

Expression of sodium/proton antiporter NhaA at various pH values in *Escherichia coli*

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Abstract

It was reported that NhaA, one of sodium/proton antiporters in *Escherichia coli*, was expressed at alkaline pH [J. Biol. Chem. 266 (1991) 21753]. In disagreement with their results, expression of an *nhaA-lacZ* fusion gene was found to be very low in an *E. coli* strain derived from MC4100 within the wide pH range from 5 to 9. When *nhaB* was deleted, the fusion gene was expressed at pH values below 8, while the expression was observed at alkaline pH after *chaA* was deleted. The internal level of sodium ions was increased by deletion of *nhaA* in strains deficient in *nhaB* and *chaA* at low and high pH values, respectively. These results suggested that *nhaA* is induced only when a low level of internal sodium ions is not kept by NhaB and ChaA. Strains used in the previous study may have low active ChaA. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Na^+/H^+ ; *nhaA*; Antiporter; pH dependence; Gene expression; *Escherichia coli*

1. Introduction

Escherichia coli has multiple extrusion systems for sodium ions, NhaA and NhaB [1–4]. In addition to these systems, ChaA can extrude sodium ions [5]. ChaA was reported to be a calcium/proton antiporter [6], but its physiological role was to transport sodium ions [5]. Verkhovskaya et al. [7,8] reported a fourth system for sodium ion extrusion that was stimulated by respiration in the presence of potassium ions. However, a mutant deficient in *nhaA*, *nhaB* and *chaA* derived from the wild-type *E. coli* had no detectable activity for sodium ion extrusion [9], suggesting that the activity of the fourth system is negligible in the wild-type *E. coli*. Since the strain used by

Verkhovskaya et al. [7] was derived from a strain having low active ChaA, the fourth system might be mediated by the altered ChaA. Thus, NhaA, NhaB and ChaA seem to be the major systems for sodium ion extrusion in the wild-type *E. coli*.

To date the structure and function of NhaA have been well studied [1–4,10]. Expression of *nhaA* was observed in alkaline medium [11] and the expression was induced by sodium and lithium ions at neutral pH [11,12]. This system could be expressed in the pH range from 6 to 9 when *nhaB* was deleted [13]. NhaA had an optimum activity at alkaline pH [14–17]. These results suggest that NhaA functions in alkaline medium. However, deletion of *nhaA* had little effect on growth in the presence of sodium ions at alkaline pH [5]. Sakuma et al. [9] reported that a mutant containing only NhaA extruded sodium ions at acid pH as well as alkaline pH, suggesting that the operation of NhaA is less dependent on pH.

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In order to clarify this disagreement, we examined conditions under which *nhaA* is expressed and its function is physiologically significant. When an *E. coli* strain derived from MC4100 [18] was used, expression of an *nhaA-lacZ* fusion gene was weak at pH values from 5 to 9, even if medium contained a high level of sodium ions. The fusion gene was well expressed within the medium pH range from 5 to 9 when both *nhaB* and *chaA* were deleted.

In contrast to MC4100, the fusion gene of a strain derived from W3133-2 [19] was expressed at high pH above 7. ChaA of W3133-2 was found to be less active [5]. Since strains used for the previous studies [11,13] were derived from W3133-2 [20], *nhaA* might be expressed at alkaline pH in such strains, due to low activity of ChaA. Thus, it has been suggested that *nhaA* expression is mainly dependent on the activity of other transport systems for sodium ion extrusion, but is less affected by pH.

2. Materials and methods

2.1. *E. coli* strains and growth media

The *E. coli* strains used are listed in Table 1. Medium KLB contained 0.5% KCl, 1% tryptone, and 0.5% yeast extract. Synthetic medium M6 contained 5 mM K₂HPO₄, 20 mM NH₄Cl, 1 mM MgSO₄, 0.1 mM CaCl₂, 0.1 mM FeSO₄, 20 µg/ml thiamine, and 1% glucose. Sixty mM 2-(*N*-morpholino)ethanesulfonic acid monohydrate (MES) for media of pH below 7, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) for media of pH 7–8, and *N*-

Tris(hydroxymethyl)-methylglycine (Tricine) for media of pH above 8 were added to KLB and M6. The medium pH was adjusted by the addition of KOH. Growth was monitored by measuring the absorbance of the medium at 600 nm. Cells were cultured under conditions which allowed to grow until the absorbance reached more than 0.8, and cells used for measurements of β -galactosidase activity and the cellular amount of sodium ions were harvested at the absorbance of 0.3–0.4.

2.2. Other methods and chemicals

Measurement of β -galactosidase activity [21], determination of the sodium ion content in the cytoplasm and medium [5], and P1 transduction [22] were carried out as described previously. Miller unit [21] was used for β -galactosidase activity. Reagents used were of analytical grade.

3. Results

3.1. Expression of *nhaA* in a strain containing *nhaB* and *chaA* at various pH values

Expression of *nhaA* was weak at pH values from 5 to 9 in TS10Z grown in medium KLB (Fig. 1A). The expression was slightly increased by the addition of 0.3 M NaCl to the medium of initial pH 7.5. TS10Z was unable to grow in the media of initial pH 7.5 containing 0.6 M NaCl and pH 9.0 containing 0.4 M NaCl. This strain could grow in the media of initial pH 5.5 and 6.5 in the presence of 0.8 M

Table 1
Escherichia coli strains used in this study

Strains	Relevant genotype	Sources and/or refs.
MC4100	<i>araD139 Δ(lac)U169 strA thi</i>	Lab. stock [18]
RK33Z	<i>melBLid ΔnhaA3 kan⁺ ΔlacZY Φ(nhaA'-lacZ)I(hyb)</i>	E. Padan [11]
TO114	W3110 <i>nhaA::kan nhaB::em chaA::cm</i>	Lab. stock [5]
TS10Z	MC4100 <i>Φ(nhaA'-lacZ)I(hyb)</i>	This study; MC4100 X P1(RK33Z)
TS12Z	MC4100 <i>chaA::cm Φ(nhaA'-lacZ)I(hyb)</i>	This study; TS10Z X P1(TO114)
TS13Z	MC4100 <i>nhaB::em Φ(nhaA'-lacZ)I(hyb)</i>	This study; TS10Z X P1(TO114)
TS14Z	MC4100 <i>chaA::cm nhaB::em Φ(nhaA'-lacZ)I(hyb)</i>	This study; TS13Z X P1(TO114)
W3133-2	<i>Δlac</i>	T. Tsuchiya [19]
TS33Z	W3133-2 <i>Φ(nhaA'-lacZ)I(hyb)</i>	This study; W3133-2 X P1(RK33Z)

kan, gene conferring kanamycin resistance; *cm*, gene conferring chloramphenicol resistance; *em*, gene conferring erythromycin resistance.

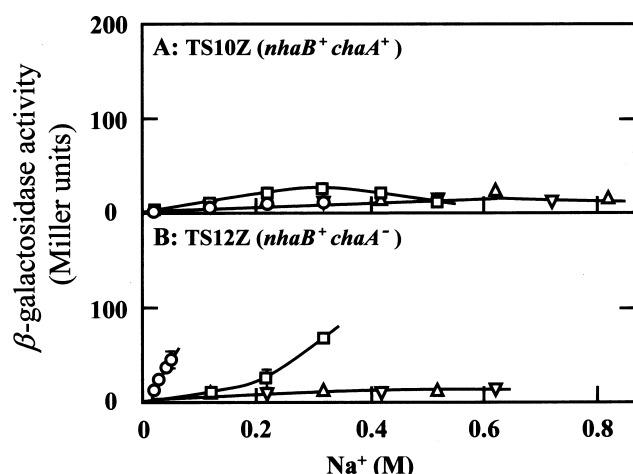


Fig. 1. Effect of *chaA* deletion on expression of the *nhaA-lacZ* fusion gene at various pH values. TS10Z (*nhaA-lacZ nhaA*⁻ *nhaB*⁺ *chaA*⁺) and TS12Z (*nhaA-lacZ nhaA*⁻ *nhaB*⁺ *chaA*⁻) were grown in medium KLB in the presence of various amounts of NaCl. When cells were harvested at the absorbance of 0.3–0.4, medium pH values were 5.1–5.3, 6.1–6.3, 7.1–7.3 and 8.3–8.5 in media of initial pH 5.5 (Δ), 6.5 (∇), 7.5 (\square), and 9.0 (\circ), respectively. Each point represents mean \pm S.D. of four measurements, but error bars of standard deviations less than 10 Miller units are not drawn.

NaCl, but the expression was again low under these growth conditions (Fig. 1A).

3.2. Expression of *nhaA* in strains deficient in *nhaB*, *chaA*, and both *nhaB* and *chaA*

Expression of the *nhaA-lacZ* fusion gene in TS12Z grown in medium KLB was less than 20 Miller units at pH values below 7, even if 0.6 M sodium ions was present (Fig. 1B). The expression in the medium of initial pH 7.5 was stimulated by the addition of 0.3 M NaCl. TS12Z could not grow in the medium of initial pH 7.5 containing 0.4 M NaCl. The expression level at pH above 8 was again increased by the addition of NaCl (Fig. 1B). It should be noted that TS12Z was unable to grow when more than 0.03 M NaCl was added to medium KLB of initial pH 9.0.

The *nhaA-lacZ* fusion gene was well expressed at pH values below 7 in TS13Z grown in medium KLB (Fig. 2A,B). The expression decreased with the increase in medium pH (Fig. 2C,D). Expression of the *nhaA-lacZ* fusion gene in TS14Z was essentially the same as that in TS13Z, except that the expression

was stimulated by the addition of lower concentrations of NaCl at pH above 8 (Fig. 2E–H). TS14Z was unable to grow in media of initial pH 9.0 and 7.5 in the presence of more than 0.03 and 0.1 M sodium ions, respectively.

3.3. Expression of *nhaA* in minimal medium

The *nhaA-lacZ* fusion gene was well expressed in TS13Z and TS14Z without the addition of NaCl in

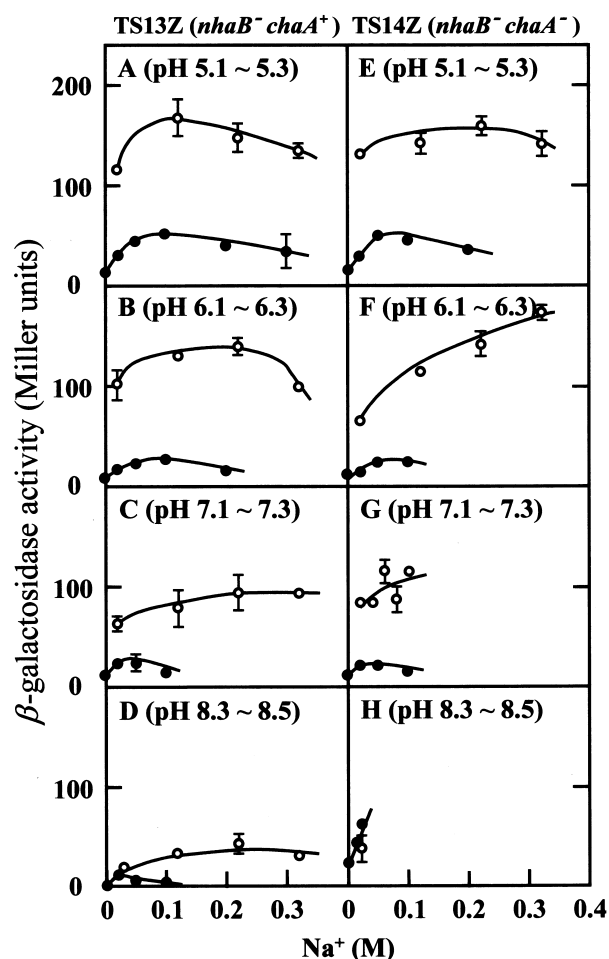


Fig. 2. Effect of *nhaB* or *nhaB* plus *chaA* deletion on expression of the *nhaA-lacZ* fusion gene at various pH values. TS13Z (*nhaA-lacZ nhaA*⁻ *nhaB*⁻ *chaA*⁺, A–D) and TS14Z (*nhaA-lacZ nhaA*⁻ *nhaB*⁻ *chaA*⁻, E–H) were grown in medium KLB (\circ) and M6 (\bullet), and harvested as described in the legend of Fig. 1. When TS14Z was grown in medium M6 of initial pH 9.0, 0.5% tryptone was added, because of a low growth rate without tryptone. Each point represents mean \pm S.D. of four measurements, but error bars of standard deviations less than 10 Miller units are not drawn.

medium KLB (Fig. 2). Since medium KLB contained 17–19 mM contaminating sodium ions, expression of the fusion gene may be induced by these contaminating ions. The expression of the *nhaA-lacZ* fusion gene was low in medium M6 containing 0.3–1.0 mM contaminating sodium ions and the expression increased at an elevated level of external sodium ions (Fig. 2). The expression levels were lower than those observed in medium KLB, but the *nhaA-lacZ* fusion gene was again expressed at acid pH in TS13Z

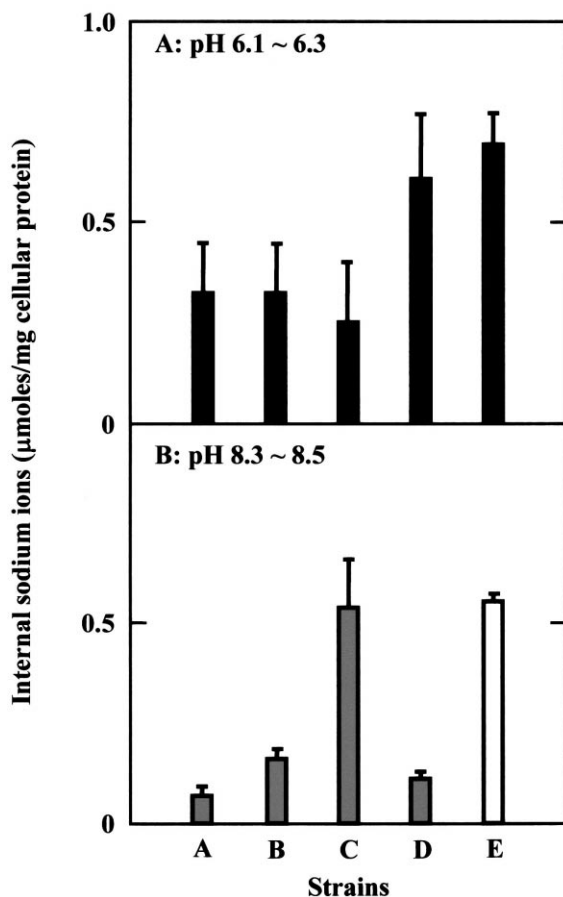


Fig. 3. The amount of internal sodium ions in various mutants. Cells grown in medium KLB of initial pH 6.5 (A) and 9.0 (B) were harvested as described in the legend of Fig. 1, and the internal amount of sodium ions was measured. Each bar represents mean \pm S.D. of four measurements. Amounts of NaCl added to medium KLB: closed bars, 0.1 M; gray bars, 0.01 M; open bars, no addition. Strains: A, MC4100; B, TS10Z (MC4100 *nhaA-lacZ* *nhaA*⁻ *nhaB*⁺ *chaA*⁺); C, TS12Z (MC4100 *nhaA-lacZ* *nhaA*⁻ *nhaB*⁺ *chaA*⁻); D, TS13Z (MC4100 *nhaA-lacZ* *nhaA*⁻ *nhaB*⁻ *chaA*⁺); E, TS14Z (MC4100 *nhaA-lacZ* *nhaA*⁻ *nhaB*⁻ *chaA*⁻).

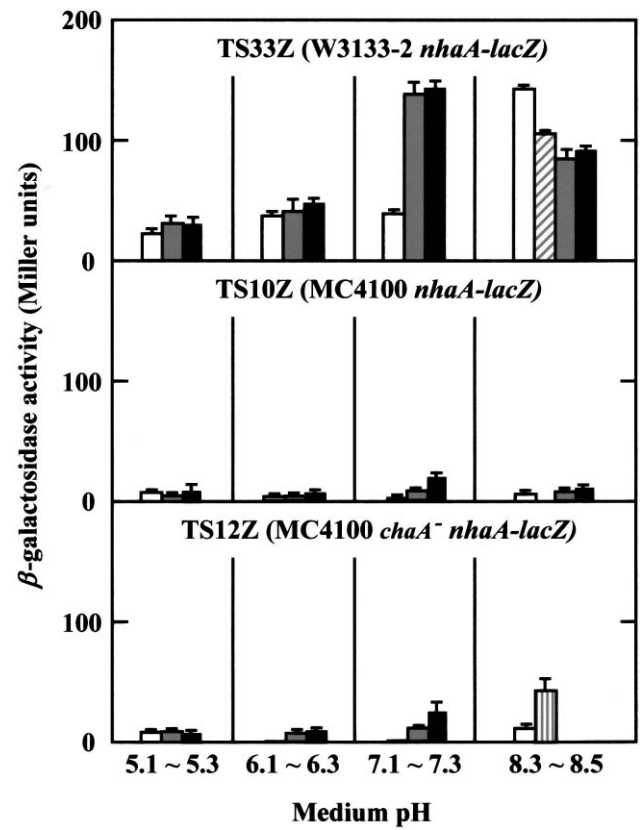


Fig. 4. Expression of the *nhaA-lacZ* fusion gene in the strains derived from W3133-2 and MC4100 at various pH values. TS33Z (W3133-2 *nhaA-lacZ* *nhaA*⁻), TS10Z (MC4100 *nhaA-lacZ* *nhaA*⁻ *nhaB*⁺ *chaA*⁺) and TS12Z (MC4100 *nhaA-lacZ* *nhaA*⁻ *nhaB*⁺ *chaA*⁻) were grown in medium KLB, and harvested as described in the legend of Fig. 1. Each bar represents mean \pm S.D. of four measurements. Amounts of NaCl added to growth medium: open bars, no addition; vertically striped bar, 0.03 M; hatched bar, 0.05 M; gray bars, 0.1 M; closed bars, 0.2 M.

(Fig. 2). TS14Z expressed the *nhaA-lacZ* fusion gene within the medium pH range from 5 to 9 (Fig. 2).

3.4. Cytoplasmic level of sodium ions in various strains

The internal level of sodium ions was almost the same in MC4100 (wild-type) and TS12Z deficient in *nhaA* and *chaA* (Fig. 3A) in acidic medium. The level was higher in strains deficient in *nhaA* and *nhaB*, TS13Z and TS14Z, at acid pH (Fig. 3A). In alkaline medium, deletion of *nhaA* or *nhaB* plus *nhaA* increased slightly the internal level of sodium ions (TS10Z and TS13Z in Fig. 3B), and the level was

high in TS12Z and TS14Z (Fig. 3B). As described above, TS14Z was unable to grow at pH above 8 when 0.01 M NaCl was added to medium KLB containing approximately 0.02 M contaminating sodium ions. These results imply that NhaA works at acid pH as well as in alkaline medium when other systems for extruding sodium ions are not operative.

3.5. Expression of *nhaA* in a strain derived from W3133-2

The *nhaA-lacZ* fusion gene was expressed in TS33Z derived from W3133-2 in medium KLB of initial pH 9.0 (Fig. 4). The expression in medium KLB of initial pH 7.5 was low, but was stimulated by the addition of 0.1 M NaCl (Fig. 4). In contrast to these pH values, the expression was weak at pH values below 7, even if NaCl was added (Fig. 4). Expression of the fusion gene was induced by LiCl in medium KLB of initial pH 7.5 in TS33Z, but the induction was again low in TS10Z and TS12Z (data not shown).

4. Discussion

In the present study, the expression level of the *nhaA-lacZ* fusion gene was found to be very low in the wild-type *E. coli*. When *chaA* was deleted, the fusion gene was expressed at high pH, while a high level of the expression was observed at low pH in strains deficient in *nhaB*. The *nhaA-lacZ* fusion gene was expressed at pH values from 5 to 9 when both *nhaB* and *chaA* were deleted. Sakuma et al. [9] showed that a mutant having only NhaA extruded sodium ions within the medium pH range from 6 to 9.

In contrast to our results, it was reported that *nhaA* was induced at alkaline pH [11]. When *nhaB* was deleted, *nhaA* was expressed in the pH range from 6 to 9 [13]. The strain used in these studies showed no growth in the presence of 100 mM NaCl at pH 8.5 when *nhaA* was deleted [23], implying that the activity to extrude sodium ions is low at alkaline pH. This may be the reason why *nhaA* was induced preferentially at high pH without the deletion of *chaA* in the previous studies. Ohyama et al. [5] suggested that the activity of ChaA to extrude

sodium ions in W3133-2 was lower than that in W3110. Strains used in the previous reports [11–13] were derived from W3133-2.

The question is why *E. coli* has NhaA. The growth rate of the wild-type W3110 decreased at an elevated level of NaCl, and the decrease was lower than that of an *nhaA* mutant derived from W3110 at pH above 8 [5]. In contrast to high pH, the growth rate of both strains declined equally with the increase in the medium level of NaCl at neutral pH [5]. The activity of ChaA to extrude sodium ions at pH 8.5 was lower than that of NhaB at pH 7.5 [9]. The internal amount of sodium ions of TS10Z (*nhaA*[−]) was almost the same as that of MC4100 (wild-type) at acid pH, but was higher than that of MC4100 at pH above 8 (Fig. 3). These data suggest that the activity of ChaA is not enough to keep a low level of internal sodium ions in alkaline medium containing a large amount of sodium ions. Therefore, the operation of NhaA may be required for growth only in sodium ion rich medium at high pH in the wild-type *E. coli*. The *nhaA* expression was low in the strain containing *nhaB* and *chaA* at alkaline pH (Fig. 1). Since the activity of NhaA is high at high pH [14–17], a low level of NhaA may be enough to complement the function of ChaA in sodium ion rich medium.

The internal amount of sodium ions in TS14Z was approximately 0.6 $\mu\text{mol/mg}$ cellular protein at both acid and alkaline pH values (Fig. 3), but *nhaA* expression was lower at alkaline pH (Fig. 2). It is possible to assume that the effective concentration of sodium ions for *nhaA* expression is affected by pH. Castle et al. [24] showed that nearly half of cytoplasmic sodium ions are bound to cellular materials. Since the amount of negatively charged molecules increases at high pH, the free concentration of cytoplasmic sodium ions should decrease at alkaline pH. This may be another possibility to account lower expression of *nhaA* at high pH.

It has been proposed that NhaB functions at pH values below 8 [9], in agreement with our present results. However, the report by Thelen et al. [25] suggested no significant change in the activity of NhaB within the wide range of medium pH from 6 to 9. They measured the activity from the amount of sodium ions extruded from cells after these ions were accumulated via a sodium/serine symporter. The magnitude of sodium ion gradient at the steady state

level is mainly dependent on a proton motive force, but not on the molecular activity of the sodium/proton antiporter. Thus, the activity of NhaB they measured may be less precise.

In conclusion, our present data suggest that NhaB and ChaA are the major systems for sodium ion extrusion functioning at low and high pH values, respectively, and NhaA functions only when a low internal level of sodium ions is not kept by other extrusion systems. Thus, our present study supports the previous proposal that bacteria use different systems for one function under different conditions [26,27].

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