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PROPERTIES OF CUTANEOUS ACETYLTRANSFERASE CATALYZING *N*- AND *O*-ACETYLATION OF CARCINOGENIC ARYLAMINES AND *N*-HYDROXYARYLAMINE

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Abstract—The role of skin for *N*- and *O*-acetylations of carcinogenic arylamine and *N*-hydroxyarylamine was studied *in vitro*. Unexpectedly high activities were observed in acetyl CoA-dependent *N*-acetylations of 2-aminofluorene (2-AF) and *p*-aminobenzoic acid (PABA) in skin cytosols of hamsters. The specific activity for 2-AF (4.52 nmoles/mg protein per min) was largely the same as that of rat liver cytosols. The cutaneous cytosols also catalyzed *N*,*N*-acetyltransfer reaction from *N*-hydroxy-4-acetylaminobiphenyl (*N*-OH-AABP) to 2-AF and acetyl CoA-dependent *O*-acetylation of 2-hydroxyamino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (*N*-OH-Glu-P-1), suggesting that hamster skin cytosol has enzymes similar to hepatic acetyltransferases. In addition, remarkably high correlations were observed between the skin and liver in the activities for *N*-acetylations of PABA and 2-AF. In a colony of Syrian golden hamsters a clear polymorphism was detected in the cutaneous *N*-acetylations of PABA and 2-AF. These animals were divided into three groups according to their activities: rapid, intermediate and slow acetylators. On the other hand, the acetylating activities in the skin and liver of these three groups showed monomorphic distribution with *N*-OH-AABP-dependent *N*,*N*-acetyltransfer of 2-AF and acetyl CoA-dependent *O*-acetylation of *N*-OH-Glu-P-1. These results, together with the detection of *N*-acetylating activity in the skin of other experimental animals and humans, suggest that skin may play an important role in the metabolism of aromatic amines and that the cutaneous acetylation in hamsters may be under the common genetic control which regulates the individual difference in the hepatic activities.

With the increase in the number of chemicals newly produced, skin becomes more easily exposed to hazardous chemicals. Recent studies suggest that skin is not only a mechanical barrier but an organ which has an activity to detoxify various types of chemicals including carcinogens [1–3]. Now various types of metabolic pathways are known to participate in the activation of carcinogenic compounds [4, 5]. Acetylation is a necessary step for the activation of carcinogenic arylamines. Although the possible role of metabolic activation of carcinogen/mutagen in the skin has been reported in the case of cytochrome P-450-dependent enzymatic reactions [1–3], no enzymatic activation through acetylation in cutaneous tissue has been reported, except for one report on rabbit skin which covered undetectable activity for the *N*-acetylation of PABA [6]. Acetyl CoA-dependent direct *O*-acetylation of *N*-hydroxyarylamines, as well as arylamine *N*-acetylation, has recently been shown as important for the metabolic activation of arylamines to induce DNA damage [7–9]. Thus, the activities of three types of the acetylating pathway, acetyl CoA-dependent *N*- and *O*-acetylations, and arylhydroxamic acid-dependent *N*,*N*-acetyltransfer, were determined to assess the role of cutaneous metabolism on the metabolic activation and inactivation of arylamines.

MATERIALS AND METHODS

Animals. Five-week-old male Syrian golden hamsters were purchased from Tokyo Laboratory Animals Science, Tokyo, Japan. Eight-week-old male and female Sprague–Dawley rats were obtained from Clea Japan, Tokyo. Nine-week-old female CD-1 mice were obtained from Charles River Japan, Inc.

Chemicals. 2-AF,§ 2-AAF and PABA were obtained from Tokyo Kasei industries (Tokyo, Japan). *N*,*N*-Diphenylamine was purchased from Wako Pure Chemicals (Osaka, Japan). Sulfhydril blocking agents, *p*-chloromercuribenzoate, *N*-ethylmaleinimide and iodoacetamide were obtained from Nakarai Chemicals (Kyoto, Japan). *N*-Acetyl PABA and *N*-propionyl PABA were synthesized using excess amounts of acetic anhydride in acetic acid and propionic anhydride in propionic acid, respectively. The reaction mixture was added dropwise into chilled water and then the precipitate (product) was isolated

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§ Abbreviations: 2-AF, 2-aminofluorene; 2-AAF, 2-acetylaminofluorene; DTT, dithiothreitol; *N*-OH-Glu-P-1, 2-hydroxyamino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole; Glu-P-1, 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole; PABA, *p*-aminobenzoic acid; *N*-OH-AABP, *N*-hydroxy-4-acetylaminobiphenyl; HPLC, high performance liquid chromatography.

by filtration. Synthetic *N*-acetyl- and *N*-propionyl-PABA showed the molecular ion at *m/z* 179 and *m/z* 193, respectively. Acetyl CoA and DTT were purchased from Sigma Chemical Co. (St. Louis, MO). *N*-OH-AABP was synthesized by acetylation of the corresponding *N*-hydroxyarylamine [10]. *N*-OH-Glu-P-1 was synthesized as reported previously [11].

Preparation of tissues. Rats and mice were stunned by a blow on the head and decapitated. Hamsters were anesthetized with dry ice and then killed by decapitation. The liver was homogenized with four to five volumes of 100 mM Tris–acetate buffer (pH 7.4) containing 1 mM DTT after perfusion *in situ* with 1.15% chilled KCl. The cytosolic fraction (105,000 *g* supernatant fraction) was obtained as described elsewhere [12]. For the preparation of skin cytosols, the back of each animal was shaved with an electric shaver and scissored out in an area of approximately 4 × 5 cm. This sheet was placed with its epidermal side down on an ice-cooled glass plate and the subcutaneous tissue was then scraped with a razor. The treated sheet of the whole skin was cut into pieces with scissors and mixed with two volumes of 100 mM Tris–acetate buffer (pH 7.4) containing 1 mM DTT and then prehomogenized with a Polytron tissue homogenizer (Kinematica GmbH, Luzern, Switzerland). Each burst was performed for 10 sec with 30 sec intervals, and 6 bursts were done for each sample. Prehomogenized skin was homogenized with a ground glass homogenizer. The cytosol of the skin was prepared by the same method described for the liver samples. Protein was determined by the method of Lowry *et al.* [13] with bovine serum albumin as the standard.

Assay procedure. For the enzymatic *N*-acetylation of 2-AF and PABA, the following system was employed. A typical incubation mixture consisted of 1.0 mM acetyl donor, 1.0 mM DTT, 50 mM Tris–HCl buffer (pH 7.0), 0.1 mM substrate amine and 0.1 mg/ml cytosolic protein in a final volume of 100 μl. The reaction was started by addition of cytosolic protein and then incubated at 37° for 10 min. To stop the reaction, 100 μl of acetonitrile was added for the *N*-acetylation of 2-AF and the mixture was centrifuged at 1500 *g* for 10 min to precipitate protein. The supernatant was subject to HPLC assay. For the *N*-acetylation of PABA, 100 μl of 1 N HCl was added to terminate the reaction and then 400 μl of ethyl acetate was added. After vortexing for 30 sec, the mixture was centrifuged at 1500 *g* for 10 min. The organic phase was taken to another tube and dried under a current of N_2 gas. The residue was resolved with eluent used in the HPLC system as described below. The principle of the assay procedure for direct *O*-acetylation of *N*-OH-Glu-P-1 was described previously [14]. Briefly, a typical incubation mixture consisted of 50 mM Tris–HCl buffer (pH 7.0), 1.0 mM DTT, 1.0 mM acetyl CoA, 0.1 mg/ml cytosolic protein and 100 μM *N*-OH-Glu-P-1. The reaction was started by the addition of the substrate and then was terminated by the addition of 600 μl of chilled methanol after incubation for 10 min at 37°. The product, Glu-P-1, was measured by the fluorescence (excitation and emission wavelengths were 376 nm and 445 nm, respectively) with a fluorescence spectrophotometer (Hitachi 650-50) [14].

HPLC conditions. For the *N*-acetylation of 2-AF, an HPLC system equipped with a JASCO Tri-Rotar V pump and UVIDEC 100-III UV spectrophotometer were used with a Nucleosil $_7C_{18}$ column (3.9 × 300 mm). The separation was performed as follows: two different ratios of the mixture of acetonitrile and 20 mM KH_2PO_4 (pH 4.5), 60:40 (v/v) and 55:45 (v/v), were used for acetyl CoA- and *N*-OH-AABP-dependent reactions at a flow rate of 1.2 ml/min, respectively. PABA and the acetylated metabolite were quantified using the same system described above, except that a mixture of 50 mM acetic acid and acetonitrile (85:15, v/v) was used as the mobile phase with a column (3.9 × 150 mm) filled with nucleosil $_7C_{18}$ at a flow rate of 1.2 ml/min. The product, 2-AAF and *N*-acetyl PABA was detected by their absorbance at 280 nm and 266 nm, respectively. *N,N*-Diphenylamine and *N*-propionyl PABA were used as the internal standard for the acetylation of 2-AF and PABA, respectively.

RESULTS

Skin and liver cytosolic acetylations of arylamines and N-hydroxyarylamine

As described in Table 1, *N*-acetylation of 2-AF

Table 1. Requirements for the *N*-acetylation of PABA and 2-AF in the hamster skin cytosol systems

System	Substrates (acetyl donor) 2-AF (acetyl CoA)	2-AF (N-OH-AABP)	PABA (acetyl CoA)
Complete*	452	152	882
—Cytosol	2	n.d.†	3
—Acetyl donor	n.d.	n.d.	n.d.

The values are the mean activity of duplicate determination (pmol/100 μl/10 min).

* Complete system consisting of 0.01 mg of cytosolic protein, 1 mM acetyl donor, 100 μM substrate, 50 mM Tris–HCl buffer (pH 7.0) and 1 mM DTT in a final volume of 100 μl.

† Less than 2 pmol/100 μl/10 min.

was dependent on both acetyl donor and skin cytosol of hamsters, and only a limited or undetectable amount of 2-AAF was formed in the absence of the skin cytosol or acetyl CoA. These results indicate that the acetyl CoA-dependent *N*-acetyltransferase exists in hamster skin cytosols. Similar requirements for cytosol and cofactor were also observed with *N*-OH-AABP-supported *N*-acetylation of 2-AF and acetyl CoA-dependent *N*-acetylation of PABA.

In the presence of 1 mM acetyl CoA, 2-AF was *N*-acetylated at the rate of 4.52 and 31.1 nmol/mg protein/min by the skin and liver cytosols, respectively (Table 2). The amount of the acetylated metabolite, 2-AAF, increased in proportion to the concentration of the skin cytosol up to 0.4 mg/ml (data not shown). In the skin and the liver cytosol, apparent K_m values for acetyl CoA were calculated to be 90 and 150 μM, respectively (Fig. 1). Cytosol of the hamster skin also had the activity for the acetyl CoA-dependent *N*-acetylation of PABA. The specific activity in the skin was approx. 18% of that in the liver (Table 2). In mammalian livers, *N*-acetylation is known to occur in the presence of arylhydroxamic acid. As described in Table 2, skin cytosol also utilized *N*-OH-AABP for the *N*-acetylation of 2-AF, although the rate was 6% of the hepatic activity. In this reaction, apparent K_m values for *N*-OH-AABP were estimated to be *ca.* 590 and 730 μM in the skin and the liver, respectively (Fig. 1).

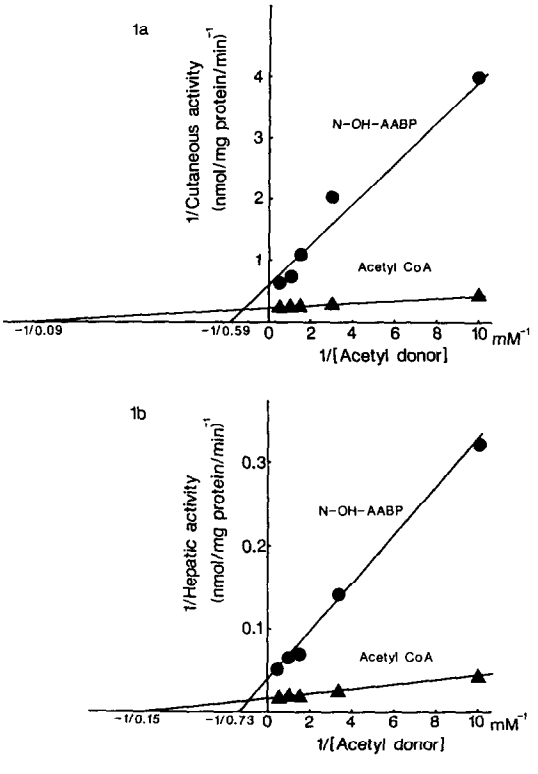

Fig. 1. Determination of K_m values and V_{max} for acetyldonors in the *N*-acetylation of 2-AF with skin (a) and liver cytosol (b) of a hamster. Two acetyldonors, *N*-OH-AABP and acetyl CoA, were used in the presence of 100 μM 2-AF respectively. Other experimental details are described in Materials and Methods.

Table 2. Hepatic and cutaneous activities for *N*-acetylation and *N,N*-transacetylation of arylamines and *O*-acetylation of *N*-hydroxyarylamine in hamster cytosols

Substrate	Activities* Liver (nmol/mg protein/min)	Skin (nmol/mg protein/min)	Ratio† S/L (%)
Acetyl CoA dependent-			
2-AF	31.1 ± 10.2	4.52 ± 2.13	15
PABA	47.7 ± 25.7	8.82 ± 5.91	18
N-OH-Glu-P-1	3.62 ± 0.41	0.30 ± 0.08	8
N-OH-AABP dependent-			
2-AF	25.6 ± 3.41	1.52 ± 0.26	6

* Data represented are the mean ± SD of 5 different individual animals.

† Percentage of cutaneous activity in hepatic activity.

Recent studies from our and other laboratories have indicated that acetyl CoA-dependent acetyltransferase catalyzes the direct *O*-acetylation of *N*-hydroxyarylamines [7, 8]. The activities were detected in the liver and several other tissues [9], but the activity in the skin had not yet been examined. Therefore, *O*-acetyltransferase activity in the skin was also quantified using *N*-OH-Glu-P-1 as a substrate. As described in Table 2, considerable activities were observed with the cytosol of the hamster skin. The specific activity in the skin corresponded to 8% of the hepatic activity.

The effect of sulfhydril blocking agents on the acetyl CoA-dependent *N*-acetylation of 2-AF was examined to determine the relationship between skin and liver acetyltransferases. As described in Table 3, sulfhydril blocking agents, *p*-chloromercuribenzoate, *N*-ethylmaleinimide and iodoacetamide, inhibited acetyltransferase activity in the skin in a manner similar to that in the liver.

Species difference in cutaneous acetyl CoA-dependent N-acetylation of 2-AF

Activity of the acetyl CoA-dependent *N*-acetylation for 2-AF in the liver and the skin of hamsters was compared with those of rats and mice. Cutaneous cytosols of all three species tested had the activity for *N*-acetylation of 2-AF in the presence of acetyl CoA. As shown in Fig. 2, the activities in livers were approx. five to six times higher than those in the respective skins in hamsters and mice, while the hepatic activity in rats was only two to three times higher than that in the skin. Thus the cutaneous activity was not much different among the three species tested, although a large difference was observed in the activity of hepatic *N*-acetylation of 2-AF.

Correlation between cutaneous and hepatic activities

Cutaneous activities of three different types of acetylation, acetyl CoA-dependent *N*-acetylation of PABA and 2-AF, *N*-OH-AABP-dependent *N,N*-

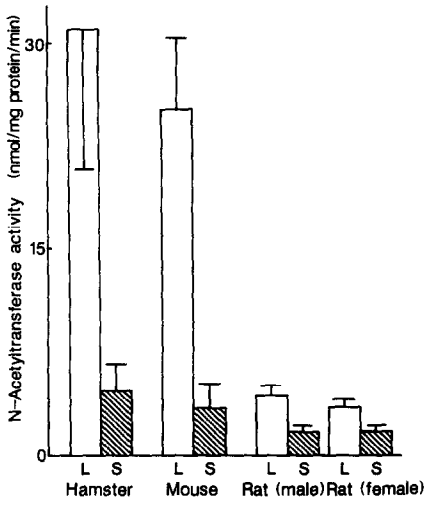

Fig. 2. Species difference in the acetyl CoA-dependent *N*-acetylation of 2-AF. Data represented are the mean value ± standard deviation of at least five determinations. L: hepatic activity; S: cutaneous activity.

acetyltransfer and acetyl CoA-dependent *O*-acetylation of *N*-OH-Glu-P-1, were examined with 19 hamsters deriving from a strain temporarily designated H_{kkl} which had been selected from some other strains by preliminary experiments. The animals were classified into three groups, slow, intermediate and rapid acetylators, according to the acetyl CoA-dependent *N*-acetyltransferase activities of PABA and 2-AF in their skins. In addition, there was a good positive correlation between their cutaneous and hepatic activities (Fig. 3a,b). On the other hand, there was no clear separation both in cutaneous and hepatic activities with *N,N*-acetyltransferase and *O*-acetyltransferase activities (Fig. 3c,d). To obtain further information on factors regulating the polymorphism, kinetical constants of the three different acetylator groups classified by acetyl CoA-dependent *N*-acetylation of PABA were determined. As shown in Fig. 4a, in *N*-acetylation of PABA, V_{max} values of slow, intermediate and rapid acetylators ranged from 0.54 to 25 nmol/mg protein/min, while K_m values for PABA were similar among all three acetylators. In contrast with *N*-acetylation of PABA, no appreciable difference was observed

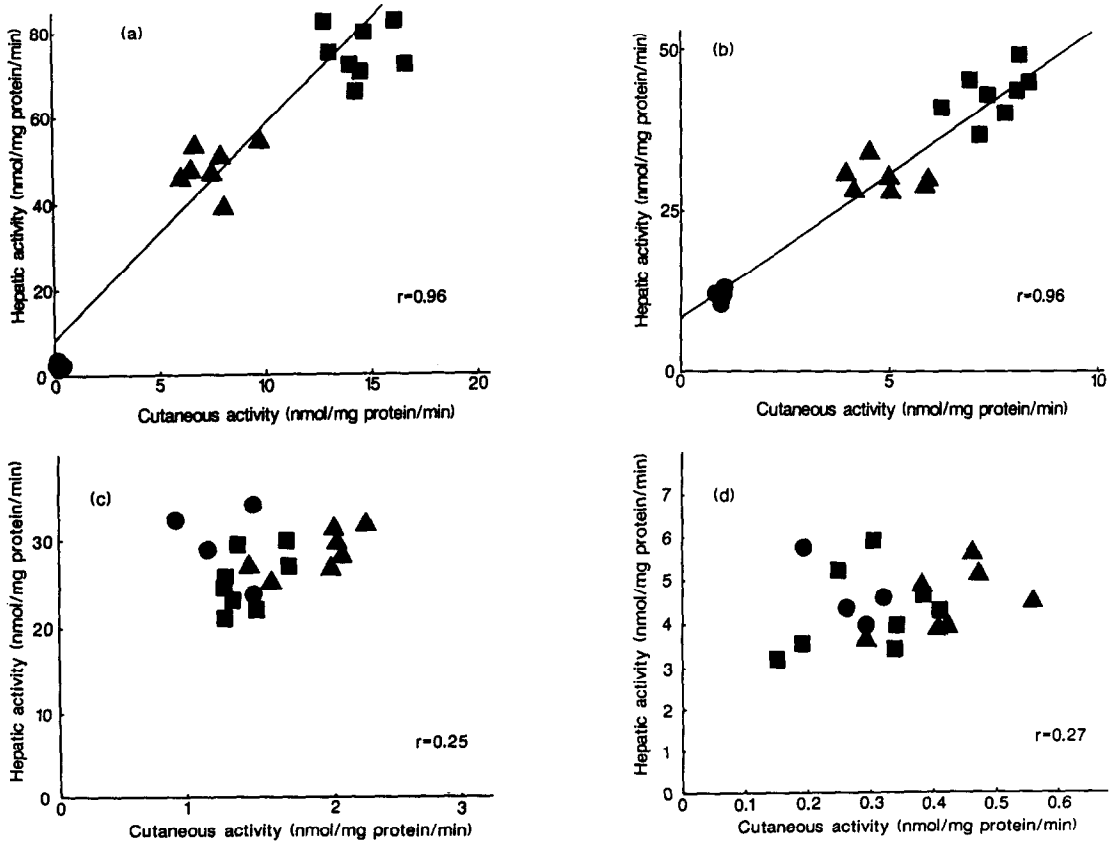

Fig. 3. Individual difference in activities of skin and liver cytosols observed in 19 hamsters. Cutaneous and hepatic activities of acetyl CoA-dependent *N*-acetylation of PABA (a) and 2-AF (b), *N*-OH-AABP-dependent *N,N*-acetyltransfer of 2-AF (c) and acetyl CoA-dependent *O*-acetylation of *N*-OH-Glu-P-1 (d) were plotted. Circle, triangle and square indicate the values of slow, intermediate and rapid acetylator of hamsters with regard to the *N*-acetylation of PABA, respectively. Experimental procedures are described in Materials and Methods.

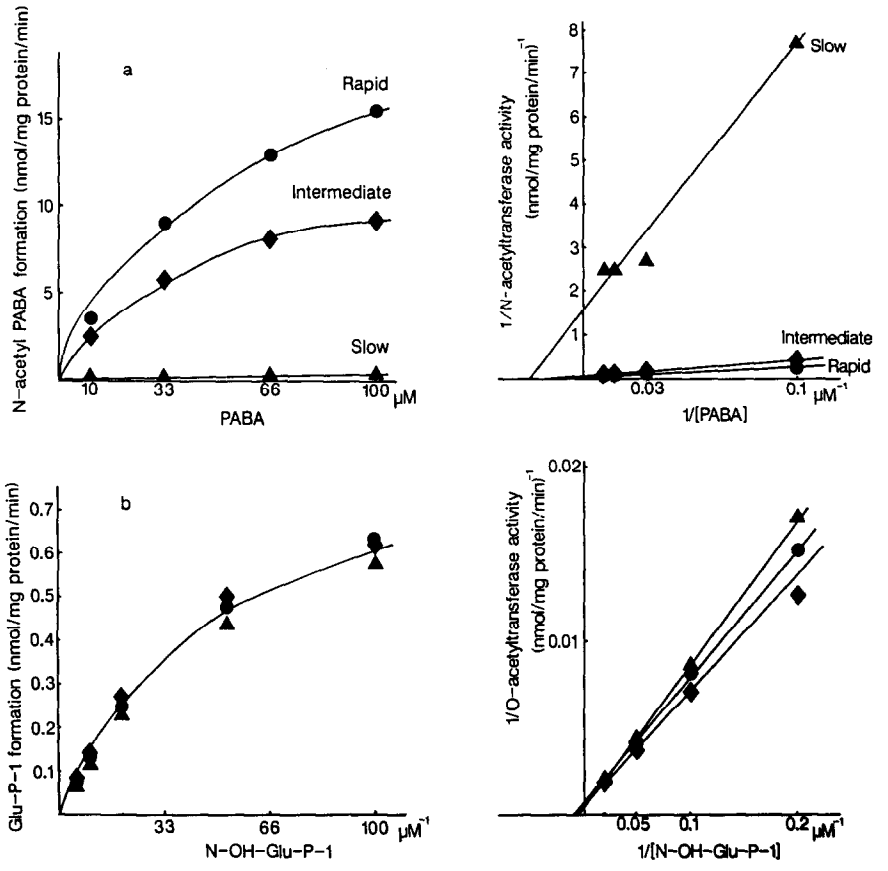

Fig. 4. Rate of acetyl CoA-dependent *N*-acetylation of PABA in hamster skin and double-reciprocal plot of concentration of PABA and the activity (a). Rate of acetyl CoA-dependent *O*-acetylation of *N*-OH-Glu-P-1 in hamster skin and double-reciprocal plot of concentration of *N*-OH-Glu-P-1 and the activity (b). Closed circles, rhombi and triangles represent rapid, intermediate and slow acetylators determined by acetyl CoA-dependent *N*-acetylation of PABA, respectively.

in V_{max} and K_m values for *O*-acetylation of *N*-OH-Glu-P-1 among the three groups in the skin (Fig. 4b).

DISCUSSION

The present study has clearly demonstrated that the skin has activity for acetylation of arylamines and *O*-acetylation of *N*-hydroxyarylamine, although the activity of *N*-acetylation of PABA was reported to be non-detectable in the rabbit skin [6]. As described in Table 2, the hamster skin showed activity for all three types of acetyltransfer reaction, the acetyl CoA-dependent *N*-acetylation of 2-AF and PABA, arylhydroxamic acid-dependent *N*,*N*-acetyltransfer and acetyl CoA-dependent *O*-acetylation, which are known to be catalyzed by hepatic cytosols. Inhibitory results with sulfhydril blocking agents indicate that both cutaneous and hepatic acetyltransferases possess functional sulfhydril group(s) in their molecules (Table 3). In addition, both cutaneous and hepatic cytosols showed similar characteristics on their acetyldonors and acetylacceptors (Fig. 1). These results indicate that skin cytosol contains the same or closely related enzyme as observed in the liver, although we have still not identified the enzyme(s) in both organs. The ratio of the cutaneous activity to the hepatic, however, varied with reactions, suggesting that the distribution of the enzymes in both organs was not equal among reac-

Table 3. Effect of sulfhydril blocking agents on the acetyl CoA-dependent *N*-acetylation of 2-AF

Inhibitors	(μM)	Liver*	Skin*
Complete system†	—	100[a]	100[b]
p-Chloromercuribenzoate	10	4	7
	100	1	4
	1000	0.2	3
N-Ethylmaleinimide	10	94	95
	100	83	90
	1000	31	35
Iodoacetamide	10	1.8	4.9
	100	0.8	4.1
	1000	0.1	1.2

* Figures are represented as % of activity compared with the complete system.

† The incubation mixture contained 50 mM Tris–HCl (pH 7.0), 1 mM DTT, 1 mM acetyl CoA, 10 μg protein of hamster skin or liver cytosol and 100 μM 2-AF in a final volume of 100 μl. Sulfhydryl blocking agents were added to the complete system.

[a] Corresponds to 53.9 nmol/mg protein/min.

[b] Corresponds to 5.1 nmol/mg protein/min.

tions tested. Assuming that the activity of cutaneous acetyltransferase is distributed homogeneously in the whole skin (*ca.* 130 cm^2), total cutaneous activities are calculated to be 9, 12, 5 and 4% of corresponding total hepatic activities for acetyl CoA-dependent *N*-acetylation of 2-AF and PABA, acetyl CoA-dependent *O*-acetylation of *N*-OH-Glu-P-1 and arylhydroxamic acid-dependent *N*-acetyltransfer of 2-AF in the hamster. Skin is an organ of direct and continuing contact with numerous potential hazardous chemicals. Therefore some chemicals including carcinogenic arylamines may be metabolized and activated through acetylation in the skin. Preliminary experiments using a human skin cytosol showed considerable arylamine *N*-acetylation activity at a level corresponding to that in rat liver. Thus, these results suggest the possibility that the skin plays a substantial role in the activation and inactivation of carcinogenic arylamines in some species including humans.

As shown in Fig. 3, a clear polymorphism which divides activities into three groups was seen in the cutaneous activities of acetyl CoA-dependent *N*-acetylation of PABA and 2-AF. This phenomenon in the skin coincides with that in the liver (Fig. 3a,b). The activities of hepatic PABA acetylation were reported to show genetic polymorphism among several strains, such as Bio. 1.5., Bio. 4.24., Bio. 41.56. and Bio. 65.67. [15]. Therefore, genetic regulation may reflect also on cutaneous *N*-acetyltransferase activities and yield the difference in cutaneous sensitivity to arylamines, especially carcinogens, although it remains unclear whether the hamsters we used in this study and the strains reported by Hein *et al.* [15] are regulated by the same genetic mechanism. Further studies on this point will be needed. It has been reported that *N*-OH-AABP-dependent *N,N*-acetyltransfer of 2-AF with hamster liver cytosol is monomorphic [16]. Indeed, monomorphic distribution of activities was observed in the skin as well as the liver (Fig. 3c), indicating that these monomorphic acetyltransfers in both organs may be controlled by the same regulation.

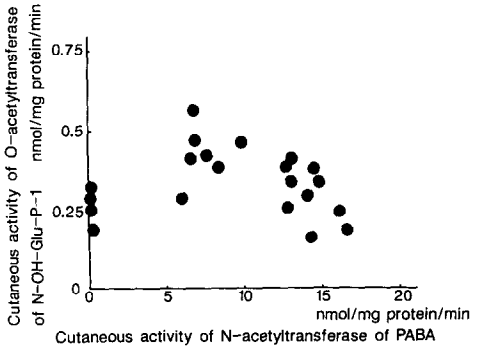

Fig. 5. Correlation between cutaneous activities of acetyl CoA-dependent *N*-acetylation of PABA and *O*-acetylation of *N*-OH-Glu-P-1. Each plot represents one individual animal. Experimental details are described in Materials and Methods.

With a polymorphic substrate, PABA, three different V_{max} values were obtained according to polymorphism, while similar K_m values were observed (Fig. 4a). These results may indicate that the polymorphism observed in *N*-acetylation of PABA with hamster skin cytosols is due to the quantitative difference in the amount of the acetyltransferase. Similar studies were also performed on hepatic activity in rabbits by Andres and Weber [17]. In contrast, there seems to be monomorphism both in the cutaneous and hepatic *O*-acetylation of *N*-OH-Glu-P-1 (Fig. 3d). Little difference in V_{max} and K_m values with this reaction was observed (Fig. 4b), and the clear separation of the activity as seen in the *N*-acetylation of PABA was not observed in the *O*-acetylation of *N*-OH-Glu-P-1 in the hamster skin (Fig. 5). Therefore, this reaction is considered to be monomorphic in the skin.

In conclusion, we have demonstrated in this study that the skin may play an important role in metabolic activation of carcinogenic arylamines and that cutaneous acetylation in hamsters may be under a common genetic control which regulates the individual difference in hepatic activities. Further study on whether skin may be an accessible organ for determination of acetylation phenotype is now in progress.

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