Rat Liver Polysome N^{α}-Acetyltransferase: Isolation and Characterization†

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ABSTRACT: Rat liver polysome N^{α}-acetyltransferase has been purified to homogeneity by a four-step procedure that utilizes ammonium sulfate precipitation, gel filtration, hydroxylapatite chromatography, and Mono Q ion exchange chromatography. The enzyme is greatly stabilized by the inclusion of EDTA and 0.01% deoxycholate in the isolation buffers. The purified enzyme has a native molecular weight of 190 000 and a subunit molecular weight of 95 000, suggesting that it is a homodimer. The enzyme shows a pH optimum of 8.0 and is strongly inhibited by Cl^-, I^-, SCN^-, and ClO_4^- and to a lesser degree by sulfate and acetate. It is unaffected by phosphate, citrate, and F^- and by Na^+ and K^+; NH_4^+ is partially inhibitory. The enzyme is also sensitive to iodoacetic acid. It is generally more similar to yeast N^{α}-acetyltransferase [Lee, F.-J. S., Lin, L.-W., & Smith, J. A. (1988) *J. Biol. Chem. 263*, 14948–14955] than to the hen oviduct enzyme, which contains a 7S RNA subunit [Kamitani, K., & Sakiyama, F. (1989) *J. Biol. Chem. 264*, 13194–13198], although the amino acid compositions are quite different.

Proteins are subject to a variety of co- and posttranslational processing steps that may affect stability, translocation, and degradation (Arfin & Bradshaw, 1988; Bradshaw, 1989). Among the earliest of these are the removal of the N-terminal initiator methionine and the addition of N^{α}-acyl modifying groups. N^{α}-Acetyl groups arising from donor acetyl-CoA entities are the predominant modifications of this type. N^{α}-Acetylation was first observed by Narita (1958) in studies on the coat protein of tobacco mosaic virus and has since been widely observed, particularly with eukaryotic cytoplasmic proteins (Jornvall, 1975; Driessen et al., 1985; Persson et al., 1985), suggesting an important, but yet undefined role(s) for this reaction. N-Acetylation can modify both α- and ϵ-amino groups and can occur both as co- and posttranslational events. The enzymes catalyzing these are probably diverse and are found in various intracellular locations (Wold, 1981; Allfrey, 1977; Rubenstein et al., 1981).

It has been suggested that N-terminal acetylation may affect biological function and/or protein stability (Jornvall, 1975). Thus the activity of pituitary hormones and endorphin-like peptides as well as hemoglobin is affected by the N-acetylation of their amino-terminal residues (Ramachandran & Li, 1967; Smyth et al., 1979; Taketa et al., 1971). At the same time the lack of acetylation can directly affect protein structure, making it more unstable to such denaturing conditions as heat (Cumberlidge & Isono, 1979; Siddig et al., 1980) or more susceptible to proteolytic modification, particularly by aminopeptidases (Jornvall, 1975). Of particular interest in this regard are the recent observations of Bachmair et al. (1986) that the nature of the N-terminal residue (penultimate to the initiator methionine) can have a marked effect on protein turnover, as mediated by ubiquitin-directed degradation. The presence or absence of acetyl groups on these residues has also been inferred to modulate turnover of proteins. The observations that mutants of hypoxanthine phosphoribosyl transferase that have unacetylated proline at the N-terminus are more unstable than those with N-acetylated alanine are illustrative of this view (Johnson et al., 1985; Johnson & Chapman, 1987). More recent studies by Lee et al. (1989b) and Mullen et al. (1989) using yeast mutants lacking in at least one N^{α}-acetyltransferase (NAT)[1] has suggested that N^{α}-acetylation is important for such broad physiological phenomena as sporulation, growth, and sensitivity to heat shock.

NATs capable of acetylating the N^{α}-amino groups of a variety of peptide and protein substrates have been identified in a large range of tissues and species (Granger et al., 1976; Pestana & Pitot, 1974, 1975a,b; Traugh & Sharp, 1977). The earliest studies were conducted with hen oviduct tissue (Tsunasawa et al., 1980), and the enzyme from this source has recently been reported to be a ribonuclear protein complex of 250 000 molecular weight (Kamitami & Sakiyama, 1989). It consists of two apparently identical protein subunits of approximately 80 kDa and one RNA particle of similar molecular mass. Lee et al. (1988) have isolated an NAT from *Saccharomyces cerevisiae* and have obtained a full-length cDNA clone and corresponding protein sequence (Lee et al., 1989a). Mullen et al. (1989) reported the same structure, also determined from DNA sequencing. This enzyme has an observed molecular mass of about 180 kDa and is composed of two identical subunits of 95 kDa. It does not apparently contain any RNA.

In this paper we describe the isolation and characterization of an N-acetyltransferase isolated from the polysomes of rat liver. It is similar in some respects to both the hen and yeast enzymes but is distinctly different in other aspects. A comprehensive study of the specificity of this enzyme with respect to both substrate sequence and length using a extended family of synthetic peptides is described in the accompanying paper (Yamada & Bradshaw, 1991). Preliminary reports of this

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[1] Abbreviations: NAT, N^{α}-acetyltransferase; MSH, melanocyte-stimulating hormone; PMSF, phenylmethanesulfonyl fluoride; DFP, diisopropyl fluorophosphate; HPLC, high-performance liquid chromatography; TFA, trifluoroacetic acid; TKM, 50 mM Tris–acetate, pH 8.0, 25 mM potassium acetate, and 5 mM magnesium acetate; PITC, phenyl isothiocyanate; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; PED, potassium phosphate buffer, pH 8.0, 1 mM EDTA, and 0.01% sodium deoxycholate; ACTH, adrenocorticotropic hormone; MAP, methionine aminopeptidase; NBS, N-bromosuccinimide; DEP, diethyl pyrocarbonate.

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work have appeared (Yamada et al., 1987, 1988; Bradshaw et al., 1987).

Experimental Procedures

Materials

Reagents used were obtained as follows: [1-^{14}C]acetyl-CoA was from Amersham, α-MSH, des-acetyl-α-MSH, and *N*-acetyl-DL-serine were from Sigma, and the molecular weight gel filtration calibration kit (bovine ribonuclease, hen egg ovalbumin, rabbit muscle aldolase, horse spleen ferritin) was from Pharmacia LKB.

Pronase, leupeptin, and soybean trypsin inhibitor were from Boehringer Mannheim. EDTA, PMSF (phenylmethanesulfonyl fluoride), DFP (diisopropyl fluorophosphate), iodoacetic acid, and pepstatin A were from Sigma. Hydroxylapatite Bio-Gel HT and Bio-Gel A-0.5m were from Bio-Rad, Sephadex G-200, Sepharose 6B, Sephacryl S-300, Ultrogel AcA44, and Mono Q HR5/5 were from Pharmacia LKB. Millex HV 4 was from Millipore, and YM10 was from Amicon. Hypersil ODS (5 μm, 2.1 × 100 mm) was from Hewlett-Packard, and Ultrasphere ODS (5 μm, 2.0 × 250 mm) was from Beckman.

Methods

N^{α}-Acetyltransferase Assay. The reaction mixture contained enzyme, 200 mM potassium phosphate buffer, pH 8.0, 2.73 nmol of [^{14}C]acetyl-CoA, and 10 nmol of substrate, adjusted to a final volume of 120 μL. The substrate used for routine assays was the synthetic octapeptide Ser-Tyr-Ser-Met-Glu-His-Phe-Arg corresponding to the amino-terminal sequence of α-MSH, generously provided by Dr. Geoff Tregear, Howard Florey Institute, Melbourne, Australia. It was prepared by the BOC-polystyrene procedure on an ABI 430A peptide synthesizer and cleaved with anhydrous HF, and the crude peptide was purified by reverse-phase HPLC. The assay reaction was initiated by the addition of enzyme to a stock solution of substrates and was incubated at 37 °C for 30 min. Activity was linear with time for at least 60 min. The reaction was stopped by the addition of TFA followed by filtration through Millex HV. The filtrate was analyzed by reverse-phase HPLC using the Hypersil ODS column on a Hitachi HPLC system. The column was eluted with a 10-min linear gradient of 10–30% acetonitrile in H_2O (0.1% TFA) at a flow rate of 0.5 mL/min and collected in 250-μL fractions. The radioactivity of each fraction was measured in a Beckman LS 7500 liquid scintillation counter. One unit of activity is defined as the amount of enzyme which transferred 10 pmol of the [^{14}C]acetyl group to the substrate per minute. Specific activity is given as units per milligram of enzyme. Approximately 10–15% of the acetyl-CoA was consumed in an average 30-min assay with 2.0 μg of pure enzyme. Figure 1 shows the elution profiles on a ODS column of assay mixtures with and without enzyme.

Polysomes from Rat Liver. Rat liver polysomes were prepared by a modified protocol of those described previously (Shires et al., 1971; Ramsey & Steel, 1976; Pestana & Pitot, 1975a,b). Male Wister rats, fasted overnight, were used in all experiments. Following sacrifice, the livers were removed and homogenized in 2 volumes of 0.25 M sucrose (1% Triton X-100) in TKM (50 mM Tris–acetate, pH 8.0, 25 mM potassium acetate, 5 mM magnesium acetate) with a Polytron homogenizer (Brinkmann Instruments) followed by centrifugation at 13000*g* for 15 min. The supernatant was filtered through cheesecloth and adjusted to 1.3% sodium deoxycholate. It was mixed by gentle stirring at 4 °C for 1 h, followed by sedimentation through two layers of 1.3 and 2.0

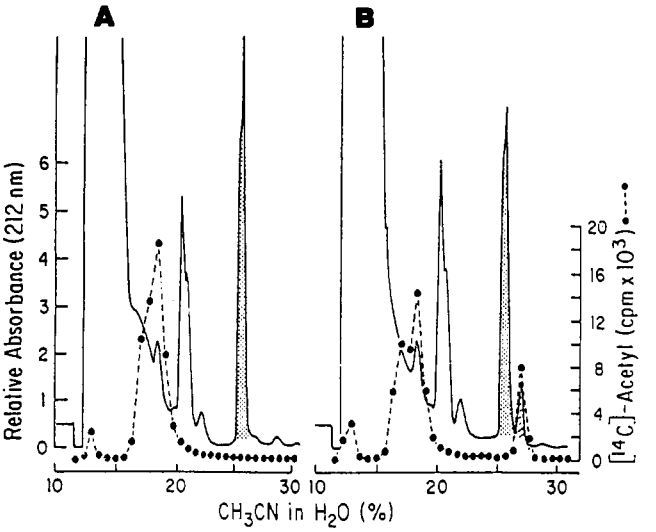

FIGURE 1: Elution profiles of the assay mixtures for determining rat liver NAT activity. Peptide (SYSMEHFR) and AcCoA were incubated with (B) and without (A) enzyme, treated with TFA, and analyzed on a Hypersil ODS column (5 μm, 2.1 × 100 mm). Elution was effected with a 10-min linear gradient of 10–30% CH_3CN in H_2O (0.1% TFA) at a flow rate of 0.5 mL/min. Radioactivity was measured on 250-μL aliquots removed from each fraction (30 s).

sucrose in TKM for 20 h at 40000 rpm in a 45 Ti rotor (Beckman). Polysomal pellets were suspended in 50 mM potassium phosphate buffer (pH 8.0) and 1 mM EDTA, homogenized, and stored at −70 °C.

Analytical Methods. Protein concentrations were measured by the dye-binding procedure of Bradford (1976) with bovine serum albumin as standard. UV and visual wavelength measurements were obtained on a Hitachi U-2000 spectrophotometer. Amino acid analyses were performed according to the PITC precolumn derivatization technique of Evert (1986) with a Beckman Ultrosphere (5 μm, 0.46 × 25 cm) and an HP 1090 liquid chromatograph (Hewlett-Packard). Protein sequence analyses were carried out by automated on-line Edman degradation (Hewick et al., 1981) with an Applied Biosystems 477A protein sequencer and an on-line 120A PTH analyzer. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed by the method of Laemmli (1970).

Results

Purification of N^{α}-Acetyltransferase

Specificity of the Assay. The principal substrate employed in these studies was a synthetic octapeptide, Ser-Tyr-Ser-Met-Glu-His-Phe-Arg, which is N^{α}-acetylated in its mature form. This sequence has been used in other studies of NAT. It has been suggested that 10 residues are minimally required for activity (Woodford & Dixon, 1979; Pease & Dixon, 1981; Kamitani et al., 1989). However, we have found that significant N-terminal acetylation can be observed with peptides as short as 4 residues, although peptides of 10–11 residues are optimal (Yamada & Bradshaw, 1991). In the peptide used, there are four potential sites for acetylation; i.e., Ser-1 could be N- or O-acetylated, and Tyr-2 and Ser-3 could be O-acetylated. To establish that the enzyme isolated was specific in its acetylation of the α-amino group, the acetylated octamer was isolated (Figure 1B) (as ^{14}C-labeled material) along with the unacetylated peptide and the amino acid composition of each was determined following hydrolysis in 6 N HCl. Each peptide gave the same composition (serine was found in a 2:1 ratio to each of the other amino acids). Subsequent analysis of the radiolabeled peptide in the protein sequencer did not

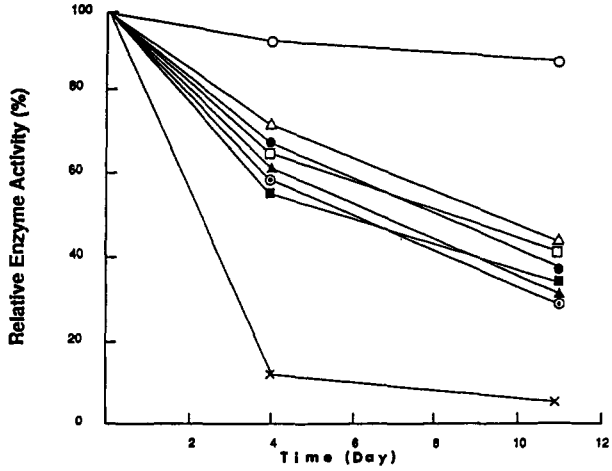

FIGURE 2: Effect of protease inhibitors on rat liver NAT activity. NAT was incubated with each reagent at 4 °C for the time indicated and activity determined as described in the text. (●) No addition; (○) 1 mM EDTA; (×) 1 mM iodoacetate; (■) 1 mM PMSF; (△) 1 mM DFP; (□) 0.1 mM leupeptin; (▲) 0.1 mM pepstatin A; (⊙) 1 mg/mL trypsin inhibitor.

yield any identifiable residues (while the other peptide gave the expected sequence). Pronase digestion of both peptides followed by amino acid analysis indicated only a single residue of serine in the labeled peptide. The unmodified peptide gave the same 2:1 ratio seen for acid hydrolysates. Quantification of the radioactivity present in the samples used for amino acid analysis indicated a molar ratio of serine:acetyl group of 1:1. Finally, the labeled acetylserine was purified from the Pronase digests by HPLC and shown to correspond to authentic N^{α}-acetyl-DL-serine. These findings established that this enzyme catalyzes only N^{α}-acetylation with this substrate.

Effect of Protease Inhibitors, Detergents, and Stabilizing Agents. Since the earliest studies (Tsunasawa et al., 1980; Pestana & Pitot, 1975a,b; Traugh & Sharp, 1977), it has been appreciated that polysome NAT is relatively unstable regardless of source. At 4 °C, the enzyme loses 30% activity at 4 days and 60% activity at 11 days (Figure 2). Using rat liver polysome extracts as the enzyme preparation, the effects of a variety of protease inhibitors, detergents, and stabilizing agents were examined to define improved isolation conditions and to provide some insight into the functional properties of the enzyme.

The effect of a variety of protease inhibitors on NAT activity is shown in Figure 2. Only EDTA significantly protected NAT, suggesting either that metal ions themselves are deleterious or that a metalloprotease or metalloproteases which are inactivated by EDTA, are responsible for the losses observed. In either case, EDTA is clearly strongly protective. Iodoacetic acid, in contrast to the other agents, markedly inhibited NAT actively, probably as the result of direct modification of the enzyme, suggesting that it is thiol sensitive.

NAT has also been found to bind to dialysis membranes, ultrafiltration membranes, and many types of chromatography resins, presumably nonspecifically. Among gel filtration resins, acrylamide resins such as Sephacryl S-300 and Ultrogel AcA44 gave low yields compared with dextran resins (i.e., Sephadex G-200 and Bio-Gel A-0.5m) and the agarose resin Sepharose 6B (data not shown). Accordingly, a number of detergents and stabilizing agents were examined for their effect on activity and as potential agents to block this absorption. Samples of polysome extract were mixed with each agent, dialyzed against a solution containing that agent, and assayed for NAT activity. Approximately 50% of the enzyme activity was recovered after dialysis when the enzyme was incubated

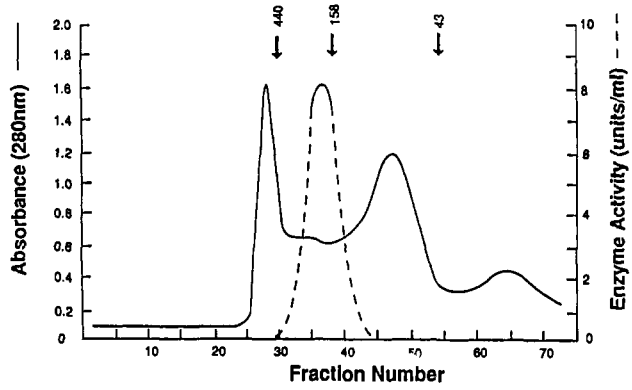

FIGURE 3: Elution profile of the purification of rat liver NAT on a column (2.6 × 100 cm) of Sephadex G-200. NAT precipitated by 75% $(NH_4)_2SO_4$ was equilibrated with 0.5 M PED buffer, loaded onto the column, and eluted at a flow rate of 25 mL/h. Fractions were collected every 15 min and assayed for 280 nM absorption and NAT activity. Fractions 33–41 were pooled for further purification. The vertical arrows note the elution positions of ferritin (440), aldolase (158), and ovalbumin (43).

with nothing and with 0.01% solutions of octyl β-glucoside, Triton X-100, and Tween 20. Similar results were obtained for sucrose (0.1 M), mannose (0.1 M), and bovine serum albumin (0.5%). Even greater losses were observed with 5% glycerol and 1 mM β-mercaptoethanol. Only deoxycholate (0.01%), which afforded a >95% recovery in activity, was effective. As a result of these experiments 1 mM EDTA and 0.01% deoxycholate were routinely included in the buffers used for the isolation of rat liver NAT.

Isolation Procedure. Rat liver NAT was purified from polysomes prepared as described above. A potassium phosphate buffer (PED), pH 8.0, containing 1 mM EDTA and 0.01% sodium deoxycholate (at various concentrations of potassium phosphate) was used throughout the procedure; all operations were performed at 0–4 °C. Volumes and activities described are for a polysome preparation from 10 rat livers that was equivalent to 100 g (wet weight).

(1) Extraction. Stored polysomes were diluted with an equal volume of 1.0 M PED to a final volume of ~70 mL and stirred gently at 4 °C for 1 h or longer to dissociate the enzyme. The mixture was centrifuged at 40 000 rpm for 30 min and the supernatant recovered.

(2) Ammonium Sulfate Fractionation. Saturated $(NH_4)_2SO_4$ (at 4 °C), adjusted to pH 8.0, was added to the extract to 35% saturation, and the mixture was allowed to stir for 60 min. The precipitate was removed by centrifugation and discarded. Additional saturated $(NH_4)_2SO_4$ solution was added to bring the supernatant to 75% saturation, followed by stirring for 60 min. The precipitate was recovered by centrifugation and resuspended in 20 mM PED to a final volume of ~5 mL.

(3) Sephadex G-200 Gel Filtration. The enzyme preparation obtained by ammonium sulfate fractionation was applied to a Sephadex G-200 column (2.6 × 100 cm, previously equilibrated with 0.5 M PED) and eluted with the same buffer at a flow rate of 25 mL/h (Figure 3). The fractions containing active enzyme were pooled (33–41). The enzyme eluted between ferritin (440 kDa) and aldolase (158 kDa), as indicated.

(4) Hydroxylapatite Chromatography. The fractions of active enzyme from gel filtration were concentrated and equilibrated with 20 mM PED on a YM10 ultrafiltration membrane and then applied to a hydroxylapatite column (1.6 × 20 cm) previously equilibrated with 20 mM PED. The column was eluted with a linear gradient of 20 mM to 0.5 M

Table I: Purification of N^{α}-Acetyltransferase from Rat Liver

purification step	total units	yield (%)	protein (mg)	sp act. (units/mg)	purification (x-fold)
polysomes	2362	100	6253	0.378	1
extract	2432	103	1775	1.37	36
ammonium sulfate fractionation (35–70%)	1462	61.9	280	5.22	13.9
Sephadex G-200	1075	45.5	49.8	21.6	57.3
hydroxylapatite	959	40.6	5.98	106	426
Mono Q	867	36.7	1.42	611	1620

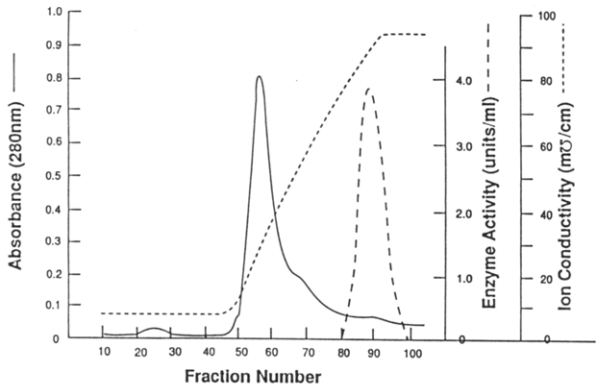

FIGURE 4: Elution profile of the purification of rat liver NAT on a column (1.6 × 20 cm) of hydroxylapatite. The active pool from the gel filtration step (Figure 3) was concentrated and equilibrated with 20 mM PED, applied to the column, and eluted with a linear gradient (20 mM to 0.5 M PED) at 1.0 mL/min. Fractions of 2 mL were collected and monitored for absorption for absorption at 280 nM, conductivity, and NAT activity. Fractions 82–97 were pooled for further treatment.

PED at 1.0 mL/min (Figure 4). Fractions (82–97) with activity were pooled.

(5) *Anion Exchange Chromatography on a Mono Q Column.* The active enzyme fractions from the hydroxylapatite chromatography were concentrated, equilibrated with 20 mM PED on a YM10 ultrafiltration membrane, and applied to a Mono Q HR5/5 column previously equilibrated with 20 mM PED. The column was eluted with a linear gradient of 20 mM to 0.5 M PED at a flow rate of 0.5 mL/min (Figure 5). Fractions 74–85 were pooled and stored at −70 °C.

This procedure, summarized in Table I, yields essentially homogeneous enzyme (Figure 5, insert) with a specific activity of 611 units/mg. It represents a 1620-fold purification with a yield of 37%. The polysomes of 10 rat livers (100 g, wet weight) produced 1.4 mg of enzyme.

Molecular Mass

The native molecular mass of rat liver polysome NAT was estimated by gel filtration on a column of Sephadex G-200, similar to that used in the purification (Figure 3), to be 190 ± 10 kDa. The standards used were horse heart ferritin, rabbit muscle aldolase, hen egg ovalbumin, and bovine pancreatic ribonuclease. SDS–PAGE analysis of the purified enzyme (Figure 5, insert) gave a molecular mass of 95 kDa, suggesting a dimeric structure associated by noncovalent forces (i.e., no disulfide bridges).

Amino Acid Analysis

The amino acid composition of rat liver N^{α}-acetyltransferase is shown in Table II. The values presented are from 48-h hydrolysates only and therefore may be low for serine, threonine, valine, and isoleucine. In addition, no precautions (other than evacuation) were taken to protect methionine and tyrosine, which may have resulted in some destruction of these amino acids as well. Half-cystine and tryptophan were not determined. Despite these limitations, it is clear that the rat

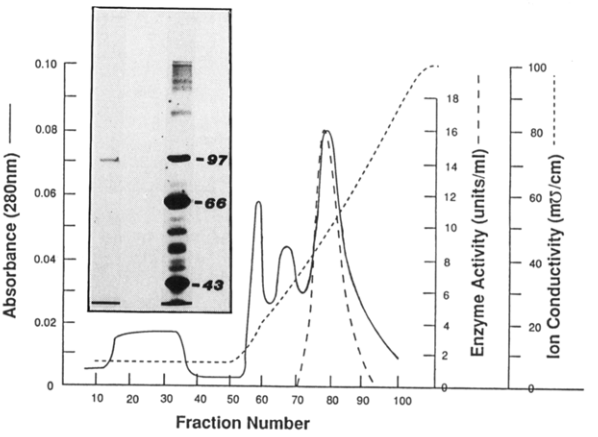

FIGURE 5: Elution profile of the purification of rat liver NAT on a column of Mono Q HR5/5. The active pool from the hydroxylapatite separation (Figure 4) was concentrated and equilibrated with 20 mM PED and applied to a column equilibrated in the same buffer. The column was eluted with a linear gradient of 20 mM to 0.5 M PED at a flow rate of 0.5 mL/min. Fractions of 0.5 mL were collected and monitored for absorption at 280 mM, ion conductivity, and NAT activity. Fractions 74–85 were pooled. The insert shows an SDS–polyacrylamide gel electrophoresis of rat liver NAT (left). The molecular weight markers (right) are phosphorylase B (97), bovine serum albumin (66), and ovalbumin (43).

Table II: Amino Acid Composition of Rat Liver N^{α}-Acetyltransferase

amino acid	rat liver[a]	yeast[b]	amino acid	rat liver[a]	yeast[b]
alanine	61	61	methionine	12	16
arginine	23	37	phenylalanine	29	41
aspartic acid	74	106[c]	proline	36	29
half-cystine	ND	8	serine	95	58
glutamic acid	111	96[d]	threonine	45	29
glycine	149	34	tryptophan	ND	8
histidine	21	12	tyrosine	10	48
isoleucine	32	44	valine	36	32
leucine	75	99			
lysine	54	96	total	863	854

[a] Values reported in residues per 95 000 molecular weight. [b] From Lee et al. (1989a) and Mullen et al. (1989). [c] Sum of aspartic acid and asparagine. [d] Sum of glutamic acid and glutamine.

liver enzyme is substantially different from that of yeast (Lee et al., 1989a; Mullen et al., 1989). No composition for the hen oviduct enzyme was reported. The most notable differences are seen in glycine, serine, threonine, and lysine. The combined aspartic acid/asparagine content is also lower in the rat enzyme as is the tyrosine content although this may be due in part to destruction as noted above. These results suggest that the two proteins may be quite different in primary sequence.

pH Optimum

The effect of pH on the activity was investigated with potassium phosphate and Tris–acetate buffers. As shown in Figure 6, the enzyme was active in a narrow range from pH 6.5 to pH 9.0, with the maximal activity observed at pH 8.0 (solid lines). The enzyme was inactive above 9 or below 6.5. However, when the enzyme was incubated at various pH values

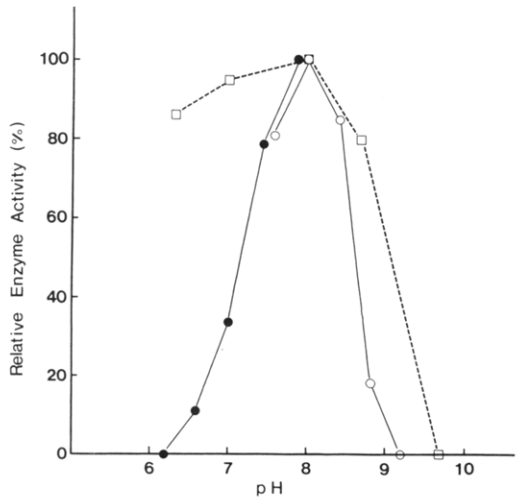

FIGURE 6: Effect of pH on the activity of rat liver NAT. The solid lines indicate enzyme activities measured at the indicated values in 0.5 M potassium phosphate (closed circles) or 0.5 M Tris–acetate (open circles). The enzyme was assayed as described in the text except the 200 mM potassium phosphate (pH 8.0) was omitted. The stability of rat liver NAT as a function of pH is given by the broken line (open squares). Enzyme solutions were incubated at 4 °C for 2 days in 0.5 M potassium phosphate buffer at the indicated pH. Following dialysis against 20 mM PED buffer containing 50% glycerol, NAT activity was measured. Activity values are given as percent of the activity observed at pH 8, which was arbitrarily assigned the value of 100%.

(in 0.5 M potassium phosphate buffer) for 48 h at 4 °C and then dialyzed to pH 8.0 (20 mM PED containing 50% glycerol to concentrate the sample), it retained 80–100% activity over the range pH 6 to pH 9 (broken line). The enzyme was inactivated at pH 10. (Under the conditions of the assay, <10% of the acetyl-CoA is hydrolyzed at pH 10, which would not affect activity at the concentration of acetyl-CoA used in the assay.) Thus the rapid decreases in activity above and below pH 8 appear to result from reversible changes in the enzyme, reflecting the titration of either active site residues (histidine and cysteine would be likely candidates) or residues that cause activity-sensitive conformation changes.

Effect of Salts on Enzyme Activity

When the enzyme was extracted from polysomes with 0.5 M KCl, it was observed that the yield was very low. Furthermore, the enzyme continued to lose activity if it was kept in 0.5 M KCl. An examination of the effect of KCl on rat liver NAT showed a linear decrease in polysome-associated activity with increasing molar concentration of KCl (data not shown). For example, at 0.4 M KCl, the preparation contained only 40% NAT activity when assayed immediately after the addition of the salt. Further decreases were observed if the sample were incubated for longer periods at the same salt concentrations.

As a result of these findings, the effect of various kinds of salts on the enzyme activity was examined. As shown in Figure 7, the enzyme is strongly inhibited by a number of anions at 0.5 M including Cl^-, SCN^-, ClO_4^-, and I^-. However, it is unaffected by F^-, phosphate, and citrate and only partially by sulfate and, perhaps, acetate. It is not influenced by Na^+ or K^+, but NH_4^+ appears to be partially inhibitory. Tris may have a slight effect as judged by the modest decrease in activity observed for 0.5 M Tris–acetate.

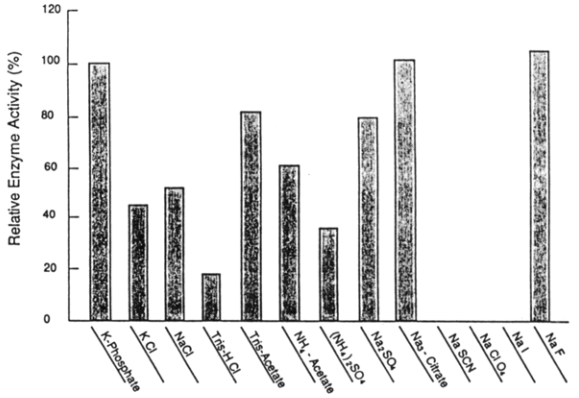

FIGURE 7: Effect of salts on rat liver NAT activity. Enzyme solutions were mixed with the indicated salts to a final concentration of 0.5 M, adjusted to pH 8.0, and incubated at 4 °C for 2 days. NAT activity was measured as described in the text except that 200 mM potassium phosphate was omitted. Activity values are given relative to samples in 0.5 M potassium phosphate, arbitrarily set at 100%.

DISCUSSION

The N^{α}-acetylation of proteins occurs in eukaryotic cells largely as a cotranslational event. However, significant activity occurs in the cytoplasm and in the ER-Golgi continuum as judged by N^{α}-acetylation events that occur posttranslationally (and usually after limited proteolytic processing) (Rubenstein et al., 1981; Smyth & Zakarian, 1980; Ramachandran & Li, 1967). In fact, the first demonstrations by Narita (1958) of the N^{α}-acetylation of tobacco mosaic virus coat protein and the N^{α}-acetylation of α-MSH (and ACTH), the sequence used for the assay in this study, are examples of modifications that arise postribosomally. The fact that α-MSH sequences are good substrates for the polysome-associated enzyme suggests that the specificities of many NATs may be similar and that the cellular origins of a preparation of the enzyme should therefore not be assumed without confirmation.

The N^{α}-acetylation of nascent polypeptide chains during protein synthesis is apparently accomplished by an enzyme(s) associated with the ribosomes. It has been determined that the addition of the acetyl moiety occurs when 20–40 amino acids have been assembled (Palmiter et al., 1978; Driessen et al., 1985), suggesting that the observed selectivity must reside in the amino-terminal sequence of the modified protein. The pattern of N^{α}-acetylation observed in situ (Huang et al., 1987; Boissel et al., 1988) and as divined from analyses of the protein sequence database (Driessen et al., 1985) strongly supports this view. As shown in the accompanying paper (Yamada & Bradshaw, 1991), examination of the substrate specificity of the homogeneous enzyme with synthetic peptides reveals the enzyme to be highly selective and to be governed primarily by the identity of the first three to four residues of the substrate. Interestingly, the enzyme apparently works in concert with methionine aminopeptidase (MAP) which removes the initiator methionine from the same nascent chains. Its specificity is also governed by the amino-terminal sequence, primarily by the residue penultimate to the methionine (Sherman et al., 1986; Huang et al., 1987). Since the bulk of N^{α}-acetylations occur on N-termini for which the methionine has been removed, it suggests that the juxtaposition of MAP and NAT is such as to give the peptidase the opportunity to act first.

The rat liver NAT purified in this study is similar in some respects to that isolated from yeast (Lee et al., 1988) and hen oviduct (Kamitani et al., 1989) but is also distinctly different in others. As shown in Table III, the rat and yeast enzymes have the same molecular weight and apparent subunit structure, i.e., they occur as apparent homodimers (see, however, below). In contrast, the oviduct enzyme is a heterotrimer composed of two protein subunits and one 7S RNA molecule, all of M_r ~80K. No RNA was found associated with the rat

Table III: Comparison of Eukaryotic N^{α}-Acetyltransferases

	yeast[a]	hen[b]	rat[c]
native molecular mass (kDa)	180 ± 10	240	190
subunits			
no.	2	2/1	2
molecular mass (kDa)	95	79/83[d]	95
pH optimum	9.0	7.8	8.0
inhibitors[e]	Cu^{2+}, Zn^{2+}, DEP, NBS	Fe^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Mg^{2+}, thiol reagents	Cl^-, I^-, NH_4^+, SCN^-, ClO_4^-, iodoacetate
p*I*	4.3	ND	(7.6)[d]
N-terminus	blocked	ND	blocked

[a] Lee et al. (1988). [b] Kamitani et al. (1989); Kamitani & Sakiyama (1989). [c] This study. [d] 7S RNA. [e] Abbreviations: DEP, diethyl pyrocarbonate; NBS, *N*-bromosuccinimide. [f] Estimated with partially purified enzyme.

liver preparation, and none was reported for the yeast enzyme (Lee et al., 1988). The rat and yeast enzymes show similar pH optima, and both have blocked N-termini. However, the amino acid compositions are distinctly different (Table II) as apparently are the measured isoelectric points. The two enzymes show distinctly different inhibitor sensitivities. The yeast enzyme is only partially inhibited by thiol reagents, i.e., *N*-ethylmaleimide, iodoacetic acid, iodoacetamide, and *p*-(chloromercuri)benzoate, while the rat liver enzyme was readily inactivated by iodoacetic acid. The distinctly weaker response of the yeast enzyme to thiol reagents is consistent with the fact that the yeast enzyme is active at pH 10 in contrast to the rat liver enzyme. This supports the view that the alkaline side of the pH optimum curve of the rat liver enzyme represents the titration of an active site thiol group and that the yeast and rat liver enzymes have different catalytic centers. The rat enzyme was directly affected by Cl^-; the yeast enzyme was not inhibited by 0.45 M KCl or NaCl (although it was affected by higher concentrations). The hen oviduct enzyme was also sensitive to thiol reagents as well as various divalent cations as was the yeast enzyme. Importantly, the three enzymes also show significant differences with respect to substrate specificity (Yamada & Bradshaw, 1991).

The NAT gene has been identified in yeast and a protein sequence predicated from the nucleic acid sequence (Lee et al., 1989a; Mullen et al., 1989). The protein subunit contains 854 amino acids corresponding to a calculated molecular weight of 98 575. The amino-terminal sequence of Met-Ser-Arg is consistent with an N^{α}-acetylated serine as the N-terminus of the native protein arising from the combined action of MAP and NAT (Arfin & Bradshaw, 1989) and leading to the blocked amino terminus observed. The sequence shows a very limited similarity to other acetyltransferase sequences (~10–15% identity).

The disruption of the single gene coding for yeast NAT (designated *nat* or *aaa1*) on chromosome IV produces a phenotype that is characterized by slow growth, defective sporulation, and heat shock sensitivity (Lee et al., 1989b; Mullen et al., 1989). Two-dimensional gel electrophoresis of the soluble proteins from these mutant cells, compared to wild type, showed that a substantial number of proteins had altered mobilities consistent with the increased positive change expected for unacetylated proteins (Lee et al., 1989c; Mullen et al., 1989). However, the reports differed on whether proteins were absent as might be expected for more rapid turnover, i.e., increased instability.

Mullen et al. (1989) also noted that this phenotype was essentially identical with that reported previously for the mutant *ard1* (Whiteway & Szostak, 1985; Whiteway et al., 1987). Further analyses suggested that the products of the *ard* and *nat* genes were linked and that they represented different subunits of the N^{α}-acetyltransferase enzyme. The *ard* gene codes for a protein composed of 238 amino acids; its sequence is unrelated to that of the *nat* product. Since a 1:1 ratio of the two gene products is apparently required, they suggested a heterodimer as the functional complex. However, since yeast NAT is isolated as a homodimer of the *nat* gene product (Lee et al., 1988), a heterotetramer of A_2B_2 composition would be more likely. Neither yeast nor rat NAT has been isolated in such a form. However, dissociation from the polysomes may result in disruption of the complex producing functional (if presumably less active) homodimers of the larger subunit. This model is consistent with the suggestion (Mullen et al., 1989) that the *ard* gene product may function to connect the NAT protein to the ribosome. Possibly, the 7S RNA of the hen oviduct enzyme may serve this function in that tissue. If this is the case, the two animal NATs would appear to be significantly different.

The yeast null mutants also reveal that there is additional NAT activity in these cells. Whether this represents another enzyme that also acts cotranslationally remains to be determined. Clearly additional NAT activity may also exist in rat liver and could explain some of the discrepancies in substrate specificity observed for the isolated rat liver enzyme (Yamada & Bradshaw, 1991) and that expected from in situ studies (Huang et al., 1987; Boissel et al., 1988).

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