Acetylation of 6-Sulphanilamidopyrimidines in the Rabbit

By S. N. COHEN and W. W. WEBER

Department of Pharmacology, New York University, School of Medicine,
New York, N.Y. 10016, U.S.A.

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*N*⁴-Acetylation is the major route of metabolism of sulphonamides in the rabbit (Smith & Williams, 1948). Wide variation occurs, however, in the extent of acetylation of sulphonamides that are very similar structurally, as has been shown in studies of the fate of various 6-sulphanilamidopyrimidines of a homologous series (Williams, 1967). Since *N*-acetyltransferase (EC 2.3.1.5), present in rabbit liver, is chiefly responsible for the acetylation of these compounds in this species (Weber, Cohen & Steinberg, 1968), we decided to determine whether the variation in the extent of acetylation *in vivo* might be attributed to differences in the rates of enzymic acetylation of these compounds.

Enzymic acetylation of 4-methoxy-, 5-methoxy-, 2,4-dimethoxy-, 2,5-dimethoxy- and 4,5-dimethoxy-6-sulphanilamidopyrimidine was studied with preparations of *N*-acetyltransferase that had been purified about 500-fold from the supernatant fraction of livers of New Zealand White rabbits. Enzyme purification was carried out by the procedure described by Weber & Cohen (1967) and the fractions used were similar to those used in our other studies on enzymic *N*-acetylation (Weber & Cohen, 1967, 1968; Cohen & Steinberg, 1967). Our previous studies with 2-sulphanilamidopyrimidines and other weakly acidic compounds showed that *N*-acetyltransferase activity was relatively greater at pH values below the *pK*_a, suggesting that the enzyme interacts preferentially with the uncharged

form of these substrates (Weber & Cohen, 1967). The *pK*_a values were determined for the 6-sulphanilamidopyrimidines (Table 1). All enzyme studies were carried out at pH 6.0, which approximates the pH for optimum activity with each of the compounds. The assay mixtures contained 0.2 μmole of acetyl-CoA, 0.03 μmole of the sulphonamide, 0.70 μmole of sodium pyrophosphate and 0.1 ml. of enzyme fraction in a total volume of 1.0 ml. Activity with each sulphonamide was measured by determining the amount of free sulphonamide remaining in each of the experimental tubes after incubation at 27° by a modification of the method of Bratton & Marshall (1939) and subtracting this from the amount of the sulphonamide in a control tube from which acetyl-CoA had been omitted.

The results of our studies *in vitro* with the substituted 6-sulphanilamidopyrimidines and the findings *in vivo* reported by Williams (1967) are summarized together in Table 1. Examination of the results obtained *in vitro* shows that there is sixfold variation in the rate at which these compounds are enzymically acetylated, with 4-methoxy-, 5-methoxy- and 2,4-dimethoxy-6-sulphanilamidopyrimidine all being acetylated at the highest rate. We wished to evaluate the relative affinity of the enzyme for each of the 6-sulphanilamidopyrimidine analogues, but *K*_m values could not be determined for either 2,5-dimethoxy- or 4,5-dimethoxy-6-sulphanilamidopyrimidine

Table 1. *Acetylation of methoxy- and dimethoxy-6-sulphanilamidopyrimidines in the rabbit*

The substituted 6-sulphanilamidopyrimidines were made available through the courtesy of Professor R. T. Williams. The method of assay of enzymic *N*⁴-acetylation is described in the text. The *pK*_a values were determined at 27°. The presentation of the results for *N*⁴-acetylation *in vivo* follows that of Williams (1967).

Substituted 6-sulphanilamido- pyrimidine	<i>N</i> ⁴ -Acetylation <i>in vitro</i> (μmole acetylated/min./ml. of DEAE-cellulose fraction)	<i>pK</i> _a	<i>N</i> ⁴ -Acetylation <i>in vivo</i>		
			Total sulphonamide excreted in 24 hr. (% of dose)	Excretion of <i>N</i> ⁴ -acetylated derivative	
				(% of 24 hr. excretion)	(% of administered dose)
4-Methoxy	0.0018	6.6	76	98	75
5-Methoxy	0.0020	6.7	82	92	76
2,4-Dimethoxy	0.0023	6.9	43	94	40
2,5-Dimethoxy	0.00072	6.5	63	7	4
4,5-Dimethoxy	0.00038	6.5	64	87	56

because of the very low rates of acetylation obtained when the concentration of either of these compounds was varied over a sufficiently broad range. Values for K_m were obtained for the three most active analogues, however, and these were in close agreement. The pK_a values for all of the analogues are separated by only 0.4 pH unit (6.5–6.9), and the differences in the rate of acetylation *in vitro* between the three most active analogues and either 2,5-dimethoxy- or 4,5-dimethoxy-6-sulphanilamidopyrimidine can therefore not be attributed to differences in the amount of any of these analogues existing in the uncharged form. However, the compounds that are acetylated most slowly *in vitro* are disubstituted, with one of the substituents in the 5-position. Thus it appears that the presence of a methoxyl group in this position, combined with one in either the 2- or the 4-position, somehow alters the interaction between the compound and the enzyme.

The results with 4-methoxy-, 5-methoxy- and 2,4-dimethoxy-6-sulphanilamidopyrimidine *in vivo* show that acetylated derivatives account for 92–98% of the excretion products of each of these analogues during the first 24 hr. after administration. Thus the compounds that were acetylated to the greatest extent *in vivo* were the ones acetylated most rapidly *in vitro*. The fact that only 43% of the dose of 2,4-dimethoxy-6-sulphanilamidopyrimidine was excreted during this interval suggests that this compound may have been relatively slowly absorbed from the gastrointestinal tract and does not alter this conclusion. Examination of the results obtained with 2,5-dimethoxy-6-sulphanilamidopyrimidine show that it is acetylated *in vitro* at a relatively low rate, and the results obtained with this compound *in vivo* are in agreement, since

only 7% of the excretion products is in the form of the acetylation derivative. However, the results obtained with 4,5-dimethoxy-6-sulphanilamidopyrimidine are different. This compound is acetylated *in vitro* more slowly than 2,5-dimethoxy-6-sulphanilamidopyrimidine, whereas the extent of its acetylation *in vivo* is much greater (87%). These results indicate that factors other than the rate of enzymic acetylation are important in determining the extent to which 2,5-dimethoxy-6-sulphanilamidopyrimidine is acetylated *in vivo*. [R. T. Williams (personal communication) recently informed us that 4,5-dimethoxy-6-sulphanilamidopyrimidine is much more lipid-soluble than 2,5-dimethoxy-6-sulphanilamidopyrimidine, and therefore would not be readily excreted until converted into the less lipid-soluble acetyl derivative. 2,5-Dimethoxy-6-sulphanilamidopyrimidine, on the other hand, can be readily excreted by the kidney without prior metabolic alteration.]

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