intermediate occurs. However, if a covalent enzyme intermediate occurs, it must form after both substrates are bound and decompose before either product leaves.

The next issue addressed was the order of binding of substrates which precedes ternary complex formation. Three major schemes are possible: (i) ordered with acetyl-CoA binding first; (ii) ordered with tryptamine binding first; and (iii) random substrate binding. To discriminate among these possibil-
ties dead end inhibitors were used (21).

Desulfo-CoA, a dead end analog of acetyl-CoA (see Fig. 4), was shown to be a linear competitive inhibitor with respect to acetyl-CoA and a noncompetitive inhibitor with respect to tryptamine. These results rule out an ordered mechanism where tryptamine binds before acetyl-CoA. In such a mechanism, desulfo-CoA would have been uncompetitive with respect to tryptamine. The desulfo-CoA experiments leave open the possibilities that there is random binding of acetyl-CoA and tryptamine or ordered binding of acetyl-CoA before tryptamine. Another interesting point is the apparent similarity in affinity which GST-AANAT displays toward acetyl-CoA (K_i = 0.51 mM) and desulfo-CoA (K_i = 1 mM). It suggests that the thioester function contributes little binding energy in the ground state complex.

Tryptophol (Fig. 4) was next evaluated as a GST-AANAT substrate/inhibitor. It was shown that replacement of the amino function of tryptamine with a hydroxy group prevents enzyme-catalyzed acetyl transfer. The lack of reactivity suggests that the nucleophilicity of the amine is critical for enzyme-catalyzed reaction because tryptophol can bind with reasonable affinity to AANAT as demonstrated by its inhibitory behavior (see below). Interestingly, the O-acetylated carnitine acetyltransferase is able to process the amino-substrate at a k_cat only 13-fold lower than the normal acyl-CoA substrate (29). This altered reactivity between the two enzymes suggests that there may be a mechanistic difference in the chemical steps catalyzed between these two classes of acetyltransferases (O and N). Trihydroxypropionate N-succinyltransferase also shows no reactivity toward the corresponding oxygen analog (30).

As expected, tryptophol proved to be a linear competitive inhibitor of GST-AANAT versus the varied substrate tryptamine. It was a clear uncompetitive inhibitor versus acetyl-CoA. Fitting the data to a noncompetitive fit gave no significant lessening of the residuals and gave a K_i that was more than 10 times higher than the K_i with a very large error (±100%). These results strongly suggest that acetyl-CoA must bind before tryptamine to the enzyme, i.e. that there is an ordered mechanism. Although this inhibitory pattern is compatible with a ping-pong kinetic mechanism, a ping-pong mechanism is ruled out by the intersecting line pattern in the two substrate kinetic analysis (Fig. 3). The ordered binding suggests either (i) a conformational change in the protein which causes the tryptamine binding pocket to become accessible only after acetyl-CoA binds or (ii) tryptamine undergoes an important, direct noncovalent binding interaction with acetyl-CoA in the enzyme active site. Differentiation between these possibilities awaits further structural studies.

CoASH was not evaluated as a reversible inhibitor in the spectrophotometric assay because it reacts with DTNB. It also forms (CoASH), in the absence of reducing agents, and the use of reducing agents in the radiochemical assay would require extensive kinetic characterization of the enzyme in the presence of such reagents, which is beyond the scope of this investigation. The reaction product N-acetyltryptamine is a noncompetitive inhibitor versus tryptamine and an uncompetitive inhibitor versus acetyl-CoA. This strongly suggests that AANAT obeys an ordered BiBi ternary complex mechanism with N-acetyltryptamine being the first product released followed by CoASH. The noncompetitive inhibition pattern versus tryptamine likely is caused by the binding of N-acetyltryptamine to both the acetyl-CoA-bound GST-AANAT form as well as the CoASH-bound GST-AANAT form. The lack of a slope effect in the inhibition of N-acetyltryptamine versus acetyl-CoA (at subsaturating tryptamine) presumably stems from the fact that the chemical step is very weakly reversible since a thioester bond (ΔG_hydrolysis of acetyl-CoA = −7.5 kcal/mol) is exchanged for an amide bond (ΔG_hydrolysis of propionamide = −2.1 kcal/mol) (21, 31).

It has not been established that any of the acetyltransferases that have been kinetically characterized are members of the motif A/B superfamily. Accordingly, demonstration that AANAT obeys a ternary complex, ordered BiBi mechanism creates a precedent for other acetyltransferases in the motif A/B superfamily, including a eukaryotic histone N-acetyltransferase believed to be important in the regulation of gene expression (32). Delineating the kinetic mechanism also develops a framework for an approach to inhibitor design. Therefore, tryptamine analog inhibitors are unlikely to be potent at blocking AANAT action at low levels of acetyl-CoA. It remains to be seen whether bisubstrate analog inhibitors can be effective.

Acknowledgment—We acknowledge gratefully W. C. Cleland (University of Wisconsin, Madison) for helpful discussions and a critical reading of the manuscript. We also express our appreciation to Eugene Koonin (National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD) for analysis of the A/B motif superfamily of acetyltransferases and valuable discussions.

REFERENCES

1. Klein, D. C., and Weller, J. L. (1970) Science 169, 1093–1095
2. Arendt, J. (1995) Melatonin and the Mammalian Pinelat, Chapman and Hall, London
3. Voisin, P., Namboodiri, M. A. A., and Klein, D. C. (1984) J. Biol. Chem. 259, 10913–10918
4. Klein, D. C., Roseboom, P. H., and Coon, S. L. (1996) Trends Endocrinol. Metab. 7, 106–112
5. Coon, S. L., Roseboom, P. H., Baler, W. J., Namboodiri, M. A. A., Coon, S. L., Koenin, E. V., and Klein, D. C. (1995) Science 270, 1681–1683
6. Bortignon, J., Wang, M. M., and Snyder, S. H. (1995) Nature 378, 783–785
7. Coon, S. L., Mazuruk, K., Bernard, M., Roseboom, P. H., Klein, D. C., and Rodriguez, I. B. (1996) Genomics 34, 76–84
8. Bernard, M., Iuvone, P. M., Cassone, V. M., Roseboom, P. H., Coon, S. L., and Klein, D. C. (1997) J. Neurochem. 69, 213–224
9. Roseboom, P. H., Coon, S. L., Baler, W. J., McCune, S. K., Weller, J. L., and Klein, D. C. (1996) Endocrinology 137, 3333–3345
10. Klein, D. C., Coon, S. L., Roseboom, P. H., Baler, W. J., Bernard, M., Gastel, J. A., Zatz, M., Iuvone, P. M., Rodriguez, I. R., Begay, V., Falcon, J., Cahill, M. G., Cassone, V. M., and Baler, R. (1997) Recent Prog. Horm. Res. 52, 307–357
11. Vatais, K. P., Weber, W. B., Bell, D. A., Dupret, J. M., Evans, D. A., Grant, D. M., Hein, D. W., Lin, H. J., Meyer, U. A., Relling, M. V., Sim, E., Suzuki, T., and Yamazoe, Y. (1995) Pharmacogenetics 5, 1–17
12. Deguchi, T. (1975) J. Neurochem. 24, 1083–1085
13. Sim, E., Hickman, D., Coroneos, E., and Kelly, S. L. (1992) Biochem. Soc. Trans. 20, 304–309
14. Weber, W. B., and Cohen, S. N. (1987) Mol. Pharmacol. 3, 266–273
15. Jencks, W. P., Gresser, M., Valenzuela, M. S., and Huneaux, F. C. (1972) J. Biol. Chem. 247, 3756–3760
16. Riddle, B., and Jencks, W. P. (1971) J. Biol. Chem. 246, 3250–3258
17. Cheon, H.-G., and Hanna, P. E. (1992) Biochem. Pharmacol. 43, 2255–2268
18. Dupret, J.-M., and Grant, D. M. (1992) J. Biol. Chem. 267, 7381–7385
19. Riddle, P. W., Blakeley, R. L., and Zernar, R. (1989) Methods Enzymol. 191, 49–60
20. Cleland, W. W. (1979) Methods Enzymol. 63, 103–138
21. Segel, I. H. (1975) Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-state Enzyme Systems, Wiley-Interscience, New York
22. Gehring, A. M., Lees, W. J., Mindiola, D. J., Walsh, C. T., and Brown, E. D. (1996) Biochemistry 35, 579–585
23. Wolfe, M. S., Lee, N. R., and Zatz, M. (1995) Brain Res. 669, 100–106
24. Thomas, K. B., Zawilska, J., and Iuvone, P. M. (1990) Anal. Biochem. 184, 228–234
25. Fajardo, N., Abreu, P., and Alonso, R. (1992) J. Biol. Chem. 267, 1–17
26. Morrissey, J. J., Edwards, S. B., and Lovenberg, W. (1977) Biochem. Biophys. Res. Commun. 77, 118–123
27. Shaw, V. W., and Leslie, A. G. W. (1991) Annu. Rev. Biophys. Biophys. Chem. 20, 363–386
28. Clore, G. W., and Goudard, R. D. (1988) Bioorg. Chem. 16, 307–334
29. Jenkins, D. L., and Griffith, O. W. (1985) Anal. Biochem. 147, 1478–1475
30. Berges, H. A. (1980) J. Biol. Chem. 255, 6160–6167
31. Sober, H. A. (1979) Handbook of Biochemistry: Selected Data for Molecular Biology, 2nd ed., p. J-184, CRC Press, Cleveland
32. Yang, X.-J., Ogryzko, V. V., Nishikawa, J., Howard, B. H., and Nakatani, Y. (1996) Nature 382, 319–324
Kinetic Analysis of the Catalytic Mechanism of Serotonin N-Acetyltransferase (EC 2.3.1.87)
Jacqueline De Angelis, Jonathan Gastel, David C. Klein and Philip A. Cole

J. Biol. Chem. 1998, 273:3045-3050.
doi: 10.1074/jbc.273.5.3045

Access the most updated version of this article at http://www.jbc.org/content/273/5/3045

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 29 references, 10 of which can be accessed free at http://www.jbc.org/content/273/5/3045.full.html#ref-list-1