BioMetals **17:** 79–86, 2004.

The respiratory inhibitor antimycin A specifically binds Fe(III) ions and mediates utilization of iron by the halotolerant alga *Dunaliella salina (Chlorophyta)*

Uri Pick
The Department of Biological Chemistry, The Weizmann Institute of Science, Rehovot 76100 Israel (Tel: (972) 8 9342732; Fax: (972) 8 9344118; E-mail: bcpick@weizmann.ac.il)

Received 23 December 2002; accepted 16 April 2003; Published online: June 2003

Key words: Antimycin A, *Dunaliella*, Fe chelator

Abstract

It is demonstrated that Antimycin A (AA), a respiratory inhibitor produced by *Streptomyces* bacteria, forms lipophylic complexes with Fe(III) ions. Spectroscopic titration indicates that Fe(III) ions interact with 2AA molecules. At growth-limiting Fe concentrations, AA mediates Fe uptake and promotes growth and chlorophyll synthesis better than other Fe chelators in the halotolerant alga *Dunaliella salina*. It is proposed that AA enhances Fe bioavailability in hypersaline solutions by formation of lipophylic Fe-AA complexes which are taken-up and utilized by the algae. The results suggest that the respiratory inhibitor AA can affect Fe metabolism in microorganisms.

Abbreviations: AA – Antimycin A; TTf – *Dunaliella* triplicated transferrin; CDTA – trans-1,2 diaminocyclohexane-N,N.N′,N′-tetraceticacid cid; DCMU – 3(3,4 dichlorophenyl) 1,1-dimethyl-urea; FCCP – carbonyl cyanide *p*-trichloromethoxyphenyl hydrazone

Introduction

The extremely low solubility of Fe(III) ions in neutral solutions is recognized as a major limitation for proliferation of phytoplankton in vast regions in oceans around the world (Coale *et al.* 1996 and others). Most of the dissolved Fe(III) in the oceans and in non-saline aquatic ecosystems is complexed by organic ligands with binding constants exceeding 10^{19} M^{-1}, which constitutes the pool of bioavailable iron for marine microorganisms (Butler 1998). This situation dictates a fierce competition for iron between different kinds of phytoplankton and bacteria in the ocean (Hutchins *et al.* 1999). Phytoplankton have evolved diverse strategies for iron acquisition, primarily via utilization of Fe(III) siderophores. Many cyanobacteria and some algae secrete catechol-type or hydroxamate-type phytosiderophores which bind ferric ions with very high binding affinity. There are indications that the complexed ferric iron may be taken up either through surface receptors, a strategy common to most bacteria, or through reduction to Fe(II) ions by ferrireductases (Trick & Kerry 1992; Benderliev & Ivanova 1994; Wilhelm *et al.* 1998; Maldonado & Price 2000). Many species of phytoplankton can utilize ferric ions that are complexed to bacterial siderophores such as ferrichrome, ferrioxamine and enterobactin. The mechanisms of iron acquisition from such high-affinity ligands are not entirely clear, but they probably include thermal, photolytic or enzymatic degradation of siderophores or reduction via ferrireductases (Soriadengg & Horstmann 1995; Kuma *et al.* 2000; Maldonado & Price 2000). A recent study of iron utilization by natural phytoplankton communities demonstrated a clear preference for different ferric ligands between prokaryote and eukaryote phytoplankton: the former exhibited a preference for ferric-siderophores whereas the latter showed prefer-

ence for ferric-porphyrin complexes (Hutchins *et al.* 1999).

A different mechanism of iron acquisition is utilized by the halotolerant alga *Dunaliella salina*. *Dunaliella* overproduces at high salinity or under iron limitation a membrane associated transferrin-like protein (TTf) which mediates efficient uptake of Fe(III) ions in saline or hypersaline solutions (Fisher *et al.* 1997, 1998).

In this study it is demonstrate that antimycin A, a respiratory inhibitor produced by *Streptomyces*, acts as a Fe(III) mediator in the halotolerant alga *D. salina*. It is shown that AA forms lipid-soluble complexes with Fe(III) which are taken up and utilized by the alga.

Materials and methods

Algae and growth conditions

Dunaliella salina (Ben-Amotz & Avron 1983) was cultured in batch cultures with periodic dilutions to be maintained in the logarithmic growth phase ($2 \cdot 10^5$-$2 \cdot 10^6$ cells ml^{-1}). The growth media contained 500 mM NaCl, 50 mM $NaHCO_3$, 5 mM KNO_3, 5 mM $MgSO_4$, 0.3 mM $CaCl_2$, 0.2 mM KH_2PO_4, 0.185 mM H_3BO_3, 7 μM $MnCl_2$, 5 μM EDTA, 2 μM $FeCl_3$, 0.8 μM $ZnCl_2$, 20 nM $CuCl_2$, 50 mM Na-tricine pH 7.5 and the indicated Fe(III)-chelates. Cell suspensions were cultured under continuous illumination (50 μmols sec^{-1} m^{-2}) in a New-Brunswick incubator shaker, model G-27 (Edison, New-Jersey) at 26 °C. Growth rates were measured in a 24-well flat-bottom microtitration plates (1.5 ml samples) for 48 to 72 h in the presence or absence of different Fe(III)-ligands under the same conditions. To initiate growth, cultures were washed twice in an iron-depleted medium and incubated at an initial concentration of $2 \cdot 10^5$cells ml^{-1} in media supplemented with the indicated concentrations of Fe(III)-ligands. Growth rates were followed by daily measurements of cell number in a Coulter counter Multisizer II (Coulter Electronics LTD, England) and of chlorophyll concentration, measured by absorption of cell extracts in 80% acetone (Arnon 1949). Effect of metabolic inhibitors on Fe^{3+} uptake and respiration were tested following 15 min pre-incubations of *D. salina* cells (10^8 cells ml^{-1}) at 23 °C in the light (50 μmols sec^{-1} m^{-2}) with the indicated inhibitors. Fe(III) uptake measurements were initiated by addition of ^{59}Fe(III)-citrate.

Assays

Iron uptake was measured by accumulation of ^{59}Fe(III) essentially as previously described (Fisher *et al.* 1998). In brief, cell samples containing $3 \cdot 10^7$ cells in 0.3 ml were incubated in the light at 23 °C for 30–60 min with the indicated concentrations of ^{59}Fe(III)-ligands (Fe:AA 1:2-4; Fe:desferal, Fe:o-phenantriline = 1:1; Fe:EDTA, Fe:CDTA = 2:5; Fe:citrate = 1:100). ^{59}Fe(III) was provided as $^{59}FeCl_3$ in 0.5 N HCl (NEN, Boston, MS). The reaction was stopped by dilution of 100 μl cell suspension into 3 ml ice-cold 'stop solution' containing 5 mM EDTA, 500 mM NaCl and 30 mM Na-MES pH 5.5, incubation for 10–15 min on ice, centrifugation at 2000*g* for 10 min and one additional wash in the same stop solution. This procedure was found to remove all externally-bound iron and/or cell-free iron (precipitated in the absence of cells) to below 5% of the cellular ^{59}Fe content. However, at Fe-AA ratios of 1:1, at concentrations exceeding 20 μM, we observed significant elevations of background (without cells) indicating Fe precipitation. Therefore, all Fe uptake measurements were carried out a Fe:AA = 1:2-4 and up to 20 μM.

Oxygen uptake was measured in a Rank-Brothers (Cambridge, UK) oxygen electrode as previously described (Shaish *et al.* 1993). Cell suspensions in fresh growth media (3 ml containing $3–5 \cdot 10^6$ cells) were incubated at 20–23 °C in a temperature-regulated and continuously mixed cell and illuminated at 200 μmols s^{-1} m^{-2}.

Fe-AA preparation, extraction and analysis

Fe-AA was prepared as follows: AA was dissolved in methanol (50 mM) and mixed with half volume of 0.1 M $FeCl_3$ dissolved in 0.1 N HCl. Purification of Fe(III)-AA for spectral analysis was performed by three consecutive extractions in chloroform:methanol:water (1:1:1) to eliminate excess Fe(III) from the chloroform phase followed by evaporation of chloroform under nitrogen and then addition of methanol. Acidic Fe-AA_a was prepared by adjustment to pH 5 with diluted HCl, basic Fe-AA_b was adjusted to pH 8.5 with 0.3 Tris base. Competition between AA and citrate for Fe(III) binding was performed by incubation of Fe(III)-AA (50 μM) with various concentrations of Na-citrate for 2 h and measurement of Fe(III)-AA concentration by absorbance at 500 nm.

Extraction of Fe(III)-AA into octanol was measured as follows: ^{59}Fe(III)-citrate (10 μM $^{59}FeCl_3$ + 2 mM citrate) was dissolved in 200 mM KCl and 30 mM Na-tricine, pH 8 with or without 300 μM AA. At the indicated time intervals, samples (200 μl) were vigorously mixed on Vortex for 2 min with 800 μl n-octanol followed by brief centrifugation to achieve phase separation. The content of ^{59}Fe(III) in the octanol phase was analyzed by scintillation counting.

Incorporation of Fe-AA into liposomes

Phospholipid vesicles were prepared by sonication of soybean phosphatidylcholine (type II-S/Sigma, 20 mg ml^{-1}) in 20 mM Na-tricine, pH 8; 50 mM KCl. Uptake experiments were initiated by addition of 30 μM ^{59}Fe(III)-AA or 10 μM ^{59}Fe(III)-citrate to liposome solutions containing 20 mM Na-tricine, 100 mM KCl, 2 mM $MgCl_2$ and 2 mg ml^{-1} phospholipid vesicles. Uptake was terminated by transferring 100 μl samples through Sephadex G-50 columns by centrifugation (Pick 1981).

Absorption spectra were measured in a Cary 5 spectrophotometer (Varian Optical Spectroscopy Instruments, Mulgrave, Victoria Australia). *The AA/Fe ratio* of Fe-AA was calculated from a spectroscopic titration of a AA with increasing Fe concentrations in methanol as follows: 500 μM AA in methanol was mixed with 50–1,000 μM $FeCl_3$. Spectra of Fe-AA mixtures and of identical $FeCl_3$ solutions (-AA) were monitored as in Figure 2. Fe-AA concentration, representing complexed AA, was calculated from the absorption at 550 nm by assuming a mM extinction coefficient ($E^{550}{}_{max}$) of 0.76 (obtained from extrapolation to $[FeCl_3]_\infty$ according to the equation $[\text{Fe-AA}]\mu M = (E^{550}/0.7) \times 500$. Fe-AA, representing complexed Fe concentration, was calculated from the difference between total Fe (Fe_t) and free Fe (Fe_{fr}) in Fe/AA solutions from the absorption at 395 nm, by assuming a mM extinction coefficient of 6.6 for $FeCl_3$ in methanol. Fe_{fr} in Fe/AA mixtures was calculated from the absorbance at 395 nm after subtracting the contribution of Fe-AA according to the empirical equation $[Fe_{fr}]\mu M = (E^{395} - 0.7E^{550}) \times 1{,}000/6.6$. AA/Fe ratio was calculated from the product Fe-AA/Fe-AA.

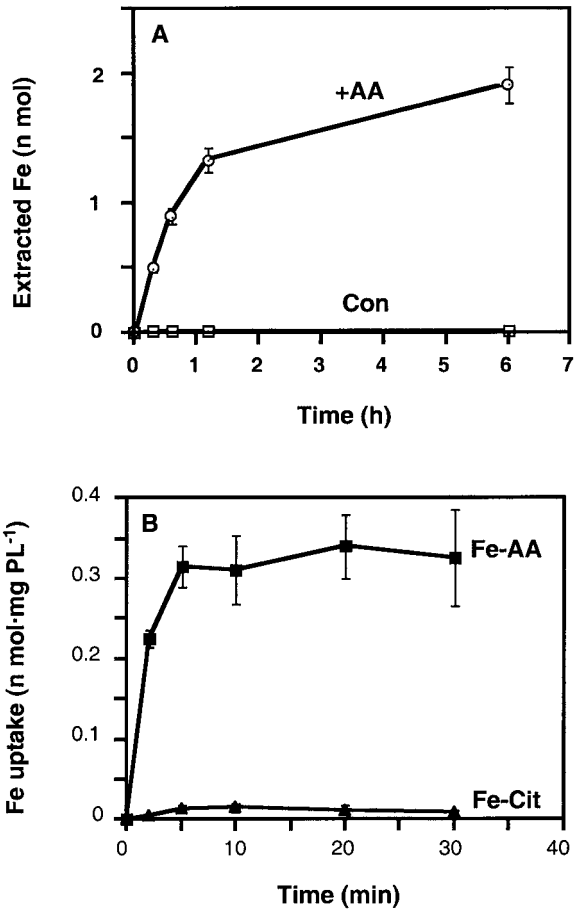

Fig. 1. Indications that AA forms lipophylic complexes with Fe^{3+}. 1A. Extraction of ^{59}Fe(III) by AA from water to octanol. Con- control without AA, +AA- with 300 μM AA. Data represent means ± SD, n = 3. 1B. Incorporation of Fe(III) from Fe-AA into liposomes. Fe-AA- with 30 μM ^{59}Fe(III)-AA, Fe-Cit- with 10 μM ^{59}Fe(III)-citrate, PL-phospholipids. (means ± SD, n = 2–4).

Results

AA forms lipophylic complexes with Fe(III) ions

AA catalyzed a time-dependent extraction of Fe(III) ions from water into octanol, an organic solvent often employed in *in vitro* membrane model studies (Figure 1A). Mixing methanolic solutions of ^{59}Fe(III) and AA with phospholipid vesicles, resulted in incorporation of ^{59}Fe(III) into the liposomes (Figure 1B). Citrate did not mediate a similar transfer of Fe(III) to liposomes or into octanol under the same conditions. These results indicate that AA forms lipophilic complexes with Fe(III) ions and can act as an efficient carrier of Fe to phospholipid membranes.

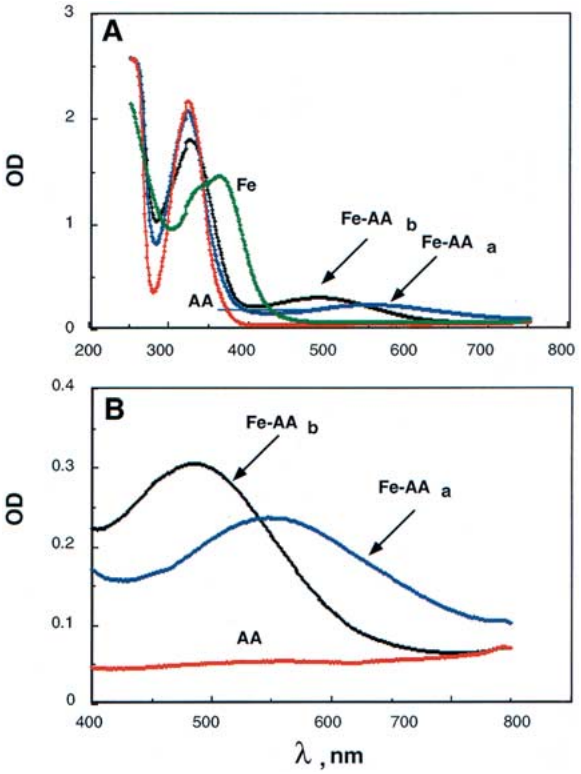

Fig. 2. Absorption spectra of Fe(III)-AA: Fe-AA_a -acid form, pH 5; Fe-$AA_{b-basicform,pH\ 8.5.Allspectraincluding}$ free AA and $FeCl_3$ (Fe) were in methanol. (A) UV + visible spectra, (B) visible spectra.

Spectroscopic analysis of Fe-AA

Mixing of acidic $FeCl_3$ with methanolic solutions of AA resulted in an intense blue color formation, indicative for chelation of Fe(III) by AA. The color changed to reddish-brown at alkaline pH (>7.0) in methanolic solutions containing >60% methanol. Figure 2, A&B depicts the spectral characteristics of Fe(III)-AA in methanol at acidic and alkaline pH. Absorption maxima of the 'acidic' and 'basic' forms of Fe(III)-AA were 555 and 480 nm, respectively, with calculated corresponding milimolar extinction coefficients of 0.76 and 1.13. Absorption changes in the UV region were also observed (Figure 2A).

Other divalent and trivalent metal salts that were tested did not give rise to similar colors with AA (not shown) and poorly competed with Fe(III) ions in formation of the Fe-AA (relative specificity Fe(III) >> Al(III) > Cu(II) > La(III) > Fe(II) > Cr(III) > Co(II) ~ Zn(II), as measured by the increase in absorption at 550 nm (not shown), suggesting that the association between Fe(III) by AA is specific to Fe(III).

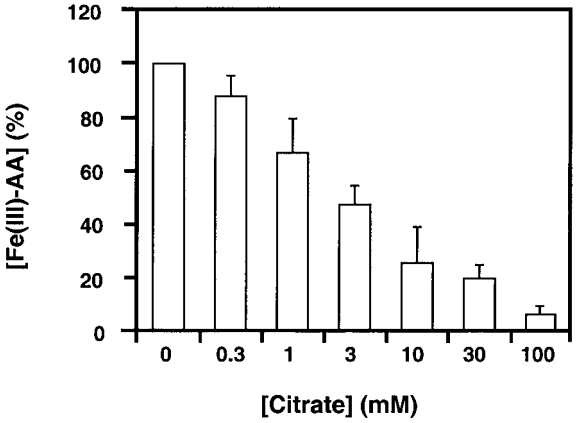

Fig. 3. Citrate/AA competition: Fe(III)-AA (50 μM) was incubated with the indicated concentrations of Na-citrate. The data represent the fraction of Fe-AA relative to the control without citrate in %. (Means ± SD, n = 3).

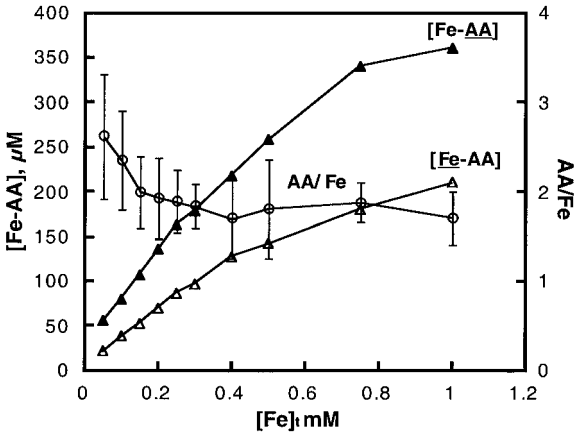

Fig. 4. Calculation of AA/Fe ratio from spectroscopic titration of Fe-AA. Solutions of 500 μM AA were mixed in ethanol with 50–1,000 μM $FeCl_3$ and the absorption spectra were measure as in Figure 2. Fe-AA representing complexed AA, was determined from the absorbance at 550 nm, and Fe-AA representing complexed Fe^{3+} was calculated from the decrease in absorption at 395 nm as explained under Materials and Methods. AA/Fe represents the calculated ratio of Fe-AA/Fe-AA (n = 3).

The apparent strength of association of Fe(III) with AA in aqueous solutions, was measured by following the decrease in absorption at 550 nm of 10 μM Fe-AA induced by various concentrations of citrate. As shown in Figure 3B, about 3 mM citrate were required to dissociate 50% of the Fe-AA complex. The final absorption was stable for at least 24 h. This result suggested that the affinity of AA for Fe(III) was several orders of magnitude higher than that of citrate anions.

An attempt to determine the ratio of AA/Fe in Fe-AA was made by spectroscopic titration of a con-

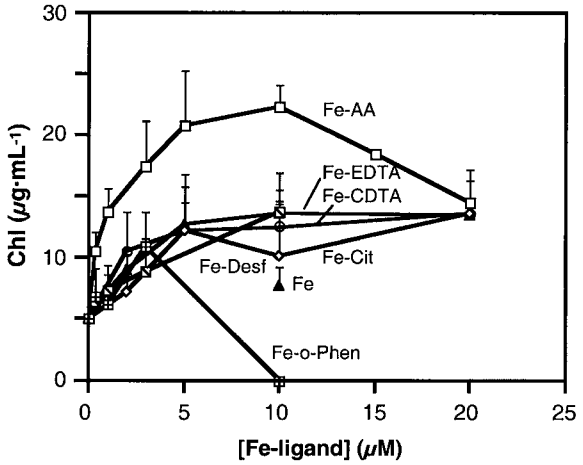

Fig. 5. Effects of different Fe(III)-chelates and Fe(III)-AA on chlorophyll content. Cit- citrate, Desf- desferral, 0-phen-*o*-phenanthroline, Fe- 10 μM Fe(III)Cl_3. Control Chl level refers to cells cultured with no added Fe(III). (means $\pm$ SD, n = 3).

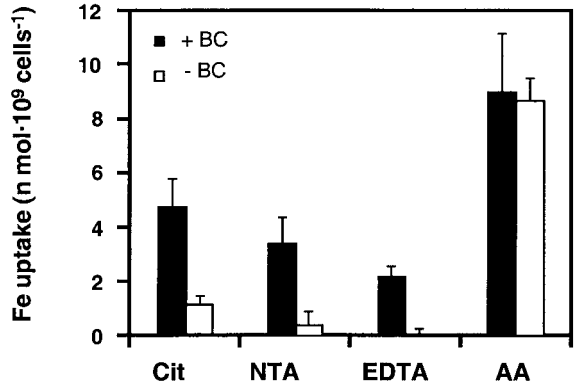

Fig. 6. Effect of bicarbonate on Fe(III) uptake from different Fe(III)-ligands. Solid bars- plus 30 mM bicarbonate, open bars- no added bicarbonate. AA- 20 μM AA/ 10 μM ^{59}Fe(III), Cit- 2 mM citrate/10 μM ^{59}Fe(III), NTA-2 mM NTA/10 μM ^{59}Fe(III), EDTA-50 μM EDTA/10 μM ^{59}Fe(III). (means $\pm$ SD, n = 3).

stant AA concentration in methanol with increasing concentrations of $FeCl_3$. The concentration of Fe-AA (corresponding to complexed AA), was determined from the absorbance at 550 nm, and the concentration of Fe-AA (corresponding to complexed Fe^{3+}) was calculated from the decrease in absorption at 395 nm (Figure 2) as explained under Materials and methods. As shown in Figure 4, the calculated ratio of AA/Fe is close to 2.

Antimycin A stimulates Fe uptake in Dunaliella salina

To test if Fe-AA can serve as a bioavailable source of Fe, it was added to Fe-depleted cultures of the halotolerant alga *D. salina* and tested for its effect on growth, synthesis of Chl, a common indicator for Fe limitation, and uptake of Fe. Ethanolic mixtures of Fe-AA, promoted synthesis of chlorophyll (Chl) better than supplementation with other Fe-chelates such as Fe-citrate or Fe-EDTA at low concentrations, but inhibited Chl synthesis at higher concentrations (Figure 5). Similarly, Fe-AA promoted growth better than Fe-citrate or Fe-EDTA at micromolar concentrations (not shown). Neither $FeCl_3$ nor AA alone were effective, indicating that the active element is a complex between AA and Fe(III) ions.

Table 1. Fe uptake from Fe(III)-AA, Fe(III)-citrate and Fe(III)-EDTA.

	Fe ligand (μM)[a]	Fe content (n mol Fe $\cdot 10^9$ cells^{-1})[b]
Fe(III)-AA	0.5	4.7 $\pm$ 0.55
Fe(III)-AA	5.0	12.7 $\pm$ 1.0
Fe(III)-citrate	1.0	2.2 $\pm$ 0.4
Fe(III)-citrate	10.0	4.9 $\pm$ 0.4
Fe(III)-EDTA	1.0	1.3 $\pm$ 0.2
Fe(III)-EDTA	10.0	2.8 $\pm$ 0.3

[a]concentrations refer to Fe(III). Fe(III):AA=1:4.
[b]*D. salina* cells were cultured for 3 h with the indicated Fe(III) ligands . Means $\pm$ SD, n = 3.

Measurements of Fe uptake by *Dunaliella* cells show that Fe(III)-AA also stimulated Fe uptake in *D. salina* cells to significantly higher levels than comparable concentrations of Fe(III)-citrate Fe(III)-EDTA (Table 1). Repeated washes of Fe-loaded cells with EDTA solutions (Fisher *et al.* 1998) did not significantly decrease Fe content indicating that the AA-mediated Fe(III) ions have been internalized.

The correlation between enhanced chlorophyll synthesis and enhanced accumulation of Fe, indicate that Fe-AA serves as an effective source of Fe for *Dunaliella*.

The observation that AA stimulates Fe uptake in *D. salina* may reflect either activation of a native iron transporter or direct mediation of Fe transport by AA itself. As mentioned above, the major Fe transport mechanism in *D. salina* is via a membrane-associated transferrin (TTf), and the characteristic feature of this mechanism is the dependence on bicarbonate ions, which are coligands Fe(III) binding in transferrins (Fisher *et al.* 1998). Indeed, as shown in Figure 6, when Fe in the assay medium was chelated by citrate, NTA or EDTA, its uptake was dependent on bicarbonate, whereas AA-catalyzed Fe uptake was

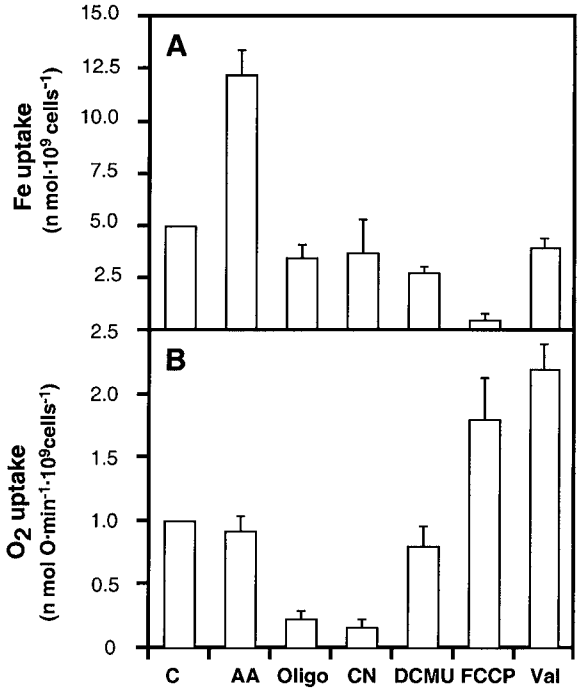

Fig. 7. Effect of metabolic inhibitors on Fe(III) uptake and respiration. C- control without inhibitors, AA- 100 μM, Olig- 200 μg oligomycin, KCN- 100 μM, DCMU- 1 μM, FCCP- 2 μM, Val- 5 μM valinomycin plus 30 mM KCl. (Means $\pm$ SD, n = 3–5).

practically independent on bicarbonate ions. These results suggest that AA does not activate Fe uptake via TTf.

Since AA is a strong respiratory inhibitor, it raises the question whether its effect on iron utilization in *D. salina* is related in any way to its electron transport inhibitory activity. As shown in Figure 7, other respiratory inhibitors (cyanide, oligimycin), a photosynthetic inhibitor (DCMU) or ionophores (FCCP, valinomycin) did not enhance Fe uptake (Figure 7A). Also in variance with respiratory inhibitors, AA did not inhibit oxygen uptake activity of intact cells (Figure 7B) suggesting that it is not an effective respiratory inhibitor in *D. salina* and that its effect on iron acquisition is not related to inhibition of respiration.

Discussion

Antimycin A as an Fe(III) ligand

The spectroscopic and solubility characteristics of AA, combined with its biological activity on the bioavailability of Fe(III) in *D. salina* strongly suggest that AA specifically interacts with Fe(III) ions

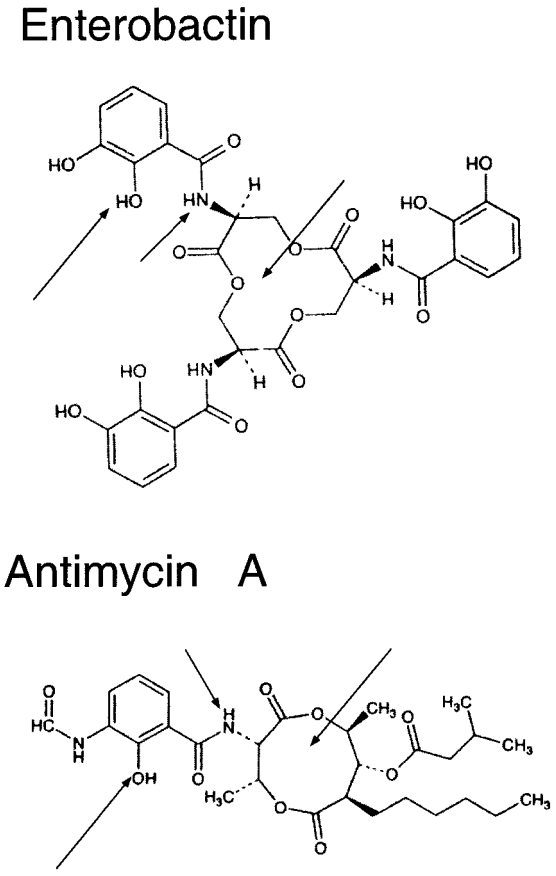

Fig. 8. Chemical structures of enterobactin and antimycin A. Arrows indicate similar chemical parts.

forming stable complexes. However, from the chemical structure of AA (Figure 8) it is evident that one molecule of AA cannot form stable complexes with Fe(III). Indeed, attempts to calculate the Fe(III)-AA stoichiometry from spectroscopic titration of AA with $FeCl_3$, yielded a ratio of 2 AA equivalents per Fe(III).

AA has a partial resemblance to enterobactin, the siderophore secreted by *E. coli*, in the chemical structure and spectral characteristics. The dilactone ring connected through an amide bond to salicylic acid derivatives in AA resembles the trilactone ring which is similarly connected to three hydroxysalicylic acid units in enterobactin except for the substitution of the formamido group in AA for one hydroxyl in enterobactin (Figure 8). Enterobactin in the absence of Fe(III) ions does not absorb in the visible spectrum, whereas Fe-enterobactin has peak absorption at 495 nm with mM extinction coefficient of 5.6. Moreover, an enterobactin hydroxysalicylate model compound, N,N-dimethyl-2,3-dihydroxybenzamidine

(DMB), interacts with Fe(III) ions in a pH-dependent manner, yielding complexes which absorb maximally at 485 nm and 580 nm in basic and acidic pH, respectively, with an apparent pK around pH 7, very similar to AA (Harris *et al.* 1979). This implies a similar association with Fe(III) ions. These similarities raise the interesting possibility that AA may have a secondary function as a Fe(III) siderophore in *Streptomyces* in addition to being a potent respiratory toxin. This prediction is intriguing in view of the fact that *Streptomyces* utilize desferrioxamines and desferrithiocin as Fe siderophores (Müller & Raymond 1984; Bergerson *et. al.* 1991).

Mechanism of AA-mediated Fe uptake in D. salina

What are the implications of these results on Fe metabolism in *D. salina*? Various species of phytoplankton can utilize bacterial siderophores as a source of Fe (Soriadengg & Horstmann 1995; Hutchins *et al.* 1999; Kuma *et al.* 2000) and at least one species of *Dunaliella, D. bardawil* utilizes bacterial factors to enhance iron acquisition (Keshtacher-Libson *et al.* 1995). However, the possibility that *D. salina* utilizes AA as a Fe(III) mediator in its natural habitat is unlikely because *Streptomyces* species are not common in hypersaline environments characteristic to *D. salina*. The most likely interpretation of the results is that AA functions as an artificial mediator of Fe(III) by forming stable lipid-soluble Fe(III)-AA complexes which penetrate through the cell membrane and can be directly utilized by the cells. Similar uptake mechanisms have been demonstrated for utilization of lipophilic organic complexes of Cu and Ni ions by aquatic organisms (Pinney & Bruland 1994; Campbell 1995).

A puzzling and potentially useful observation is the finding that AA hardly inhibited respiration in *D. salina* and affected growth only at relatively high concentrations. A possible reason for the resistance to AA may be that it is neutralized by the algae, for example by cleavage of the dilactone ring by esterases. The inhibition observed at high concentrations of Fe(III)-AA (>10 μM), which was correlated with accumulation of Fe, may be either due to inhibition of respiration or to Fe toxicity which is associated with the production of reactive oxygen species (ROS) as amply demonstrated in many organisms. The low sensitivity of *D. salina* to AA may have practical applications for selective elimination of predators and contaminations from commercial cultures of *Dunaliella*, and possibly of other algae. Supplementation with low concentrations of AA may have a dual function, both in improving Fe(III) acquisition and in protecting algal cultures against predators and contamination.

Acknowledgements

This work was supported by grants from the Israel Science Foundation (Nu. 623/98-1), from Minerva Foundations (Munich, Germany) and the Wilstäter Center of Photosynthesis Research. The author wishes to thank Prof. Abraham Shanzer from The Department of Organic Chemistry at The Weizmann Institute of Science for helpful suggestions and for critical reading of the manuscript.

References

Arnon DI. 1949 Copper enzymes in isolated chloroplasts: Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol* **24**, 1–15.

Ben-Amotz A, Avron M. 1983 On the factors which determine massive ß-carotene accumulation in the halotolerant alga *Dunaliella bardawil. Plant Physiol* **72**, 593–597.

Benderliev KM, Ivanova NI. 1994 High-affinity siderophore-mediated iron transport system in the green alga *Scenedesmus incrassatulus*. *Planta* **193**, 163–166.

Bergerson RJ, Weigand J, Dionis JB, Egli-Katmakka M, Frei J, Huxley-Tencer A, Peter AA. 1991 Evaluation of desferrithiocin and its synthetic analogs as orally effective iron chelators. *J Med Chem* **199**, 2072–2078.

Butler A. 1998 Acquisition and utilization of transition metal ions by marine organisms. *Science* **281**, 207–209.

Campbell PGC. 1995 Interactions between trace metals and aquatic microorganisms: A critique of the free-ion activity model. In: Tessier A & Turner DR eds. *Metal speciation and bioavailability in aquatic systems*. New York: Wiley-Interscience; 45–102.

Coale KH, Johnson KS, Fitzwater SE, Gordon RM, Tanner S, Chavez FP, Ferioli L, Sakamoto C, Rogers P, Millero F, Steinberg P, Nightingale P, Cooper D, Cochlan WP, Landry MR, Constantinou J, Rollwagen G, Trasvina A, Kudela R. 1996 A massive phytoplankton bloom induced by an ecosysem-scale iron fertilization experiment in the equatorial Pacific ocean. *Nature* **383**, 495–501.

Fisher M, Gokhman I, Pick U, Zamir A. 1997 A structurally novel transferrin-like protein accumulates in the plasma membrane of the unicellular green alga *Dunaliella salina* grown in high salinities. *J Biol Chem* **272**, 1565–1570.

Fisher M, Zamir A, Pick U. 1998 Iron uptake by the halotolerant alga *Dunaliella salina* is mediated by a plasma membrane transferrin. *J Biol Chem* **273**, 17553–17558.

Harris WR, Carrano CJ, Cooper SR, Sofen SR, Avdeef AR, McArdie JV, Raymond KM. 1979 Coordination chemistry of bacterial iron transport compounds. 19. Stability constants and electrochemical behavior of ferric enterobactin and model complexes. *J Am Chem Soc* **101**, 6097–6104.

Hutchins DA, Witter AE, Butler A, Luther III GW. 1999 Competition between marine phytoplankton for different chelated iron species. *Nature* **400**, 858–861.

Keshtacher-Libson E, Hadar Y, Chen Y. 1995 Oligotrophic bacteria enhance algal growth under iron-deficient conditions. *Appl. Environ Microbiol* **61** 2439–2441.

Kuma K, Tanaka J, Matsunaga K, Matsunaga K. 2000 Effect of hydroxamate ferrisiderophore complex (ferrichrome) on iron uptake and growth of a coastal marine diatom, *Chaetoceros sociale*. *Limnol Oceanogr* **45**, 1235–1244.

Maldonado MT, Price NM. 2000 Nitrate regulation of Fe reduction and transport by Fe-limited Thalassiosira oceania. *Limnol Oceanogr* **45**, 814–826.

Müller G, Raymond KN. 1984 Specificity and mechanism of ferrioxamine-mediated iron transport in *Streptomyces pilorus. J Bacteriol* **160**, 304–312.

Pick U. 1981 Liposomes of a large trapping capacity prepared by freezing and thawing of sonicated phospholipid mixtures. *Arch Biochem Biophys* **212**, 186–194.

Pinney JT, Bruland KW. 1994 Uptake of lipophilic organic Cu, Cd, and Pb complexes in the coastal diatom *Thalassiosira weissflogii. Environ Sci Technol* **28**, 1781–1790.

Shaish A, Avron A, Pick U. 1993 Are active oxygen species involved involved in induction of β-carotene in *Dunaliella bardawil*? *Planta* **190**, 363–368.

Soriadengg S, Hortsmann U. 1995 Ferrioxamine-B and ferrioxamine-E as iron sources for the marine diatom *Phaeodactylum tricornutum*. *Mar Ecol Prog Ser* **127**, 269–277.

Trick CG, Kerry R. 1992 Isolation and purification of siderophores produced by cyanobacteria, *synechococcus* sp PCC-7942 *and Anabaena variabilis* ATCC-29413. *Curr. Microbiol* **24**, 241–245.

Wilhelm SW, MacAuley K, Trick CG. 1998 Evidence for the importance of catechole-like siderophores in the iron-limited growth of cyanobacteria *Limnol Oceanogr* **43**, 992–997.