Biochemistry

OVEREXPRESSION, PURIFICATION, AND CHARACTERIZATION OF RECOMBINANT HUMAN ARYLAMINE N-ACETYLTRANSFERASE 1

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Human arylamine *N*-acetyltransferase 1 (NAT1) has been overexpressed in *E. coli* as a mutant dihydrofolic acid reductase (DHFR) fusion protein with a thrombin sensitive linker. An initial DEAE anion-exchange chromatography resulted in partial purification of the fusion protein. The fusion protein was cleaved with thrombin, and human rNAT1 was purified with a second DEAE column. A total of 8 mg of human rNAT1 from 2 L of cell culture was purified to homogeneity with this methodology. Arylamine substrate specificities were determined for human rNAT1 and hamster rNAT2. With both NATs, the second order rate constants (*kcat*/Kmb) for *p*-aminobenzoic acid (PABA) and 2-aminofluorene (2-AF) were several thousand-fold higher than those for procainamide (PA), consistent with the expected substrate specificities of the enzymes. However, *p*-aminosalicylic acid (PAS), previously reported to be a human NAT1 and hamster NAT2 selective substrate, exhibits 20-fold higher specificity for hamster rNAT2 (*kcat*/Kmb 3410 μM-1sec-1) than for human rNAT1 (*kcat*/Kmb 169.4 $\mu M^{-1}sec^{-1}$). *p*-aminobenzoylglutamic acid (pABglu) was acetylated 10-fold more efficiently by human rNAT1 than by hamster rNAT2. Inhibition studies of human rNAT1 and hamster rNAT2 revealed that folic acid and methotrexate (MTX) are competitive inhibitors of both the unacetylated and acetylated forms of the enzymes, with KI values in 50-300 μM range. Dihydrofolic acid (DHF) was a much poorer inhibitor of human rNAT1 than of hamster rNAT2. The combined results demonstrate that human rNAT1 and hamster rNAT2 have similar but distinct kinetic properties with certain substrates, and suggest that folic acid, at least in the non-polyglutamate form, may not have an effect on human NAT1 activity *in vivo*.