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The Effects of Vitamin E on Arylamine *N*-Acetyltransferase Activity in Strains of *Helicobacter pylori* From Peptic Ulcer Patients

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Abstract—Arylamine *N*-acetyltransferase (NAT) activities with 2-aminofluorene (2-AF) and *p*-aminobenzoic acid (PABA) were determined in the bacterium *Helicobacter pylori*. Cytosols or suspensions of *H. pylori,* with or without specific concentrations of vitamin E co-treatment, showed different percentages of 2-AF acetylation. The data indicated that there was increased NAT activity associated with increased levels of vitamin E in *H. pylori* cytosols and intact bacteria. For the cytosol and intact bacteria examinations, the apparent values of K_m and V_{max} were increased when vitamin E was added to the reaction mixtures for 2-AF and PABA acetylation, respectively. This report is the first demonstration to show that antioxidant agents (vitamin E) can promote *H. pylori N*-acetyltransferase activity.

Keywords: vitamin E; 2-aminofluorene; *N*-acetyl-2-aminofluorene; *p*-aminobenzoic acid; *N*-acetyl-*p*-aminobenzoic acid; *N*-acetyltransferase.

Abbreviations: 2-AAF = 2-acetylaminofluorene; 2-AF = 2-aminofluorene; EDTA = ethylenediaminetetraacetic acid; BSA = bovine serum albimin; DMSO = dimethyl sulfoxide; DTT = dithiothreitol; CFU = colony-forming units; OD = optical density; PMSF = phenylmethylsulfonylfluoride; PABA = *p*-aminobenzoic acid; N-Ac-PABA = acetyl-*p*-aminobenzoic acid; NAT = *N*-acetyltransferase.

INTRODUCTION

Exposure to environmental and occupational chemicals is recognized as an important cause of chemical carcinogenesis. *N*-acetylation is thought to play an important role in arylamine metabolism. Two *N*-acetyltransferase (NAT) enzymes have been identified and isolated from mammalian tissues, designated NAT1 and NAT2, catalyse *N*-acetylation of various arylamines (Hein *et al*., 1991; Minchin *et al*., 1992; Weber, 1987). NAT2, and most recently NAT1, has been shown to be a polymorphic enzyme that segregates individuals into rapid and slow acetylator phenotypes (Vatsis and Weber, 1993; Weber and Hein, 1985). For humans, there are statistical and epidemiological studies linking rapid acetylation to increased risks of colorectal cancer (Ilett *et al*., 1987; Lang *et al*., 1987; Roberts-Thomson *et al*., 1996) and slow acetylation to increased risks of bladder cancer (Cartwright *et al*., 1982). Individual genetic variations in humans for the regulation of the *NAT* loci have been implicated as important factors in determining susceptibility to environmental and occupational arylamine carcinogens (Lang *et al*., 1987; Weber and Hein, 1985). Recently, other investigators have demonstrated that intestinal microflora plays an important role in the formation of *N*-acetyl derivatives from arylamines in dogs (Okumu *et al*., 1995). Thus, it is suspected that normal flora in the human digestive system may be involved in the metabolism of arylamines.

Colonization of the human gastric mucosa by *Helicobacter pylori* was discovered in 1983 and, in addition to HCl secretion, is a major contributor to the development of peptic ulcer disease (Goodwin and Carrick, 1991; Marshall, 1983; Graham, 1989). *H. pylori* has already been shown to survive and grow in the stomach of humans. Recently, there has been increasing evidence that persistent infections

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with *H. pylori* can be a risk factor for the development of gastric adenocarcinoma (Blaser and Parsonnet, 1994; Correa, 1991). Other investigators have demonstrated that chronic atrophic gastritis is a precursor lesion of gastric cancer, and chronic gastritis might be induced or exacerbated by *H. pylori* (Dixon, 1989; Kato *et al*., 1992; Marshall, 1986). Evidence has also shown that gastritis, peptic ulcer, and gastric cancer are related to the presence of this bacterium (Marshall *et al*., 1988).

Vitamin E (α-tocopherol) is an antioxidant and acts against some cancers, including inhibition of chemical carcinogenesis in some animal studies (Byers and Perry, 1992; Dorgan and Schatzkin, 1991; Odukoya *et al*., 1984). The possible role of vitamin E in the enhancement or inhibition of colon tumours in mice has also been reported (Dorgan and Schatzkin, 1991). In addition to its antioxidant property, vitamin E influences cellular responses to oxidative stress through modulation of signal transduction pathways (Azzi *et al*., 1992). Vitamin E has been found to inhibit *in vitro* brain protein kinase C activity (Mathonery and Azzi, 1989). Vitamin E protected human skin fibroblasts against the cytotoxic effects of UV in tissue culture (Kondo *et al*., 1990). But other reports have demonstrated that rats or mice with intestinal tumours induced by repeated doses of 1,2-dimethylhydrazine showed higher tumour incidence when fed a diet with added vitamin E as compared with a vitamin E-deficient diet (McIntosh, 1992; Temple and El-Khatib, 1987). There is no available information about vitamin E affecting bacterial NAT activity. Thus, the present study was focused on the effects of vitamin E on NAT activity in *H. pylori*.

MATERIALS AND METHODS

Chemicals and reagents

Vitamin E (α-tocopherol), ethylenediaminetetraacetic acid (EDTA), 2-aminofluorene (2-AF), 2-acetylaminofluorene (2-AAF), *p*-aminobenzoic acid (PABA), acetyl-*p*-aminobenzoic acid (N-Ac-PABA), carnitine acetyltransferase, Tris, leupeptin, bovine serum albumin (BSA), phenylmethylsulfonylfluoride (PMSF), acetyl carnitine, dithiothreitol (DTT) and acetyl-coenzyme A were obtained from Sigma Chemical Co. (St Louis. MO, USA). Acetonitrile, dimethyl sulfoxide (DMSO) and potassium phosphates were obtained from Merck Co (Darmastadt, Germany). All of the chemicals used were reagent grade. The Bio-Rad protein assay kit was obtained from Bio-Rad Laboratories (Hercules, CA, USA).

Helicobacter pylori

H. pylori was clinically isolated from patients who visited the department of medicine (China Medical College) for endoscopy, as described in previous studies Chung *et al*. (1997). The optical density (OD) of the intact bacterial cell suspensions was determined in triplicate by using a Beckman Spectrophotometer DU 6401. The colony-forming units (CFU) were derived from a standard curve correlating OD. with plate counts.

Preparation of bacterial cytosols

About 10×10^{10} bacterial colony forming units (CFU) were washed twice in cold phosphate buffered saline then placed immediately in 1 ml lysis buffer [20 mM Tris–HCl, pH 7.5 (at 4°C), 1 mM DTT, 1 mM EDTA, 50 μM PMSF and 10 μM leupeptin]. Then the cell suspensions were disrupted in a sonicator following by centrifugation for 30 min at 10,000 ***g***. The supernatant was kept on ice until assayed for NAT activity.

NAT activity determination

The determination of Ac-CoA-dependent *N*-acetylation of 2-AF were as described by Chung *et al*. (1997). Incubation mixtures in the assay system consisted of a total volume of 90 μl: tissue cytosol, diluted as required in 50 μl lysis buffer (20 mM Tris–HCl, pH 7.5, 1 mM DTT, 1 mM EDTA, 1 mM acetyl carnitine) and 2-AF or PABA at known concentrations for substrate. The reactions were started by addition of 20 μl AcCoA. The controls had 20 μl water added in place of AcCoA. The final concentration of PABA or 2-AF was 0.1 mM, and that of AcCoA was 0.5 mM. The reaction mixtures were incubated at 37°C for 10 min and stopped with 50 μl 20% trichloroacetic acid for PABA reactions and 100 μl acetonitrile for 2-AF reactions. All experimental reactions and controls were run in triplicate. The amounts of acetylated product and remaining non-acetylated substrate were determined by HPLC (Chung *et al*., 1997). An aliquot of the NAT reaction mixtures was injected onto a C18 reversed-phase column (Spherisorb 4.6×250 nm) of a Beckman HPLC (pump 168 and detector 126), and eluted at a flow rate of 1.2 ml/min. For PABA and N-Ac-PABA, the solvent system was 50 mM acetic acid–CH_3CN (86:14) with detection at 266 nm. The retention time of PABA was 4.0 min and that of N-Ac-PABA was 6.5 min. For 2-AF and 2-AAF, the solvent system was 20 mM KH_2PO_4, pH 4.5/CH_3CN (53:47) with detection at 280 nm. The retention time was 6.5 min for AAF and 9 min for AF. All compounds were quantitated by comparison of the integrated area of the elution peak with that of known amounts of standards. NAT activity is expressed as nmol acetylated per min per mg of cytosolic protein.

Protein determination

Protein concentrations in the cytosols of *H. pylori* were determined by the method of Bradford (1976) with BSA as the standard. All of the samples were assayed in triplicate.

Table 1. Effects of vitamin E on *H. pylori* NAT activity in cytosols

Vitamin E concn	AAF (nmol/min/mg protein	*N*-Acetyl-PABA (nmol/min/mg protein)
Control	0.91 ± 0.12	0.75 ± 0.08
0.04 mM	0.96 ± 0.12	0.80 ± 0.10
0.4 mM	[a]1.18 ± 0.16	[a]0.92 ± 0.11
4 mM	[b]1.46 ± 0.28	[b]1.18 ± 0.16
40 mM	[c]1.64 ± 0.22	[c]1.29 ± 0.24
400 mM	[d]1.70 ± 0.25	[d]1.32 ± 0.28

H. pylori cytosol was incubated in the presence of various concentrations of vitamin E (0.04, 0.4, 4, 40 and 400 mM). The NAT activity was determined as described in Materials and Methods. Values are means $\pm$ SD of three individual experiments. [a]Differs between 0.4 mM vitamin E and control; $P < 0.05$. [b]Differs between 4 mM vitamin E and control; $P < 0.01$. [c]Differs between 40 mM vitamin E and control; $P < 0.005$. [d]Differs between 400 mM vitamin E and control; $P < 0.001$.

Table 2. Effect of vitamin E on *H. pylori* NAT activity in intact bacteria

Vitamin E concn	AAF (nmol/min/mg protein)	*N*-Acetyl-PABA (nmol/min/mg protein)
Control	0.75 ± 0.14	0.68 ± 0.10
0.04 mM	0.80 ± 0.12	0.71 ± 0.12
0.4 mM	[a]0.88 ± 0.11	[a]0.80 ± 0.09
4 mM	[b]0.96 ± 0.20	[b]0.89 ± 0.14
40 mM	[c]1.07 ± 0.23	[c]1.01 ± 0.22
400 mM	[d]1.12 ± 0.27	[d]1.07 ± 0.24

H. pylori was incubated in the presence of various concentrations of vitamin E (0.04, 0.4, 4, 40 and 400 mM). The NAT activity was determined as described in Materials and Methods. Values are means $\pm$ SD of three individual experiments. [a]Differs between 0.4 mM vitamin E and control; $P < 0.05$. [b]Differs between 4 mM vitamin E and control; $P < 0.01$. [c]Differs between 40 mM vitamin E and control; $P < 0.005$. [d]Differs between 400 mM vitamin E and control; $P < 0.001$.

Time-course study measuring the effects of vitamin E on NAT activity in H. pylori

The reaction mixtures (containing 0.087 mM 2-AF) contained 3×10^9 cells in the presence or absence of vitamin E (40 mM, which is a normal concentration in humans) were incubated at 37°C for 0.25, 1, 2 and 4 hr, respectively. The NAT activity was assayed as described above.

Effects of various concentrations of vitamin E on NAT activity of H. pylori

Vitamin E was dissolved in DMSO at various concentrations ranging from 0.04 to 400 mM. The reaction mixtures consisted of 50 ml cytosols diluted as required, 20 ml recycling mixture containing 2-AF or PABA at selected concentration for substrates, and 10 μl vitamin E (at selected concentration). The reactions were started by addition of 20 μl Ac-CoA. The control reactions had 20 μl distilled water in place of Ac-CoA. Following these steps, the NAT determination was determined as described above for the effects of vitamin E on NAT activity in *H. pylori* (Chung *et al*., 1997).

Effects of vitamin E on the kinetic constants of NAT from H. pylori

Cytosols of *H. pylori* were co-treated with or without a specific concentration of vitamin E (40 mM) and the NAT activity determined as described above. All of the reactions were run in triplicate. For the intact bacterial studies, 3×10^9 bacterial cells in trypticase soy broth were incubated with arylamine as the substrate (22, 45, 67.5, 90, 112.5, 135 mM 2-AF or PABA) with or without a specific concentration of vitamin E (40 mM) for 96 hr at 37°C in an aerobic atmosphere (5% O_2, 10% CO_2, 85% N_2). At the conclusion of incubation, the cells and media were removed and centrifuged. For the experiments with 2-AF, the supernatant was immediately extracted with ethylacetate–methanol (95:5), the solvent evaporated, and the residue redissolved in methanol, and assayed by HPLC. For the experiments with PABA, the supernatant was direct assayed by HPLC (Chung *et al*., 1997). All of the samples were run in triplicate.

Statistical analysis

Statistical analysis of the data was performed with an unpaired Students *t*-test. The kinetic constants were calculated with the Cleland HYPER Program (Cleland, 1967) that performs linear regression using a least-squares method. The velocity 1/V) versus substrate (1/S) data were linearized by plotting (1/S) *v*. (1/V).

RESULTS

The possible effects of vitamin E on NAT activity in *H. pylo*ri in cytosols and intact bacteria were examined by HPLC assessing the percentage of acetylation of 2-AF and PABA. Cytosols or suspensions of *H. pylori*, with or without specific concentrations of vitamin E co-treatment, showed different percentages of 2-AF and PABA acetylation. A comparison of the relative cytosolic and intact bacterial NAT activity, with or without specific concentrations of vitamin E, are presented in Tables 1 and 2. The data indicated that there was increased NAT

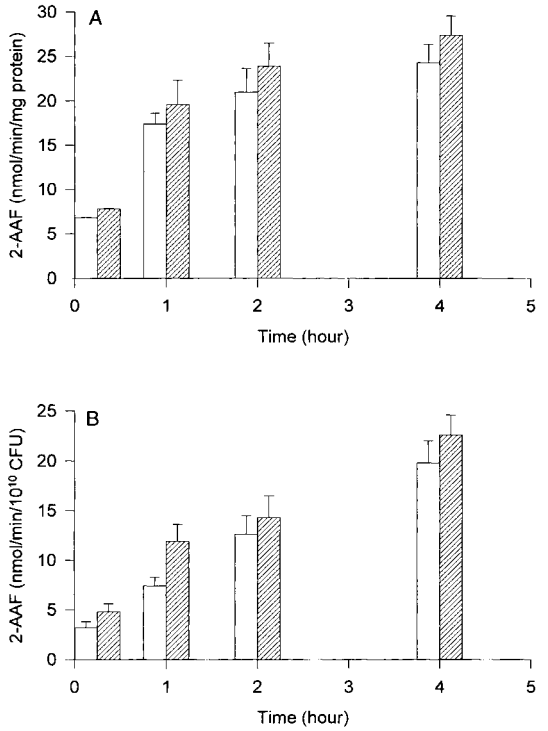

Fig. 1. Time-course effects of 40 mM vitamin E on NAT activity in *H. pylori*. Reaction mixtures (with or without 40 mM vitamin E) [(A) for cytosol examination and (B) for intact bacteria examination] were incubated at 37°C and assayed at 0.25, 1, 2 and 4 hr, respectively. Time-course effects were determined as described in Materials and Methods. Open bar = control; Shaded bar = vitamin E-treated.

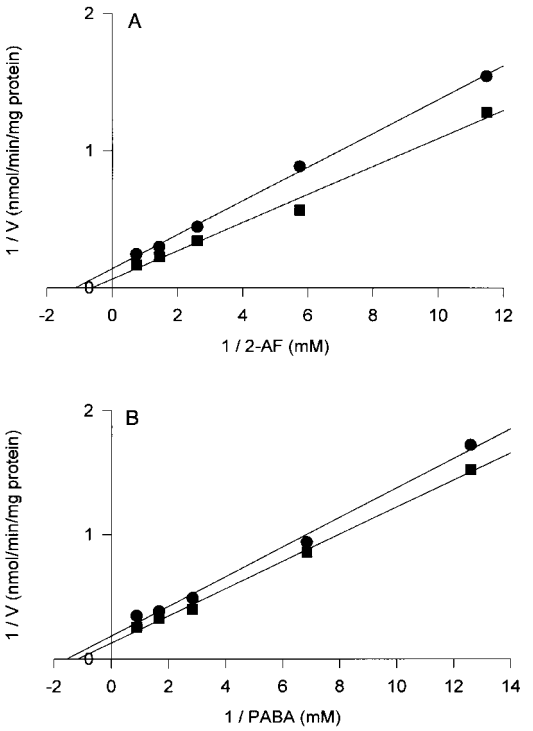

Fig. 2. Lineweaver–Burk double reciprocal plot of NAT in *H. pylori* by cytosol examination. Recycling mixtures containing 0.087, 0.174, 0.384, 0.696 and 1.392 mM 2-AF (A) and PABA (B) were used. Cytosols were prepared as described in Materials and Methods. Lineweaver–Burk double reciprocal plots were measured by using linear regression analysis of reciprocal substrate concentrations plotted against reciprocal initial velocities. ■ = control. ● = vitamin E-treated.

activity associated with increased vitamin E levels in cytosols and intact bacteria, that is, the higher the concentration of vitamin E in the reaction mixtures, the higher the promotion of NAT activity.

To determine the time-course effect of 40 mM vitamin E on NAT activity both in cytosols and intact bacteria, reaction mixtures with or without 40 mM vitamin E were incubated at 37°C and assayed at different time intervals. The results are shown in Fig. 1(a, b). For the cytosol examination, the amounts of acetylated product increased $6.0 \pm 0.8\%$, $8.4 \pm 0.9\%$, $9.6 \pm 0.7\%$, $12.2 \pm 0.8\%$ for the 0.25, 1, 2 and 4-hr incubations for 2-AF acetylation, respectively. For the intact bacteria examination, the amounts of acetylated product increased $16.8 \pm 2.2\%$, $28.4 \pm 6.1\%$, $19.6 \pm 1.4\%$ and $24.8 \pm 2.6\%$ for the 0.25, 1, 2 and 4-hr incubations for 2-AF acetylation, respectively. Similar results are found in PABA, which is used as substrate in cytosol and intact bacteria examinations (data not shown).

The kinetic constants determined for *H. pylori* NAT using 2-AF and PABA as substrates with or without 40 mM vitamin E are shown in Figs 2 and 3 and given in Tables 3 and 4. For the cytosol examination, the apparent values of K_m and V_{max} were 0.90 ± 0.09 mM and 5.18 ± 0.28 nmol/min/mg protein, respectively, for 2-AF, and 0.69 ± 0.07 mM and 4.33 ± 0.27 nmol/min/mg protein, respectively, for PABA. However, when vitamin E was added to the reaction mixture, the apparent values of K_m and V_{max} were increased 1.0- and 0.72-fold, respectively, for 2-AF and 0.32- and 0.51-fold, respectively, for PABA. For the intact bacteria examination, the apparent values of K_m and V_{max} were 0.70 ± 0.07 mM and 3.12 ± 0.26 nmol/min/10×10^{10} CFU, respectively, for 2-AF, 0.45 ± 0.06 mM and 1.17 ± 0.09 nmol/min/10×10^{10} CFU, respectively, for PABA. However, when vitamin E was added to the reaction mixture, the apparent values of K_m and V_{max} were increased 0.22- and 0.34-fold, respectively, for 2-AF, and 0.24-and 0.13-fold, respectively, for PABA.

DISCUSSION

There are many events which are likely to be factors for the observed effects of vitamin E on NAT activity in *H. pylori*. First, the NAT enzyme exists in many kinds of experimental animals including humans, and NAT has been shown to be involved in metabolism of arylamine carcinogens (Grant *et al.*, 1992; Minchin *et al.*, 1992; Weber, 1987).

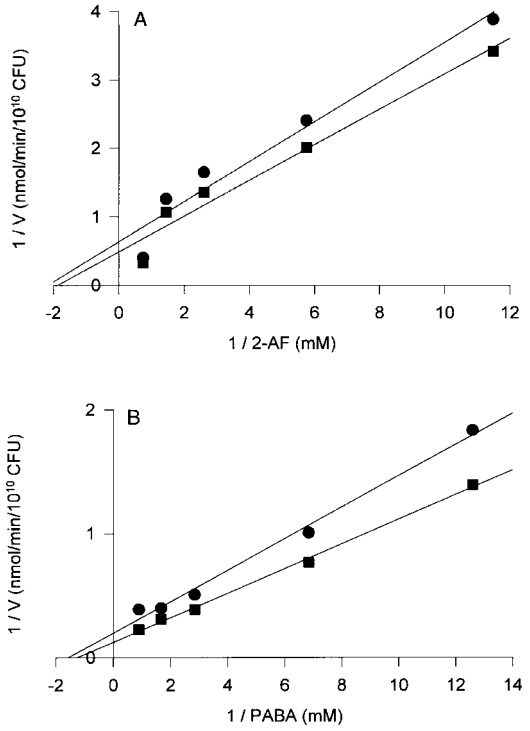

Fig. 3. Lineweaver–Burk double reciprocal plot of NAT in *H. pylori* by intact bacteria examination. Recycling mixtures containing 0.087, 0.174, 0.384, 0.696 and 1.392 mM 2-AF (A) and PABA (B) were used. Intact bacteria were prepared as described in Materials and Methods. Lineweaver–Burk double reciprocal plots were measured by using linear regression analysis of reciprocal substrate concentrations plotted against reciprocal initial velocities. ■ = control. ● = vitamin E-treated.

Secondly, some enzymes of enteric bacteria are known to contribute to the metabolic activation of chemical carcinogens in animal studies (Kinouchi *et al*., 1993; Larsen, 1988). Thirdly, it has been reported that several species of dog intestinal bacteria exhibit *N*-acetylation activities towards arylamines in varing degrees (Okumura *et al*., 1995). Fourthly, earlier studies demonstrated that *H. pylori* contains NAT activity (Chung *et al*., 1997). Recently, the present author's data also showed that vitamin C promotes NAT activity in *Klebsiella pneumoniae*. Fifthly, other investigators have already demonstrated that attenuation of NAT activity is associated with several disease processes (Weber, 1987; Weber and Hein, 1985). There are no available reports that address vitamin E effects on NAT activity in *H. pylori*. Thus, the present studies were focused on the effects of vitamin E on NAT activity in *H. pylori* cytosols and intact bacteria.

According to other reports, the microflora may be involved in metabolism of chemical compounds in human or animal intestine. The intestinal microflora in mice have been reported to play an important role on absorption and metabolic activation of 1-nitropyrene (Kinouchi *et al*., 1993). But in humans, 1-nitropyrene can be metabolized to 1-aminopyrene and *N*-formyl-1-aminopyrene by human microflora (Manning, 1986), and the acetyltransferase enzyme of human enteric bacteria may contribute to the metabolic activation of 2-amino-3-methylimidazo[4,5-*f*]quinoline (Watanabe *et al*., 1992). Other investigators have demonstrated that some anaerobic and facultative bacteria normally associated with the mammalian intestine could perform *N*-acetylation, as well as *N*-formation, of arylamine (Okumu *et al*., 1995). The nature of the bacterial enzyme responsible for *N*-formation remains unknown. Thus, *H. pylori* may be involved in the metabolic activation or detoxification of the arylamine carcinogen in humans, because the present author's earlier study demonstrated that *H. pylori* could acetylate 2-AF and PABA *in vitro* and intact bacteria cells (Chung *et al*., 1997).

Vitamin E has been widely studied for its effects on biological functions. The data presented here clearly demonstrated that vitamin E did affect *H. pylori* NAT activity, in concentrations from 0.004 to 400 mM in cytosols and in intact bacteria examinations. Vitamin E did increase the formation of acetylated products of 2-AF and PABA by *H. pylori*. The results show that vitamin E had a dose-dependent effect on the NAT activity in *H. pylori*, that is, the higher the concentration of vitamin E, the higher the promotion of NAT activity. The promotion of vitamin E to the acetylation of 2-AF is slightly higher than with PABA. Substrate specificities for NATs are different, but overlapping. For example, in humans, NAT1 acetylates PABA and *p*-aminosalicyclic acid, whereas, NAT2 does not. NAT2 acetylates sulfamethazine, whereas, NAT1 shows negligible activity towards this substrate. However, both enzymes show significant activity with carcinogenic arylamines, such as 2-AF. Although several intestinal bacteria show different

Table 3. Kinetic data for acetylator of 2-AF in *H. pylori*

	Cytosol		Intact bacteria	
	K_m (mM)	V_{max}(nmol/min/mg)	K_m(mM)	V_{max}(nmol/10 × 10^{10}CFU)
Control	0.90 ± 0.09	5.18 ± 0.28	0.70 ± 0.07	3.12 ± 0.26
Vitamin E	[a]1.82 ± 0.27	[b]8.69 ± 0.35	[b]0.85 ± 0.08	[d]4.16 ± 0.28

Values are means ± SD of three independent experiments. The acetyl CoA and vitamin E concentrations were 0.1 mM and 40 mM, respectively. The kinetic constants were calculated from the modified HYPER Program of Cleland (1967). [a]Differs between 40 mM vitamin E and control; $P < 0.001$. [b]Differs between 40 mM vitamin E and control; $P < 0.05$.

Table 4. Kinetic data for acetylator of PABA in *H. pylori*

	Cytosol		Intact bacteria	
	K_m(mM)	V_{max}(nmol/min/mg)	K_m(mM)	V_{max}(nmol/10×10^{10}CFU)
Control	0.69 ± 0.07	4.33 ± 0.27	0.45 ± 0.06	1.17 ± 0.09
Vitamin E	[a]0.90 ± 0.08	[b]6.53 ± 0.22	[b]0.56 ± 0.08	[d]1.33 ± 0.12

Values are means $\pm$ SD of three independent experiments. The acetyl CoA and vitamin E concentrations were 0.1 mM and 40 mM, respectively. The kinetic constants were calculated from the modified HYPER Program of Cleland (1967).[a]Differs between 40 mM vitamin E and control; $P < 0.01$.[b]Differs between 40 mM vitamin E and control; $P < 0.05$

N-acetylation activities for 2-AF and PABA, most of these bacterial species show higher activity for acetylation of 2-AF than for PABA. The difference between these two types of activity may be due to bacterial species variations for NAT activity. Currently, there are no available reports that demonstrate whether bacterial NAT belongs to NAT1 or NAT2, and no bacterial NAT sequences have been reported, although several reports have demonstrated that bacteria contain NAT activity using arylamine as the substrate.

The results in the time-course study showed that the promotion rates of vitamin E slightly increases as the incubation time continues. There are two reasons for choosing 40 mM vitamin E for the reported examinations. First, NAT activity in *H. pylori* was promoted about 50% after co-treated with vitamin E. Secondly, the amounts of vitamin E (40 mM) was close to the normal concentration (12–35 mM) in human (Stuart, 1982). As vitamin E did promote NAT activity in *H. pylori*, the kinetic constants were also affected. For the cytosol examinations, co-treatment with vitamin E indicated that the apparent values of K_m and V_{max} increased 0.22- and 0.54-fold for 2-AF, and 0.26- and 0.58-fold for acetylation of PABA. For the intact bacteria examination, co-treatment with vitamin E indicated that the apparent values of K_m and V_{max} increased 0.10- and 0.44- fold for acetylation of 2-AF, 0.12- and 0.49-fold for acetylation of PABA. The results from kinetic studies showed that vitamin E increased the apparent K_m and V_{max} values in both assay systems. These results suggest that vitamin E promotes the *N*-acetylation of both substrates at high exposure levels.

Vitamin E has been demonstrated to inhibit chemical carcinogenesis in some but not all animal studies. Both inhibition and enhancement of colon tumours in mice have been reported (Linder, 1985). Vitamin E increases the rate of substrate acetylation by NAT, and it may or may not increase the risk of tumour formation. It may depend on whether the host was exposed to the arylamine carcinogens. If the host was exposed to the arylamine carcinogen then the increased NAT activity may lead to the increased risk of tumour formation. Other reports have demonstrated that elevated levels of NAT activity may be associated with increased sensitivity to the mutagenic effects of many arylamines (Einisto *et al*., 1991). Other studies have demonstrated that attenuation of NAT activity is associated with several disease processes (Weber, 1987; Weber and Hein, 1985). This needs further investigation. Four patients were offered orally vitamin E 400 (IU/soft gells twice per day for 4 days), followed by the isolation of the bacteria then *H. pylori* were examined *in vitro* for NAT activity. The results demonstrated that vitamin E did increase NAT activity in two patients ($P < 0.05$) (data not shown), but two strains isolated from another two patients did not show significant differences between the vitamin E treated group and the group that was not treated with vitamin E. Humans may take vitamin E as a dietary supplement and may be exposed simultaneously to arylamine carcinogens through food consumption (Wakabayashi *et al*., 1992); further investigations are needed to determine the enhancing effect of vitamin E on *H. pylori* NAT activity. Vitamin E could promote the metabolism of arylamine carcinogens in humans. Therefore, this finding is very important and offers information about the role of an antioxidant agent (vitamin E) which may or may not protect 2-AF-induced carcinogenesis.

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