To elucidate the molecular basis of human N-acetylation polymorphism, cDNA clones encoding human liver N-acetyltransferases (EC 2.3.1.5) were isolated from λgt10 cDNA libraries using the 32P-labeled cDNA of rabbit liver N-acetyltransferase recently cloned in this laboratory. Three types of cDNAs (D-14, O-7, and D-24) were isolated and their nucleotide sequences were determined, from which the amino acid sequences of human N-acetyltransferases were deduced. All the cDNAs coded for 290 amino acids. Between D-14 and O-7 cDNAs, there was only a single-base substitution in the coding region, which replaced glutamic acid in D-14 cDNA for glycine in O-7 cDNA. There were considerable differences between O-7/D-14 and D-24 cDNAs, with 80% homology in amino acid sequences. When the cDNAs were inserted into pEF321 expression vector and introduced into Chinese hamster ovary cells, N-acetyltransferase activity was expressed in three groups of the transfected cells. The activity in cells transfected with D-14 cDNA was only 9–17% of the activity in O-7 cells. Immunoblot analysis of the transfected cells indicated that the difference in the enzyme activity between O-7 and D-14 cells was possibly due to a difference in the amount of enzyme proteins. The substrate specificity of the expressed enzymes indicated that O-7 and D-14 cDNAs code for polymorphic N-acetyltransferase whereas D-24 cDNA codes for monomorphic enzyme. Southern blot analysis indicated that the polymorphic and monomorphic N-acetyltransferases were encoded in separate genes and that there was restriction fragment length polymorphism with KpnI digestion in the polymorphic N-acetyltransferase gene.

A familial accumulation of isoniazid-induced nerve disturbance led to the discovery of N-acetylation polymorphism in man, giving rise to the terminology “slow” or “rapid” acetylation, determined by a low or high arylamine N-acetyltransferase gene. This led to the discovery of N-acetylation polymorphism in man, giving rise to the terminology “slow” or “rapid” acetylation, determined by a low or high arylamine N-acetyltransferase gene. The primary structures of the cDNAs and their expression in mammalian cells have indicated that the polymorphic and monomorphic N-acetyltransferases were products from different genes and suggested that the difference in activity between two forms of the polymorphic N-acetyltransferase might be due to a single amino acid substitution of the enzyme protein.

MATERIALS AND METHODS

Cloning of N-Acetyltransferase cDNAs—Total RNA was extracted from human livers obtained at autopsy using the guanidine thiocyanate method (5), and poly(A)+ RNA was selected separately from two liver specimens as described previously (7). One-hundred ng of the cDNAs ligated with 1 μg of λgt10 arms resulted in 3 × 106 independent recombinant phages. The λgt10 cDNA libraries were screened with the cDNA of rabbit liver N-acetyltransferase, which was 32P-labeled by a multiprime labeling system (Amersham, United Kingdom) to a specific activity of 5 × 106 cpm/μg. Nitrocellulose filters were prehybridized at 42 °C for 24 h in a solution containing 5 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.4), 5 × Denhardt’s, 5 × Denhardt’s, and 0.1% SDS, 100 μg/ml denatured salmon sperm DNA. The filters were hybridized with the 32P-labeled rabbit cDNA in the same solution at 42 °C for 24 h and then washed in 2 × SSC containing 0.1% SDS at 42 °C for 30 min twice.

Nucleotide Sequence Analysis—Positive phages were cloned, and the restriction sites of DNAs were analyzed. Restriction fragments were subcloned into pUC18 vector and sequenced by the dyeodeoxy method (8).

Expression of cDNAs in CHO Cells—The cDNAs were inserted into the EcoRI site of pEF321 expression vector provided by Dr. S. Sugano, The Institute of Medical Science, The University of Tokyo in the sense orientation under the control of human elongation factor (9). To adjust the length of the 5'-noncoding region of O-7 and D-14 cDNA, the EcoRI-Neol fragment (115–140) of O-7 cDNA was ligated into the same site of D-14 cDNA. The transfection into CHO cells was performed as described previously (10). Cloned cells were cultured in 10-cm dishes to confluence, harvested, and sonicated in 250 μl/dish of 50 mM potassium phosphate buffer, pH 7.0. After centrifugation at 15,000 × g for 30 min, the supernatant was used for determination of the enzyme activity.

The abbreviations used are: SDS, sodium dodecyl sulfate; CHO, Chinese hamster ovary; kb, kilobase, RFLP, restriction fragment length polymorphism.

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**Fig. 1. Restriction maps, and nucleotide and deduced amino acid sequences of cDNAs encoding human liver arylamine N-acetyltransferases (a, O-7 and D-14 cDNAs; b, D-24 cDNA). bp, base pairs. Nucleotide residues are numbered in the 5' to 3' direction starting with the initiation ATG as number 1. The deduced amino acid residues are numbered beginning with the initiating methionine. The nucleotide and amino acid residues in D-14 which differ from O-7 cDNA are shown at the upper lines in the open box. One-letter amino acid notation was used. The poly(A) signal (AATAAA) in the 3' non-coding region is underlined.**

The assay of N-acetyltransferase activity.

**Assay of N-Acetyltransferase Activity—**N-Acetyltransferase activity with 2-aminofluorene, procarcinamide, p-phenetidine, and 5-methoxystyptamine substrates was determined by radiochemical assay as described previously (11). The enzyme activity was corrected for the extraction efficiencies of the products. N-Acetyltransferase activities for sulfamethazine and p-aminobenzoic acid was determined spectrophotometrically with 15 nmol amine substrates and 50 nmol of acetyl-coenzyme A (12). The reactions were carried out at 37 °C for 30 min.

**Northern Blot Analysis of Transfected Cells—**Total RNA was extracted from the transfected cells by the hot acid phenol-SDS method (10). The RNA was electrophoresed on a 1% agarose gel containing formaldehyde and transferred to a GeneScreen Plus (Du Pont-New England Nuclear) (14). The filters were hybridized at 42 °C in 50% formamide, 1 M NaCl, 10% dextran sulfate, 1% SDS, 150 ng/ml of 32P-labeled cDNA, or the D-24 specific cDNA 32P-labeled using the multiprime labeling system. Genomic DNA was prepared from the liver as described (14). The DNAs (10 μg) were digested with 30-40 units of BamHI, KpnI, and EcoRI at 37 °C for 5 h, then incubated for 2 h with the 100-fold diluted ascite obtained from a mouse immunized with the O-7 fusion protein. The filter was washed with TBS containing 0.1% Triton X-100 and incubated with 125I-labeled anti-mouse IgG(Fab')2 (2 μCi/ml). Radioactive bands were detected by autoradiography.

**RESULTS AND DISCUSSION**

We have previously isolated the cDNA coding for arylamine N-acetyltransferase from chicken liver (7). Using the chicken cDNA and an oligonucleotide corresponding to the amino acid sequence of rabbit N-acetyltransferase reported by Andres et al. (21), we have lately isolated the cDNA for rabbit N-acetyltransferase (7). The rabbit cDNA was employed as a probe for the screening of human XgtlO cDNA libraries prepared from the poly(A)+ RNAs of two human livers obtained at autopsy. Out of 3 × 10^6 independent recombinant phages in each library, 8 and 16 positive clones, respectively, were isolated. Restriction enzyme analysis revealed that one of the

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cDNA libraries contained three types of cDNAs (O-7, 7 clones; D-14, 8 clones; and D-24, 1 clone) and the other contained two types (O-7, 7 clones; D-24, 1 clone), although the insert sizes varied from 0.6 to 1.3 kb. Restriction maps (Fig. 1, a and b) indicated that O-7 cDNA differed from D-14 only in a BamHI site, whereas there were considerable differences in the restriction sites between D-24 and O-7/D-14 cDNAs. The nucleotide sequences of the longest of the three types of cDNAs were analyzed, from which amino acid sequences were deduced (Fig. 1, a and b). There were 1,276 nucleotides in O-7, 1,210 nucleotides in D-14, and 1,319 nucleotides in D-24 cDNA. D-14 cDNA was 66 nucleotides shorter than O-7 cDNA at the 5'-noncoding region. An open reading frame probably begins at the initiating ATG at positions 1-3 and end at the translation termination codon TAG at positions 871-873. All three cDNAs coded for 290 amino acids. There was 80% homology in the amino acid sequences between O-7/D-14 and D-24 cDNAs. Between O-7 and D-14 cDNAs there was only a single-base substitution at position 857, replacing glycine in O-7 cDNA for glutamic acid in D-14 cDNA at position 286. The calculated molecular weights of the proteins were 33,542 for O-7, 33,787 for D-14, and 33,614 for D-24 cDNA. The 3'-noncoding regions ended at an EcoRI site.

The cDNAs were inserted into plasmid pEF321 expression vector and introduced into CHO cells with pEF321-Neo. Following selection with G418, stable transfected cell lines were isolated, and N-acetyltransferase activity was determined in the crude supernatants of cell homogenates by using procainamide as a substrate. N-Acetyltransferase activity was expressed in three groups of the transfected cells (Fig. 2a), whereas cells transfected with pEF321-Neo plasmid alone showed no activity. The cells transfected with O-7 or D-24 cDNA expressed a high enzyme activity, except for the D-24 cells. In contrast, all the cell lines transfected with O-7 or D-24 cDNA showed a lower N-acetyltransferase activity, from 9 to 17% of the activity in O-7 cells. Northern blot analysis of the transfected cells revealed an almost equivalent expression of N-acetyltransferase mRNA except in D-24-8 cells which showed a low N-acetyltransferase activity and a low level of mRNA (Fig. 2b). To estimate the quantities of enzyme protein, immunoblot analysis was performed using mouse antibody raised against the O-7 fusion protein. A protein band at 31 kDa corresponding to N-acetyltransferase was clearly detected in the cells transfected with O-7 cDNA, whereas the band was hardly detectable in the cells transfected with D-14 cDNA. This result suggests that the difference in N-acetyltransferase activity between O-7 and D-14 cells was possibly due to the difference in the amount of enzyme proteins. It could be that the translation efficiency was different between O-7 and D-14 cDNAs or that the substitution of an amino acid in D-14 enzyme resulted in its rapid degradation.

To examine which of the cDNAs corresponded to the polymorphic and monomorphic N-acetyltransferases, the substrate specificities of the enzymes expressed from the cDNAs were analyzed (Table I). The enzymes expressed from O-7 and D-14 cDNAs acetylated sulfamethazine very efficiently, but p-aminobenzoic acid not at all. In contrast, p-aminobenzoic acid was acetylated very efficiently by the enzyme expressed from D-24 cDNA.
Northern blot analysis was performed using poly(A)+ RNA from three human livers with \(^{32}P\)-labeled O-7- and D-14-specific cDNA fragments (Fig. 3a). The O-7-D-14-specific probe hybridized intensely to a mRNA of approximately 1.5 kb, whereas the D-24-specific probe hybridized only slightly to a mRNA of similar size. This result indicates that the mRNA for the polymorphic N-acetyltransferase is abundant in human liver, while the monomorphic mRNA is sparse.

Southern blot analysis was performed with genomic DNA prepared from human livers using the whole O-7 cDNA as a probe (Fig. 3b). The DNAs from three subjects showed almost identical fragment patterns with digestion by BamHI, EcoRI, HindIII, PvuII, EcoRV, and BglII (data for the last four enzymes are not shown), whereas restriction fragment length polymorphism (RFLP) was observed with KpnI digests. In subjects 1 and 3, 5.0- and 5.5-kb bands were detected, while the 5.0-kb band was not observed in subject 2. To identify which of the genes give rise to the RFLP pattern, O-7- and D-24-specific cDNA probes were employed. Each probe detected different bands in digestions by three different enzymes, indicating that the polymorphic and monomorphic liver N-acetyltransferases are encoded in two separate genes. A RFLP of KpnI digests was detected in human genomic DNAs with the O-7-specific probe. We have analyzed genomic DNAs from four livers obtained at autopsy and leukocyte DNAs from 12 healthy volunteers. All the DNAs so far examined showed one of the two RFLP patterns. Since the rapid acetylator is predominant in Japanese (26), it might be that one type of the RFLPs represents the rapid acetylator homozygote gene, while the other represents the heterozygote gene. In this respect, it would be of importance to elucidate the correlation between the RFLP patterns of genomic DNA and the two types of cDNA with or without a BamHI site. A considerable difference in enzyme activity was observed between cells transfected with O-7 and with D-14 cDNAs. This observation suggests the possibility that the D-14 and O-7 cDNAs correspond to the slow and rapid acetylator phenotypes, respectively. Further studies are required to elucidate the relationship between the RFLP patterns of genomic DNA, the presence or absence of a BamHI site in the cDNAs, and a high or low N-acetyltransferase activity.

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