Prodigiosins as a New Group of $\text{H}^+/\text{Cl}^-$ Symporters That Uncouple Proton Translocators*

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We reported previously (Kataoka, T., Muroi, M., Ohkuma, S., Waritani, T., Magae, J., Takatsuki, A., Kondo, S., Yamasaki, M., and Nagai, K. (1995) FEBS Lett. 359, 53–59) that prodigiosin 25-C uncoupled vacuolar $\text{H}^+\text{-ATPase}$, inhibited vacuolar acidification, and affected glycoprotein processing. In the present study we show that prodigiosins (prodigiosin, metacycloprodigiosin, and prodigiosin 25-C) inhibit the acidification activity of $\text{H}^+\text{-ATPase}$ chloride dependently, but not membrane potential formation or ATP hydrolysis activity, and suggest that they promote $\text{H}^+/\text{Cl}^-$ symport (or $\text{OH}^-/\text{Cl}^-$ exchange, in its equivalence) across vesicular membranes. In fact, prodigiosins displayed $\text{H}^+/\text{Cl}^-$ symport activity on liposomal membranes. First of all, they decreased the internal pH of liposomes depending on the external chloride, and raised it depending on the internal chloride when external buffer was free from chloride. Second, their effect was electroneutral and not seriously affected by the application of an inside positive membrane potential generated by $\text{K}^+$ and valinomycin. Finally, they promoted the uptake of $[^36]\text{Cl}$ from external buffers with concomitant intraliposomal acidification when external pH was acidic relative to liposome interior. As prodigiosins hardly inhibit the catalytic activity (ATP hydrolysis) unlike well known $\text{OH}^-/\text{Cl}^-$ exchangers (for example, tributyltin chloride), they should provide powerful tools for the study of molecular machinery and cellular activities involving transport of protons and/or chloride.

Prodigiosins are a group of red pigments produced by microorganisms like $\text{Streptomyces}$ and $\text{Serratia}$ (1). They show a wide variety of biological activities involving selective inhibition of T cell proliferation induced by concanavalin A (2, 3), immunomodulation in immunized mice (4–6), and suppression of the bone resorption by osteoclasts (7). We reported previously that prodigiosin 25-C raises lysosomal pH and suppresses glycoprotein processing due to its uncoupling effect on vacuolar (lysosomal) $\text{H}^+\text{-ATPase}$ without showing apparent protonophoric activity (8). Although prodigiosin 25-C also affects mitochondrial F-ATPase and produces swelling of mitochondria besides Golgi, it did not affect the cellular ATP level, showing relatively specific perturbation of vacuolar pH when treated in vivo, which makes it a promising drug for the analysis of vacuolar function.

In the present paper, we show that $\text{H}^+/\text{Cl}^-$ symport activity is expressed even on liposomal membranes and present evidence that prodigiosins are a new group of $\text{H}^+/\text{Cl}^-$ symporters (or $\text{OH}^-/\text{Cl}^-$ exchangers, in their equivalence) that uncouple (dissociate) proton translocation from otherwise coupled catalysis (for example, ATP hydrolysis) in such a way that they inhibit acidification but neither catalysis nor membrane potential formation. A preliminary account of this work was presented in the 22nd Meeting of the Japan Bioenergetics Group (9).

EXPERIMENTAL PROCEDURES

Materials—Prodigiosin was prepared from the culture broth of $\text{Serratia marcescens}$ as described (10) and metacycloprodigiosin and prodigiosin 25-C were obtained from $\text{Streptomyces hiroshimensis}$ (11). Rats (Wistar, male) were obtained from Sankyo Labo Service (Tokyo, Japan). Fluorescein-labeled dextran (FD) was either synthesized according to Ref. 12 or supplied in the form of fluorescein isothiocyanate-dextran (M, 70,000) from Sigma. Triton WR-1339 was obtained from Ruger Chemical Co. (Irvington, NJ). n-Octyl-β-D-thioglucoide was purchased from Dojin (Kumamoto, Japan). Protease inhibitors of microbial origin were obtained from the Peptide Research Institute (Osaka). Bafilomycin A1 was kindly provided by Professor K. Altendorf (University of Osnabruck, Germany). Phenol/phospholipids were obtained from Sigma (Sigma type II-S phosphatidylincholine from soybean phospholipids) and used without purification. Pyranine (8-hydroxy-1,3,6-pyrenetricarboxylic acid) was supplied by Dojin (Kumamoto, Japan). Triton WR-1339 was obtained from Roter Chemical Co. (Rochester, NY). P-Nitrophenylphosphatase was supplied in the form of fluorescein-dextran (3.8 MBq/ml, 103 μCi/ml). Cleared™ was obtained from NEN Life Science Products Inc. (Wilmington, DE) through Dai-ichi radio isotope (Tokyo). Nigercin, carbonyl cyanide p-trifluoromethoxyphenylhydrazone, MES, HEPES, tetramethylammonium hydroxide (TMAH), sodium gluconate, and glucono-6-lactone were supplied by Sigma. Other reagents were purchased as commercial products mostly from Sigma.

Preparation of Lysosomes (Dextranosomes) and Solubilization of Lysosomal V-ATPase—Preparation of fluorescein-dextran loaded lysosomes (FD-dextranosomes) from rat liver was performed as described (13), with the exception that the Percoll washout procedure was omitted and the lysosomal layer in the Percoll gradient was used for the experiments. Bovine chromaffin granules were obtained as described (14). Lysosomal V-ATPase was solubilized from membrane ghosts of Triton-

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‡‡ The abbreviations used are: MES, 2-(N-morpholino)-ethanesulfonic acid; diS-C3-(5), di(propidium)iodocarbocyanine iodide; DTT, dithiothreitol; F-ATPase, F-type $\text{H}^+\text{-ATPase}$; FD, fluorescein-labeled dextran; rε, tetramethylammonium chloride; V, internal pH gradient; oxonol-V, bis(3-phenyl-5-oxoisoxazol-4-yl)pentamethine oxonol (Oxonol-V) and dipropylthiodicarbocyanine iodide (diS-C3-(5)) were from Nippon Kanko Shikiso (Okayama). Chlorine 36 ($^{36}\text{Cl}$) was obtained from Amersham as sodium chloride solution (3.8 MBq/ml, 103 μCi/ml). Cleared™ was obtained from NEN Life Science Products Inc. (Wilmington, DE) through Dai-ichi radio isotope (Tokyo). Nigercin, carbonyl cyanide p-trifluoromethoxyphenylhydrazone, MES, HEPES, tetramethylammonium hydroxide (TMAH), sodium gluconate, and glucono-6-lactone were supplied by Sigma. Other reagents were purchased as commercial products mostly from Sigma.

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Prodigiosins

![Chemical structures of prodigiosins](Image)

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Prodigiosins have a general structure of methoxybipyrrolypropromethene with hydrocarbon side chain(s) attached to the "right-hand" pyrrole ring. In this study, we used prodigiosin, metacycloprodigiosin, and prodigiosin 25-C (Fig. 1) but present the results of metacycloprodigiosin in most cases because the results were almost the same among the three prodigiosins. In the previous paper (8), we showed that prodigiosin 25-C increased lysosomal pH in cultured cells as a result of its uncoupling activity on the lysosomal H$^+$-ATPase (V-ATPase), although prodigiosin 25-C uncoupled F-ATPases, too. Fig. 2A shows the dose responses of the effect of metacycloprodigiosin, as compared with bafilomycin A$_1$, on the acidification, bafilomycin A$_1$-sensitive ATPase, and membrane potential formation of lysosomal V-ATPase. Metacycloprodigiosin (and prodigiosin), like prodigiosin 25-C, inhibited acidification with an IC$_{50}$ of 5 - 20 nM (at 170 µg of protein/ml; corresponding to 30 - 120 pmol/mg of protein) but hardly affected ATPase activity up to ~1 µM (>50 ~ 100 times that of the IC$_{50}$ inhibition activity). This contrasts with the effect of bafilomycins which inhibit both acidification and ATPase activities with an IC$_{50}$ of about 1 nM (Fig. 2B; for bafilomycin A$_1$). Furthermore, prodigiosins did not affect the membrane potential formation of V-ATPase either (Fig. 2A, for metacycloprodigiosin), which also contrasts with the effect of bafilomycins (IC$_{50}$ inhibition of membrane potential formation ~1 nM) (Fig. 2B; for bafilomycin A$_1$). This feature is unique to prodigiosins and not shared by the other basic substances tested so far in our laboratory. The insensitivity to prodigiosins of ATPase and membrane potential formation was shared also with F-ATPases (mitochondrial and bacterial H$^+$-ATPases).

**RESULTS**

Prodigiosins Inhibit Acidification, but Affect Neither ATP Hydrolysis nor Membrane Potential Formation Mediated by V-ATPase

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The inability of prodigiosins to inhibit membrane potential formation suggests that they functionally: 1) inhibit transformation of ΔΨ (driven by V-ATPase) into ΔpH (31); 2) change the participating ion from H⁺ to another cation, either through ionophoric activity (of say nigericin, a H⁺/K⁺ exchange ionophore) or by changing the ion specificity of the ATPase (like (V or F)-type Na⁺/ATPase or respiratory Na⁺ pump) (32–34); or 3) change the participating ion from H⁺ (or OH⁻) to another anion either through ionophoric activity (for example, TBT, a OH⁻/Cl⁻ exchanger (35)), or by changing the ion specificity of the ATPase (like the relationship between halorhodopsin and bacteriorhodopsin (36)). But, the prodigiosins did not show any ionophoric activity against H⁺/K⁺ exchange ionophores, even in the presence of K⁺-valinomycin added to the assay buffer to dissipate ΔΨ and to eliminate possible participation of chloride channel(s) (2,3). On the basis of these observations, we next tested if prodigiosins show H⁺/Cl⁻ symport (or OH⁻/Cl⁻ exchange) activity was suspected. In fact, their activity to inhibit vesicular acidification required chloride (with no obvious saturation), even in the presence of K⁺-valinomycin added to the assay buffer to dissipate ΔΨ and to eliminate possible participation of chloride channel(s). On the basis of these observations, we next tested if prodigiosins show H⁺/Cl⁻ symport (or OH⁻/Cl⁻ exchange) activity on phospholipid membranes using liposomes that are devoid of proton pump protein.

**H⁺/Cl⁻ Symport (OH⁻/Cl⁻ exchange) Activity of Prodigiosins on Liposomes**

Prodigiosins Produced Chloride Gradient-dependent Perturbation of Intraliposomal pH—Depending on the preliminary observations, we performed a direct assay of the H⁺/Cl⁻ symport activity of prodigiosins by measuring the effect of prodigiosins on the chloride-dependent change of internal pH of the liposomes (Fig. 3). In Fig. 3A, liposomes containing the pH-sensitive fluorescent probe pyranine (24) prepared in KCl were diluted 40-fold in external buffer containing either gluconate (sodium gluconate) or chloride (NaCl). In control experiments, Me₂SO or EtOH (solvent control), added instead of prodigiosins or TBT, did not produce significant change in the fluorescence, and the addition of MES-TMAH produced a rapid decrease in fluorescence to a certain extent (indicative of the presence of non-trapped extraliposomal pyranine) followed by a slow gradual decrease of fluorescence (indicating acidification of the liposome interior), suggesting limited permeability of liposomal membranes to protons (or hydroxyl anions). On the other hand, metacycloprodigiosin (1 μM), like TBT (100 nM), produced a rapid increase in the pyranine fluorescence (indicating internal alkalization) in gluconate buffer but not in chloride buffer. However, the addition of MES-TMAH (pH 6.0) produced a marked decrease of pyranine fluorescence (indicating intraliposomal acidification) in prodigiosin- or TBT-treated liposomes relative to control (Me₂SO or EtOH), and the rate of this decrease was greater in chloride than in gluconate buffer.

Likewise, when pyranine-containing liposomes prepared in a buffer containing potassium gluconate (and MgSO₄) in place of KCl (and MgCl₂) were diluted in external buffer containing either gluconate (sodium gluconate) or chloride (NaCl), metacycloprodigiosin (1 μM), like TBT (100 nM), produced a rapid decrease in the pyranine fluorescence (indicating internal acidification) in chloride more than in gluconate (Fig. 3B). The addition of MES-TMAH (pH 6.0) produced a further decrease of pyranine fluorescence in prodigiosin- or TBT-treated liposomes relative to control (Me₂SO or EtOH), and the rate of this decrease was greater in chloride than in gluconate buffer. But also note that the rate of this decrease was not zero in gluconate, and greater in prodigiosin- than TBT-treated liposomes. Essentially the same results were obtained with prodigiosin 25-C and prodigiosin (see below). In additional control experiments, 1 μM NH₄Cl did not show such activity, while nigericin produced a quick fluorescent decrease indicative of intraliposomal acidification due to external H⁺/internal K⁺ exchange.

**Dependence of the Prodigiosin-mediated Liposomal Acidification on External Chloride Ion**—In Fig. 4A, pyranine-containing liposomes prepared in potassium gluconate were diluted 40-fold in external buffer consisting of different ratios (1:0, 1:1, 1:3, 1:7, and 0:1) of NaCl and sodium gluconate. The results clearly show that both metacycloprodigiosin and TBT acidified intraliposomal pH almost linearly with no obvious saturation dependent on the chloride concentration as summarized in Fig. 4B: again note that the rate of the acidification in gluconate was greater in prodigiosin- than TBT-treated liposomes. This result is also consistent with the ionophoric nature of intraliposomal acidification driven by prodigiosins. In order of effectiveness, the inhibitors ranked as follows: TBT > prodigiosin, metacycloprodigiosin > prodigiosin 25-C: the effective concentrations in chloride buffer for 50% of maximum fluorescence


quenching in 10 s were 1.0, 0.7, and 9.0 nmol/mg phospholipid for prodigiosin, metacycloprodigiosin, and prodigiosin 25-C, respectively, as compared with 23 pmol/mg phospholipid for TBT.

**Electroneutral Nature of the Prodigiosin-catalyzed H\(^+\)/Cl\(^-\) Symport**—In Fig. 5, pyranine-containing liposomes prepared in KCl (and MgCl\(_2\)) at pH 6.0 were first diluted 40-fold in external buffer (20 mM HEPES-TMAH, pH 7.5) containing either 25 mM sodium gluconate or NaCl and the pyranine fluorescence was recorded as described under “Experimental Procedures.” A, Cl\(^-\)-containing liposomes. **MES-TMAH** (pH 6.0), 50 mM; **TX-100**, 0.2%; **Triton X-100**; **Meta**, 100 nM metacycloprodigiosin; **TBT**, 1 \(\mu\)M; **DMSO**, 1% Me\(_2\)SO (solvent control for metacycloprodigiosin) in sodium gluconate buffer (dashed line); **EtOH**, 1% EtOH (solvent control for TBT) in sodium gluconate buffer (dashed line). B, Cl\(^-\)-free liposomes. **MES-TMAH** (pH 6.0), 50 mM; **TX-100**, 0.2%; **Triton X-100**; **Meta**, 1 \(\mu\)M metacycloprodigiosin; **TBT**, 100 nM; **EtOH**, 1% EtOH (solvent control for TBT) in sodium gluconate buffer (dashed line); **DMSO**, 1% Me\(_2\)SO (solvent control for metacycloprodigiosin) in NaCl buffer (dashed line); **EtOH**, 1% EtOH (solvent control for TBT) in NaCl buffer (dashed line).

**Prodigiosin-mediated Chloride Transport in Liposomes**

**FIG. 3.** Chloride gradient-dependent changes of intraliposomal pH produced by metacycloprodigiosin and TBT. Liposomes containing pyranine, prepared in a buffer (20 mM HEPES-TMAH, pH 7.5) containing either (A) KCl (25 mM KCl, 2.5 mM MgCl\(_2\)) (Cl\(^-\)-containing liposomes) or (B) potassium gluconate (25 mM potassium gluconate, 2.5 mM MgSO\(_4\)) (Cl\(^-\)-free liposomes) were diluted 40-fold in external buffer (20 mM HEPES-TMAH, pH 7.5) containing either 25 mM sodium gluconate or NaCl and the pyranine fluorescence was recorded as described under “Experimental Procedures.” A, Cl\(^-\)-containing liposomes. **MES-TMAH** (pH 6.0), 50 mM; **TX-100**, 0.2%; **Triton X-100**; **Meta**, 100 nM metacycloprodigiosin; **TBT**, 1 \(\mu\)M; **DMSO**, 1% Me\(_2\)SO (solvent control for metacycloprodigiosin) in sodium gluconate buffer (dashed line); **EtOH**, 1% EtOH (solvent control for TBT) in sodium gluconate buffer (dashed line). B, Cl\(^-\)-free liposomes. **MES-TMAH** (pH 6.0), 50 mM; **TX-100**, 0.2%; **Triton X-100**; **Meta**, 1 \(\mu\)M metacycloprodigiosin; **TBT**, 100 nM; **EtOH**, 1% EtOH (solvent control for TBT) in sodium gluconate buffer (dashed line); **DMSO**, 1% Me\(_2\)SO (solvent control for metacycloprodigiosin) in NaCl buffer (dashed line); **EtOH**, 1% EtOH (solvent control for TBT) in NaCl buffer (dashed line).
metacycloprodigiosin and TBT, although slight differences are noted between metacycloprodigiosin and TBT in pH dependence. The temperature dependence of the uptake of $^{36}\text{Cl}$ into liposomes indicated an activation energy of about 15–16 kcal/mol for both metacycloprodigiosin and TBT which matched with the value reported for TBT (41).

**DISCUSSION**

In recent years, there have been several important findings regarding the mechanism of energy transduction, some relating to structural biology (for F$_F^0$F$_1$-ATPase (42) and cytochrome oxidase complexes (43, 44)), others to combined molecular fluorescence micromanipulation technology (45) (showing molecular spin of H$^+$-ATPase molecules). Nevertheless, one important aspect of energy transduction, “energy coupling,” remains to be clarified. From a biochemical point of view, specific modifiers of energy transduction would represent a breakthrough, just as uncouplers opened the way to the chemiosmotic hypothesis of Mitchell in 1961.

Prodigiosins were initially thought of as candidates of such modifiers to help elucidate the energy transduction mechanism: prodigiosins inhibited the acidification mediated by various H$^+$-translocating ATPases without inhibiting ATP hydrolysis or membrane potential formation (8) (Fig. 2), unlike ordinary uncouplers of oxidative phosphorylation or ATPase inhibitors. However, evidence has been accumulating that prodigiosins have an ionophoric nature and we demonstrated in the present study that prodigiosins act as a H$^+$/$\text{Cl}^-$ symporter (or OH$^-$/Cl$^-$ antiporter, in its equivalence) on liposomes, by showing: 1) alkalinization and acidification of intraliposomal pH depending on the outward and inward chloride gradient, respectively; and finally, 3) the pH gradient (outside acid)-dependent uptake of radioactive chloride into liposomes in parallel with intraliposomal acidification.

These results on the whole favor the idea that the uncoupling effect of prodigiosins is due to their H$^+$/$\text{Cl}^-$ symport activity.
across biological membranes. In fact, their effective concentrations for H\(^+/\)Cl\(^-\) symporting activity on liposomes mediated by prodigiosins and TBT. Uptake of \(^{36}\)Cl into liposomes was performed according to “Experimental Procedures.” Briefly, potassium-loaded liposomes (50 mg/ml) containing 500 \(\mu\)M pyranine prepared in 20 mM HEPES-TMAH (pH 7.5), 5 mM potassium gluconate and 2.5 mM MgSO\(_4\) were centrifuged through Sephadex G-25 equilibrated with 20 mM MES-TMAH (pH 6.0) and 5 mM KCl and the eluate was used for the experiments as liposome suspension (28 mg of phospholipids/ml). The assay buffer contained 20 mM MES-TMAH (pH 6.0), 5 mM KCl, and Na\(^{36}\)Cl (0.1 \(\mu\)Ci/ml). 250 \(\mu\)l each of the above liposome suspension and assay buffer were mixed and the reaction started at 37 °C. At 1 min, 5 \(\mu\)l of either prodigiosin (1% Me\(_2\)SO as solvent control) or TBT (1% EtOH as solvent control) was added to the assay buffer to attain the indicated final concentrations. Mean ± S.D. of triplicates are plotted. Fluorescence assays were performed as described under Fig. 3, after dilution of 40 \(\mu\)l of the above liposome suspension into the above assay buffer. DMSO, 1% Me\(_2\)SO; EtOH, 1% EtOH; PG, prodigiosin; 25-C, prodigiosin 25-C; Meta, metacycloprodigiosins; TX-100, 0.2% Triton X-100.

The \(^{36}\)Cl uptake of TBT on liposomes (Figs. 4 and 6) is not clear. It may reflect difference of permeability against the ionophore between liposomes and organelar membranes due to compositional differences (for example, absence or presence of proteins).

The \(^{36}\)Cl symport (or OH\(^-\)/Cl\(^-\) antiport) activity of prodigiosins also explains several previous findings: 1) that the ATP level was hardly affected by prodigiosin 25-C; 2) mitochondrial swelling induced by prodigiosin 25-C, and so on. As prodigiosins did not affect the membrane potential formation, they are not expected to affect ATP formation in mitochondria where the proton motive force is stored mainly in the form of transmembrane potential. In this regard, prodigiosins are quite similar to the traditional OH\(^-\)/Cl\(^-\) exchangers, triorganotins (for example, TBT), which inhibit vesicular acidification but hardly affect membrane potential formation in reconstituted cytochrome oxidase proteoliposomes (46) nor ATP formation in the photosynthesis driven by halorhodopsin (47). Like prodigiosins, triorganotins induce mitochondrial swelling (48, 49),
which is explained by the uptake of osmotically active Cl\(^-\) in exchange for respiration-induced OH\(^-\) (equivalent to coupled transport of Cl\(^-\) with respiration-induced H\(^+\)). However, triorganotins act, at the same time, as uncouplers of oxidative phosphorylation and inhibit mitochondrial ATP formation, because they inhibit the F-ATPase molecules themselves through SH-protecting reagent (for example, N-ethylmaleimide)-sensitive binding (46, 50–54). In fact, the effect of TBT on the acidification by H\(^+\)-ATPases was not suppressed by the presence of large amounts of liposomes, contrary to the effect of prodigiosins.\(^4\) Golgi swelling might be due to ΔpH-induced accumulation of osmotically active protonated weak bases (prodigiosins) within the Golgi apparatus. Golgi, rather than lysosomes, swell possibly because Golgi membranes are less permeable to K\(^+\) (19, 55), resulting in more Cl\(^-\) uptake than K\(^+\) extrusion.

The nature of prodigiosins as H\(^+\)/Cl\(^-\) symporters also suggests that there are additional translocators or biological activities affected by prodigiosins: they will include respiratory proton pumps, vacuolar H\(^+\)-translocating pyrophosphatases, ATP-dependent transhydrogenases of NADP\(^+\) and NADH, H\(^+\)- or Cl\(^-\)-transporting bacterio/halo-rhodopsins, H\(^+\)(OH\(^-\))- or Cl\(^-\)-coupled symporters or antiporters, and Cl\(^-\)-pumps. Prodigiosins may prove useful for the clarification of ion transport mechanisms.

What is the mechanism of H\(^+\)/Cl\(^-\) symporter (or OH\(^-\)/Cl\(^-\) antiporter) of prodigiosins? Organotins are considered to support the exchange of halides with OH\(^-\) across membranes due to their covalent bonding (co-ordination) with halides on one side of the membrane accompanied by their hydrolyses on the other side (41, 57, 58). Prodigiosins, on the other hand, probably bind with halides electrostatically in accordance with Hofmeister series (possibly helped by the stabilizing effect of hydrogen-bonding and/or charge transfer (59, 60) interactions of protonated nitrogen in prodigiosins), resulting in the formation of lipophilic ion pairs which facilitate proton-coupled transmembrane transport of halides as do phase transfer catalysts (61, 62). In this sense, prodigiosins are probably H\(^+\)/Cl\(^-\) symporters rather than OH\(^-\)/Cl\(^-\) exchangers: protonated prodigiosins are less likely to bind OH\(^-\), especially at acidic pH. In addition, prodigiosins show anion specificity similar to that of TBT but with some important differences: for example, gluconate prefers prodigiosins to TBT (Figs. 3 and 4). Studies on the crystallographic structure of prodigiosin salts as well as on the structure-activity relationship will help clarify the essential structures and the action mechanism of these new H\(^+\)/Cl\(^-\) symporters.

Several other new H\(^+\)/Cl\(^-\) symporters (or OH\(^-\)/Cl\(^-\) exchangers) have been reported recently. They include thallium chloride (Tl\(^{3+}\), e.g. TlCl\(_3\)) (63), bepridil (64), Hg\(^{2+}\) and Cu\(^{2+}\) (65), sapphyrin (66), and cryptate (67). Also, the number of new Cl\(^-\)-sensing ionophores has been accumulating: they include Mn\(^{2+}\)-sensing ionophores (39, 40), pamamycin (68), mercury organic compounds (ETH 9018 (69)), besides methyltributylammonium chloride, tetrabutylammonium chloride, and tridecylmethylammonium chloride, some of which may exhibit H\(^+\)/Cl\(^-\) symporting (OH\(^-\)/Cl\(^-\) antiporting) activity, too.

The activity of prodigiosins presented in this article suggests that these anion-exchange compounds constitute a new group of probes for the analysis of vacuolar function, as their effects are relatively selective to vacuolar pH \textit{in vivo}, hardly affecting the cellular ATP level. Namely, we can expect that they affect all aspects of cellular functions involving V-ATPase, including endocytosis, exocytosis, and intracellular trafficking as well as cell growth, cell differentiation, and cell death (apoptosis). In fact, we have already reported suppressive activity of prodigiosins on various aspects of immunity and bone resorption (2–7). Also, we can expect prodigiosin-specific reactions,

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because prodigiosins do not seriously affect membrane potential formation: for example, they selectively inhibit ΔpH-dependent uptake of certain neurotransmitters (like dopamine or histamine) without affecting Δψ-dependent uptake of other neurotransmitters (like glutamate) into synaptic vesicles (70). In summary, we have now three different types of vacuum perturbators in hand: 1) weak bases and acidic ionophores which raise the pH and induce swelling of the vacuum system; 2) prodigiosin group H⁺/Cl⁻ symporters that affect only vacular pH; and 3) bafilomycin group antibiotics (including concanamycins and destruxins) that affect both pH and membrane potential formation. By proper application of these probes, we will be able to clarify much more extensively the unknown functions of the vacuum system. Furthermore, the H⁺/Cl⁻ symporter activity of the compounds reported in this study may actually be a sort of “missing link” and should be taken seriously, especially in regard to therapeutic drugs.

Finally, prodigiosins are also interesting from the point of view of organic synthesis, because they are small enough for chemical modifications aimed at providing more active and less toxic compounds. Although their synthetic history is long (71–74), a simple and elegant way to synthesize prodigiosins was reported recently (75) which will help in the synthesis of new active derivatives.

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