

Review

Marine biodiversity and chemodiversity: a tale of many different stories, with a dicey outcome

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Abstract

Marine biodiversity as we see it today on our planet is the result of some four billion years of evolution of life. If the initial stages of this story are conjectural, all life forms have always been associated with the presence of water and its associated chemistries. With metabolisms adapting to forms of energy progressively made available, with the conquest of new media and new territories, biodiversity has made quantum jumps and suffered occasional natural catastrophes. Under stabilized climatic eras, marine communities generate around natural architectures, sometimes of colossal dimensions, such as kelp beds or coral reefs. Complex niches appear in order to accommodate life forms having different trophic requirements, some life forms serving as food or as substrate or as carrier to other life forms. Chemical diversity is well reflected by the sophistication of the metabolic repertoire and its associated transcriptomic syntax, in a molecular language necessarily elaborate to support biodiversity on a sustainable basis. Yet today this stability can be harmed by human overcontrol of the environment, for example by producing a vast number of new chemicals which compete with natural molecules in an unfavorable way. Finally, chemical elements or molecules which have a special role in the marine world will be given as examples of 'evolutionary singularities'.

Keywords: Marine biodiversity; chemical diversity; marine chemical singularities; evolution.

Résumé

La biodiversité marine telle que nous l'observons aujourd'hui est le résultat d'environ 4 milliards d'années d'évolution. Si les étapes initiales de cette histoire sont hypothétiques, toutes les formes de vie ont été associées à la présence d'eau et à ses chimies associées. Avec des métabolismes s'adaptant aux formes d'énergie progressivement disponibles, avec la conquête de nouveaux milieux et de nouveaux territoires, la biodiversité a réalisé des sauts quantiques et subi de nombreuses catastrophes naturelles. Dans des conditions climatiques stabilisées, des communautés marines ont émergé autour d'architectures naturelles, parfois de dimensions colossales, comme les lits de varech ou les récifs de corail. Des niches complexes sont apparues, accueillant des formes de vie aux exigences trophiques différentes, certaines servant de nourriture ou de substrat ou de transporteur à d'autres formes de vie. La diversité chimique est bien reflétée par la sophistication du répertoire métabolique et la syntaxe transcriptomique qui lui est associée, dans un langage moléculaire suffisamment élaboré pour supporter la biodiversité sur une base durable. Aujourd'hui, cependant, cette stabilité peut être mise en défaut par l'action de l'homme sur l'environnement, par exemple en produisant une énorme quantité des nouvelles molécules susceptibles d'entrer en compétition d'une façon défavorable avec les molécules naturelles. Enfin, on présentera comme des exemples 'de spécificités évolutives' des éléments chimiques ou des molécules qui ont un rôle spécial dans le monde marin.

Mots clefs: Biodiversité marine; chimiodiversité; spécificités chimiques marines; évolution.

1. Introduction

Evolution can be regarded as a continuous process with its chemical, biological and cultural components unfolding from one another, and in constant interaction. The anthropic influence on the biodiversity and chemodiversity of planet earth is a consequence of the uprise of human culture and of its demands on natural resources. A definite turn was taken in the second half of the 19th century with industrialization, in particular with the exploitation of fossil hydrocarbons as energy sources and later for the synthesis of organic polymers. At the same time, Darwin showed that biodiversity and chemodiversity are linked and are the result of constant interactive processes. Now, our contribution to biodiversity with genetically modified organisms and to chemodiversity with the synthesis of new molecular structures is enormous and is exceeding our capacity for proper recycling and disposal. The impact of fossil energy utilization may also lower the fitness of natural ecosystems and their resilience limits to constant accumulation of wastes from anthropic activities is often reaching a point of no-return.

2. Chemodiversity came first, then biodiversity followed

Planet earth was formed some 4.6 billion years ago, from the accretion of solar matter which *nuclear chemistry* had already differentiated into all known atoms since the Big Bang. The young planet's degassing atmosphere was not oxygenated but condensation reactions and gradual cooling yielded liquid water which formed the original ocean in which *inorganic ions* of the outer magma solubilised as salts. The Hadean atmosphere was reducing, and the formation of the essential bricks of life depended on *radical chemistry*. The rise of *organic chemistry*, i.e. carbon-based, corresponded to a definite enrichment in chemodiversity with the development of chirality and the making of simple amino acids, monosides, nucleic acids and lipids. Differentiation of chemical microenvironments from the "primitive soup" was necessary for life-essential processes. *Biochemical reactions* might have appeared in vacuoles within the confines of self assembling lipids. *Polymer building* might have been favored by inclusions of charged clays acting as *catalysts*, while electron transfer processes across vacuole membranes occurred. Self-replication of such micro-environments and self vs. non-self differentiation (recognized as life-defining conditions), can be considered as the premises of biodiversity. Early prokaryotes in Archean times were anaerobic and depended upon reductive sulfur-iron chemistry for energy, yet they quickly differentiated according to available niches - *biodiversity* was born. Direct use of sunlight as energy source and the emergence of appropriate electron-transfer machinery allowed cyanobacteria to thrive and produce oxygen which enriched the atmosphere to levels that became toxic to themselves. The engulfment of photosynthetic bacteria into phagotrophic hosts, known as primary endosymbiosis, may have been the answer: the chemoautotrophic Archea host enjoyed access to an extra energy source, whereas the cyanobacteria was able to get rid of its cell wall, becoming a plastid with its genome reduced to the necessary minimum. Secondary and tertiary endosymbioses gave rise to plant, algal and fungal lineages. Mitochondria are probably the remnants of a proteobacterium-like bacterial symbiont, and it is believed by some that the nucleus and organelles observed in extant eukaryotic cells are the result of successive similar fusion-acquisition scenarios. Distant cell-cell communication and competition between the new entities called for the use of specific chemical effectors, which added to the existing repertoire of *natural products*. Multicellularity became possible with the coming of the eukaryotic mode of life, sometimes undergoing complex life cycles with alternating sexual and asexual phases. Sessile marine organisms, plants and invertebrates extensively rely on secondary metabolites for defense and access to life-essential resources. They also depend on contact or proximal interactions with mutualistic or symbiotic prokaryotes to ensure that each event in the course of their existence (larval/spore dispersal and settlement, metamorphosis, proper growth form and general fitness) is optimized. Indeed the synthesis of bioactive molecules extracted from marine invertebrates (sponges, tunicates etc.) is dependent upon the presence of the proper bacterial microflora. Attempts to isolate these associated strains usually fail, and an optimal microenvironment as provided by the natural host is necessary for quorum-sensing conditions to be reached, and thenceforth for the production of the molecules or the expression of participating enzymes to occur. In complex and biodiverse ecosystems, such as in coral reefs, chemodiversity is optimal but the natural equilibrium is fragile.

Marine communities generate around natural architectures, sometimes of colossal dimensions, such as kelp beds or coral reefs. Complex niches appear in order to accommodate life forms having different trophic requirements, some life forms serving as food or as substrate or as carrier to other life forms. Each entity uses its own chemical "vocabulary" (metabolomics) in response to physiological status and to stresses generated by climatic and biotic interactions. The underlying "grammatical" rules (transcriptomics) reflect the genome's ability to maintain and perpetuate the species.

3. Man-made biodiversity, chemodiversity and cultural evolution: the last 150 years

Ever more sophisticated volatile and diffusible molecules are generated to address specific medical, cosmetic or biotechnological needs. New structures are invented using combinatorial chemistry. Pharmacophores are copied and modified from natural products, or produced at industrial scale by genetic engineering (genetically modified organisms). All are products of our *cultural evolution*. Chemodiversity has now reached a level that has never been attained before, yet disposal and recycling aspects have been neglected. As a result, improper degradation of toxic or bioactive components of urban effluents, generate a negative impact on wildlife fitness and biodiversity.

Research is now concentrating more on how ecosystems function in order to find ways to slow down their degradation, rather than to see them as resources to exploit. Natural product chemists are now more interested in why secondary metabolites are produced and in how they function in their natural setting, rather than only considering them as potential drugs. The integration of genetics, transcriptomics, proteomics and metabolomics brings life sciences and chemistry together to defend the noblest of all causes: our environment and its diversity. Singularities in evolution are now spotted out by evolutionists, and the next two sections are devoted to two elements, vanadium and iodine, which have each had a fundamental role in the evolution of life and chemodiversity, among other examples.

4. Vanadium as a case study

4.1. 'Heavy metals' as pollutants

The term "heavy metal" is scientifically nonsensical (Duffus, 2002) and belongs to the fuzzy terminology used by environmentalists. These elements (which are not inevitably "heavy" or strong Lewis electron acceptors) cause metabolic dysfunctions to organisms that incorporate them under an absorbable form and accumulate them in their tissues. Not only is the consumer's own fitness affected but also that of its offspring: it is the entire food chain that ends up being threatened as the culprit element is being accumulated under its toxic form(s) in ever greater concentrations, to sub-lethal or lethal levels. This type of pollution is clearly dominated by anthropic activities, as witnessed by countless reports on fluvial, estuarine and harbor contamination evidencing a net negative impact on the exposed biodiversity.

4.2. 'Heavy metals' as essential oligo-elements

A less common use, yet just as ill-defined, of the term "heavy metals" makes reference to a series of di- or multivalent cationic oligo-elements that are accumulated quite specifically by some marine plants and invertebrates, in order to perform metabolic tasks which are not always fully understood, notwithstanding their known toxicity at low concentrations in other organisms (Miramand & Unsal, 1978). Such is the case for vanadium which can be actively concentrated from seawater in specific cells or organs of some groups of ascidians (Mishibata *et al.*, 2002) or passively and at much lower concentrations in the body wall or test of holothurians and sea-urchins (Miramand *et al.*, 1982). In the case of didemnid and tunicate ascidians, detoxification is carried out by transforming the pentavalent V^V toxic form of vanadium into a non-toxic, usable V^{III} form as blood pigment or protein cofactor. More specifically, vanadium is concentrated from seawater up to ten million times in the vacuoles of specific blood cells (signet ring cells, Mishibata *et al.*, 2002) called vanadocytes, where it is maintained in a non-toxic reduced state (3+) as complexes with sulfate ions, *e.g.* $[V(H_2O)_6]^{3+}$ or $[V(H_2O)_5(SO_4)]^+$, as depicted in a simplified form in Figure 1.

The sulfate ions were recently found to be actively incorporated into the vacuoles (via a Na^+ dependant sulfate transporter protein AsSUL1 in the ascidian *Ascidia sydneiensis samea* (Ueki *et al.*, 2009). Using K-edge XAS spectroscopy on a different tunicate model (*Ascidia ceratodes*), Franck *et al.* (2008) determined V and S speciation in different blood cell types (including vanadocytes) and in blood plasma, wherefrom they postulated a novel redox mechanism for vanadium reduction in ascidians, which involves a vanadium reductase. The sequence is as follows: (i) a vanadate complex is organized in the catalytic pocket of the metalloenzyme from picking up oxidized (V^V) $H_2VO_4^-$, (ii) a first reduction to vanadyl V^{IV} then (iii) a second reduction to vanadic V^{III} form occur, followed by (iv) hydrolytic dissociation of the $[V(H_2O)_6]^{3+}$ reduced form, leaving the catalytic pocket ready to pick up a new vanadate ion. The redox activity reported here for vanadium is quite original and unique in vanadium-protein assemblies, allowing the intracellular use of vanadium, at the expense of resorting to a multi-step vanadate reductase cycle. Future investigations will undoubtedly highlight this biochemical mechanism as yet another singularity akin to the use of cellulose in the ascidians, which are regarded as a "deviant" phylum since whole-genome comparisons were made against other chordate lineages (Dehal *et al.*,

2002). In brown algae, vanadium is used both extracellularly in its available form (V^V) as the cofactor of apoplasmic vanadium-dependant haloperoxidases (V-HPO), thereby eluding toxicity problems, and intracellularly probably via appropriate chelators, as cofactor of cytoplasmic haloperoxidases. Algal V-HPOs are redox-inert (V^V) with a catalytic site featuring a coordinate chemistry identical to that of acid phosphatases (Hemricka *et al.*, 1997, Littlechild *et al.*, 2002). Indeed, the similarity between vanadate and phosphate is the favoured explanation for vanadate accumulation in vertebrate bones, and hence for the vanadate complex as non-heme cofactor of peroxidases, in lieu of phosphate.

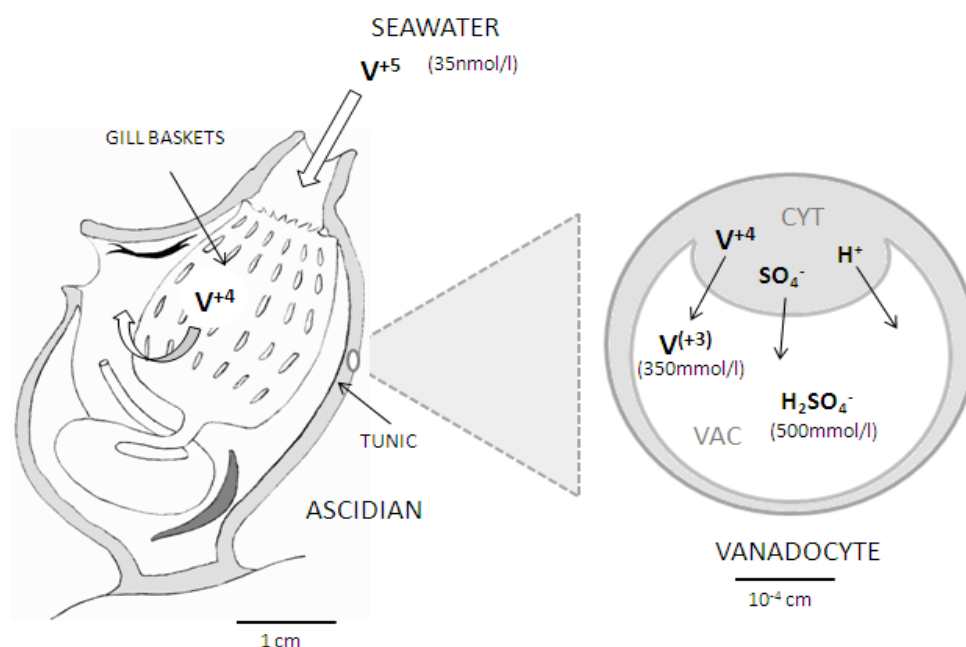


Figure 1. Vanadium accumulation pathway in tunicates. In seawater, vanadium ions are in the +5 (V^V) oxidation state as HVO_4^{2-} or as $H_2VO_4^-$ ions, at very low (35nM) concentrations. Past the branchial baskets, most of the vanadium is incorporated and reduced to the +4 oxidation state (VO^{2+} ; V^IV). When concentrated into the vacuoles (VAC) of the vanadocytes from the cytoplasm (CYT), vanadium is further reduced to the +3 oxidation state (V^{3+} ; V^{III}) in a highly protonated (pH= 1.9) environment in which V^{III} and sulfate ions coexist as counterions, forming complex cations (adapted and simplified from Mishibata *et al.*, 2002, p. 431).

4.3. When 'heavy metals' are not 'oligo' elements anymore

As of now, there is no clear explanation to account for the presence of such high levels of vanadium in the blood of ascidians (very unusual chordates), nor for the co-occurrence of other "soft" and "hard" transition metals such as Cr, Mn, Co, Ni, Mo and W. Tissue acidity (pH < 2) appears to be the major chemical defense in tunicates that concentrate sulfuric acid in their vanadocytes and in the tunic, (Odate & Pawlik, 2007 on *Phallusia nigra*), rather than heavy metal toxicity due to bioaccumulation in the tunic which is directly exposed to predation (Parry, 1984). This lack of insight into the significance of such bioaccumulation phenomena observed in many filter-feeding marine invertebrates comes at a time when the "heavy metal" input into the marine environment, *e.g.* vanadium generated by the burning of fossil fuels, the discards of oil refineries and bauxite treatment, far exceeds the estimated natural recycling capabilities (Hope, 2008).

5. Iodine as a case study

5.1. Halogens as ion makers

Halogens share with "heavy metals" their ability to be concentrated to many times environmental levels, by filter feeding invertebrates and by macrophytic algae, without being involved as permanent structural components. Whereas "heavy metals" are regarded as "those ecologically disturbing cationic elements" that are scattered on the left hand side of the periodic table, halogens belong to a discrete and chemically coherent group of highly reactive anionic elements that have a central role in trans-membrane communication that is so important in marine life processes.

5.2. Evolutionary importance of iodine

The molecular mass of iodine (126.90 U) is the highest by far of all elements used in biological systems, including metals, yet the evolutionary importance of iodine is quite unique. Early prokaryotes (LUCA) had to carry out the conversion of toxic environmental iodate into iodine which could be readily incorporated in the cytoplasm and perform a number of essential catalytic functions, *e.g.* by coupling with essential organic molecules. The ubiquity of iodinated tyrosines (*e.g.* mono- and di-iodotyrosine or MIT and DIT) across extant eukaryotic phyla evidences their general function as endocrine molecules involved in cell-cell communication (in plants) as well as time-coordinated and dose-dependent developmental changes in eukaryotes, as reviewed by Crockford (2009). Tyrosin halogenation is believed to occur spontaneously, *i.e.* not by enzyme mediation (Morrison & Schonbaum, 1976), which may explain the evolutionary importance of MIT and DIT. If thyroid hormones (TH), which are built from the same components as MIT and DIT (Fig. 2), are well characterized in vertebrates as signaling molecules that perform fundamental roles as regulators of development, metabolism, growth and differentiation, there is increasing evidence that THs also operate in invertebrates. Heyland & Moroz (2005) postulate that THs function as cross-kingdom hormones that are produced by phytoplankton, ingested or absorbed by invertebrate larvae (*e.g.* veligers of molluscs, pluteus of echinoderms) at the next trophic level (zooplankton), where they act as regulators of development, specifically during metamorphosis and larval settlement. Early chordates seem to have the ability to synthesize TH hormones in the endostyle, an organ regarded as homologous to the vertebrate thyroid. There is now enough evidence to establish the persistence and coherence of THs across phyla, from marine forms to terrestrial forms, possibly right from the rise of oxygen in the post-archean atmosphere some 2.5 billion years ago, as already proposed for MIT and DIT. The prevalence of iodine chemistry in very early life forms has thus led to interesting speculations as its cornerstone role as a catalyst and inorganic antioxidant (without which the emergence of multicellularity would never have taken place (Crockford, 2009). Indeed, iodine is regarded as an additional biomarker towards the presence of life in extraterrestrial environments, along with traces of water presence and fossilized bacterial biofilms.

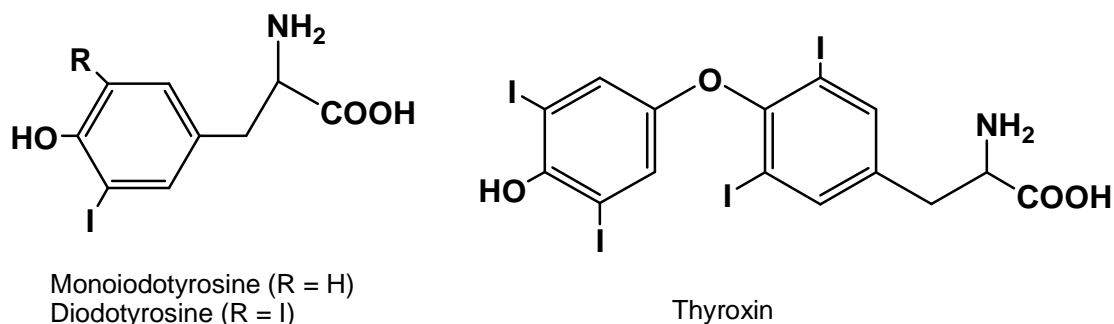


Figure 2. Mono- (MIT) and diiodotyrosine, (DIT), and TH (thyroxine and derived).

5.3. Iodine as antioxidant and antimicrobial agent

In order to survive, sessile marine organisms have to face regular (*e.g.* tidal, circadian and seasonal) fluctuations and occasional catastrophic changes in their physico-chemical environment, as well as to resist biotic stress (predation and competition for essential resources).

Long exposures to air and sunlight would be detrimental to macroalgae living on the intertidal and upper subtidal zones of temperate seashores, which also host fish, mollusks and echinoderms as potential herbivores. Several mechanisms allow brown algae to withstand direct exposure to air and sunlight. Hydration is maintained by the production of mannitol (slimy mannose-derived diol) which also acts as an antiadherent against fouling by epiphytes, while strong light-absorbing (*i.e.* highly conjugated pigments) and reactive oxygenated species (ROS) detoxicating molecules (*i.e.* highly reactive nucleophilic polyphenols and polyunsaturated fatty acids) provide protection against genotoxic and radical damage. In situations of acute stress (*e.g.* microbial infection following mechanical damage due to herbivore browsing), local overproduction of hydrogen peroxide *i.e.* oxidative stress occurs, followed by vanadium-dependant bromoperoxidase (V-BrPO) mediated production of volatile iodinated and brominated compounds, the whole process performing a coordinated antimicrobial - antioxidant action.

5.4. The biochemical aspects

The speciation of halogenated effluxes in brown algae is far from being as diverse as that of red algae, and it mostly involves iodine and bromine. Monohalomethane (*e.g.* CH₃I) generation is straightforward and involves the transferase - mediated nucleophilic attack of the corresponding halide (*e.g.* iodine) at the electrophilic CH₃⁺S site of S-adenosyl methionine (SAM) (Amashi *et al.*, 2006). Other halomethanes involve the prior oxidation of halide ions as *e.g.* hypohalogenous acids (HOI, HOBr) by V-BrPO in presence of photosynthetically-generated hydrogen peroxide, then methylation into di- (*e.g.* CH₂I₂) or polysubstituted halomethanes, including a variety of hetero-substituted forms (*e.g.* CHBr₂I).

5.5. The evolution of a specificity for iodine in kelps

The catalytic site of vanadium-dependant chloroperoxidase (V- ClPO) can oxidize chloride, bromide and iodide ions in the presence of hydrogen peroxide, but V- IPO (iodoperoxidase) can only oxidize iodide anions under the same conditions (Butler & Sandy, 2009). Gene expression studies in the kelp *Laminaria digitata* led Cosse *et al.* (2008) to formulate an evolutionary hypothesis for iodine specificity in some Laminariales. Duplication from a common ancestral gene may have led to the emergence of two haloperoxidase gene families (Colin *et al.*, 2005) that are expressed in a tightly coordinated manner in defense responses: iodine metabolism seems to be up-regulated at early stages following elicitation to replenish iodine losses while some V-BrPO members are likely to be solicited for anti-oxidant protection and cell wall strengthening. Laminariales are indeed peculiar in their ability to concentrate iodine at blade level at 3.10⁴ to 3.10⁵ times seawater concentration, yet there is no clear answer as to why Laminariales have developed (or retained?) such an important and extensive metabolic dependence to iodine, as compared to most halogen-using marine biota.

5.6. The iodine cycle

Kelp beds act as iodine sinks by accumulating iodine as iodide (I⁻) in the peripheral tissues of the thallus. Direct restitution of iodide ions as molecular iodine (I₂) which turns into atomic I by photolysis, or as V-HPO-mediated volatile haloalkanes (VHOC) is photooxidized as OI by ozone occurs especially during daytime and at low tides (Kupper *et al.*, 2008). Halogenated alkanes are an important component of the biogeochemistry of the temperate marine boundary layer or MBL (reviewed in La Barre *et al.*, 2010, in press), in particular due to the release of I₂ and of photo-labile iodocarbons from macro- and micro-algae. Kelp beds generate a great variety of volatile haloalkanes when exposed. Methyl diiodide CH₂I₂ is the dominant organic iodine species released from kelps (Carpenter *et al.*, 1979; 1980) and it has been suggested as an initiator of the iodine nanometric particles that function as cloud-forming nuclei (McFiggans *et al.*, 2004).

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