Algae
Volume 16(1): 21-24, 2001

[Minireview]

Potential Utilization of Various Molecules from Micro- and Macro-Algae: Goals and Procedures

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Recent advances have been done in the identification, isolation and biological evaluation of use of several kinds of molecules from various algal sources. We aim to give the state of some of the current research done in this domains on both polysaccharides from the cell wall and lipids which are both known as possible sources for valuable molecules with therapeutic effects.

Key Words: antiproliferative, biological activity, fucan, lipid, microalgae, polysaccharide, seaweed

SULFATED POLYSACCHARIDES FROM BROWN SEAWEEDS

The present application of seaweeds rest on some specific chemical and physical properties of phycocolloids. We will essentially focus on fucans for which we have shown a heparin-like activity but it presents a very interesting property: it has a long life effect, larger than that of the classical low molecular weight heparin (LMWH).

In seaweed the cell walls are made up of different polysaccharides (i.e., cellulose, alginates, carrageenans or fucans) and proteins. These polysaccharides have been the subject of intense research during the past 30 years, mainly because they form highly viscous solutions or strong gels that remain stable in the presence of many additives.

Fucan is a collective term corresponding to a family of sulphated polysaccharides found in brown seaweed. In a preliminary study, we isolated a fucoidan fraction from the brown seaweed *Ascophyllum nodosum* (Mauray *et al.* 1995). This fraction with a molecular weight of about 20 kDa showed interesting antithrombotic properties, after intravenous administration to rabbits, relative to unfractionated heparin (UFH). Recent studies, done in collaboration with CNRS and Paris V University, on low-molecular weight heparins (LMWH) have shown that these compounds are at least as effective and safe as UFH and could be considered as good candidate in the prophylaxis and treatment of deep venous thrombosis.

In this study, a low-molecular weight (LMW) fucoidan of 8 kDa was obtained by chemical degradation of high-molecular-weight fucan extract (Fig. 1). The antithrombotic and anticoagulant activities of this new compound

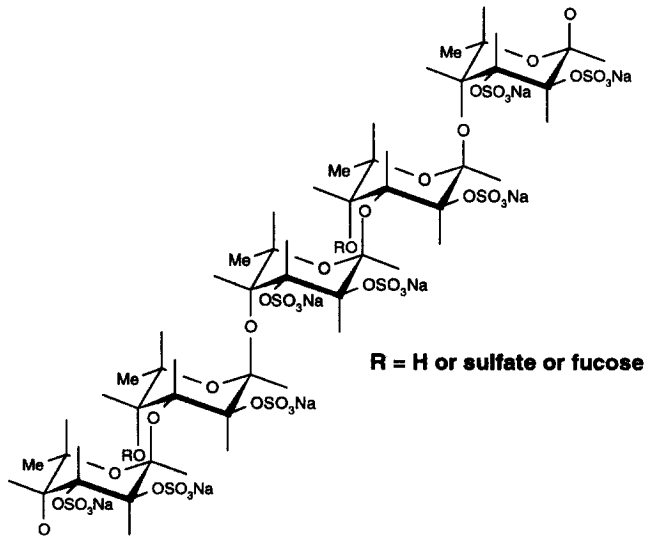

Fig. 1. LMW fucoidan of 8 kDa obtained by chemical degradation of high-molecular-weight fucan extracted from the brown seaweed *Ascophyllum nodosum*.

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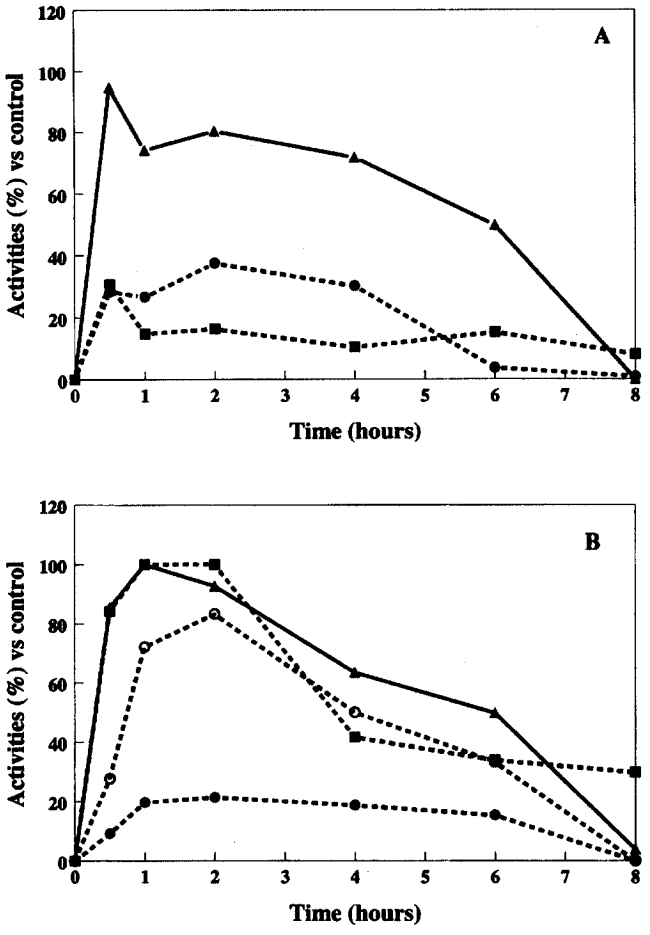

Fig. 2. Time-course of ex vivo anticoagulant and antithrombotic activities of LMW fucoidan (A) at 20 mg/kg (ED_{80}) and dalteparin (B) at 200 anti-Xa IU/kg (dose close to the ED_{80}). The compounds were administered by the subcutaneous route at various times before thrombus induction by bovine factor Xa, in the rabbit. Blood was collected just after thrombosis induction for ex vivo assays: TCT — ■ — ; APTT — ● — (CK Prest Stago); antithrombotic activity — ▲ — and for dalteparin anti-Xa activity — ○ —. Each result is the mean of experiments performed on groups of 5 to 7 treated and control animals. The values are expressed as the percentage variation versus control.

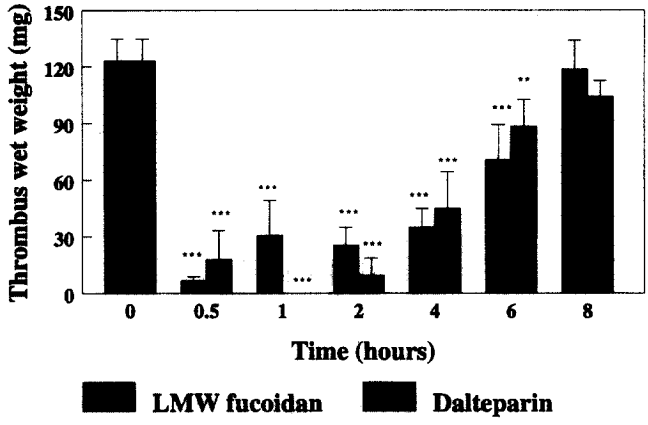

Fig. 3. Time-course of the venous antithrombotic effects in Wessler's model using factor Xa as the thrombogenic agent, following a subcutaneous injection of 20 mg/kg LMW fucoidan or 200 anti-Xa IU/kg dalteparin (antithrombotic ED_{80}) in the rabbit. Each result is the mean of experiments performed on groups of 5-7 treated and control animals. Data are mean ± SEM wet thrombus weights. **: $p \leq 0.01$, ***: $p \leq 0.001$ vs control.

Table 1. Haemorrhagic effects of LMW fucoidan and dalteparin in rabbits

Treatment	Dose (mg/kg) (anti-Xa IU /kg)	Bleeding time (sec)	Ratio	N
Control	0	129.1 ± 13.7	1	8
LMW fucoidan	100	189.6 ± 17.2	1.47	7
Dalteparin	1000	253.4 ± 67.0	1.96	7

were compared to those of a LMWH, dalteparin, following subcutaneous administration to rabbits (Millet *et al.* 1999). This LMW fucoidan exhibited dose-related venous antithrombotic activity, with an ED_{80} of about 20 mg/kg, 2 h after a single subcutaneous injection (Fig. 2). Its activity was comparable to that of dalteparin (close to 200 anti-Xa IU/kg) and was maximal 30 min after a single subcutaneous injection (Figs 2-3). The activity remained stable (about 70%) from 1 to 4 h after injection, and disappeared by 8 h. The effects of LMW fucoidan and dalteparin on the bleeding time 2 h after subcutaneous injection at approximately 5 times their ED_{80} values were evaluated. At this dose, the percentage increase in the bleeding time was lower with fucoidan (47%) than with dalteparin (96%), corresponding to a bleeding time with drug/control bleeding time ratio of 1.47 for LMW fucoidan and 1.96 for dalteparin. However, owing to individual variations in bleeding times, this difference was not significant (Table 1). LMW fucoidan thus has potent antithrombotic activity and a potentially weaker haemorrhagic effect (i.e. a smaller prolongation of the bleeding time) than dalteparin.

LIPIDS FROM MICROALGAE

In the lipid domain, we will present results on the metabolism, the production and the biological activities of lipids in different microalgae.

Microorganisms have historically proven to be an exceptionally rich source of biologically active metabo-

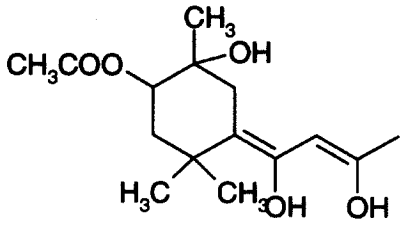

Fig. 4. Degradation product of carotenoids from the marine diatom *Skeletonema costatum*.

lites. These metabolites have been important biomedically as leads to new pharmaceutical compounds. However, due to these previous investigations when new species are examined, the risk of rediscovery of previously described factors has greatly increased. To reduce the rate of reisolation of known factors, the examination of groups of microorganisms poorly investigated in the past constitute the major strategy. For this reason, during the last decade the screening of microalgae, especially the cyanobacteria (blue green algae) has received ever increasing interest for antibiotics and pharmacologically active compounds.

Our first studies were realised on the marine diatom *Skeletonema costatum* that is mass cultivated on the French west coast to feed larvae in aquaculture. The screening of biologically active lipids in this algae led to extraction and purification of two active principles. The first one was a mixture of polyunsaturated fatty acids (PUFA), which possessed an antibiotic effect directed against *Vibrio anguillarum*, a pathogen bacteria in aquaculture (Naviner *et al.* 1999). The second principle was studied for its effect on asynchronous cells of a human non-small-cell bronchopulmonary carcinoma line (NSCLC-N6). Cell growth appeared to be inhibited in the G1 phase of the cell cycle, and kinetic studies in the pre-treated cells showed that this growth arrest was irreversible. These events are related to a terminal maturation induction (Bergé *et al.* 1997). The active principle was further purified and identify as a degradation product of carotenoids (Roussakis *et al.* 2000).

The second part of our work is the improvement of the biosynthesis of known active lipids by microalgae. We studied the change occurring when the conditions of culture are modified. We especially addressed the following question: how to promote the synthesis of one kind of polar lipid : the sulfoquinovosyldiacylglycerol (SQDG) which have been described as a potent antiviral agent.(Gustafson *et al.* 1989) ? We have shown that a deficiency in phosphate led the algae to modify their

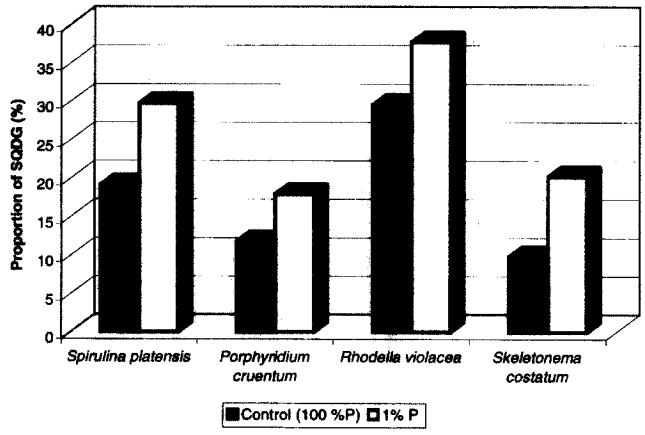

Fig. 5. Proportions of SQDG in different microalgae under two supplementations of phosphates

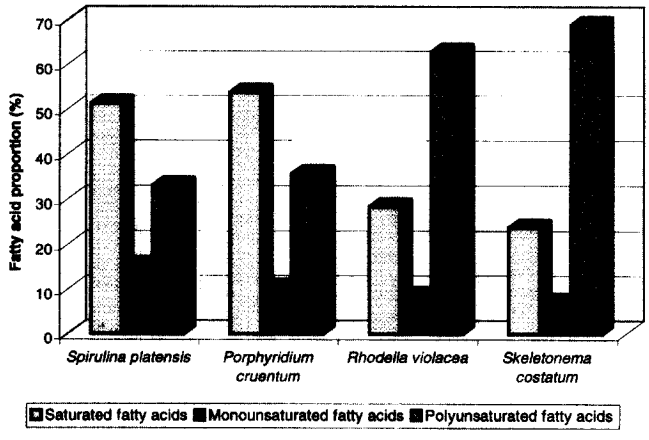

Fig. 6. Fatty acids composition of SQDG in case of deficiency of phosphates

membrane composition in order to maintain the global charge of the membrane (Fig. 5). Thus, the sulfoquinovosyldiacylglycerol increased in the same proportion as the phospholipides reduced. We have also study the global fatty acid composition of the SQDG (Fig. 6) with particular attention to the polyunsaturated one (PUFA). We have then selected 2 microalgae: the red one named *Rhodella violacea* for its high proportion of SQDG under deficiency of phosphates (up to 38%) and the relatively high proportion of PUFA in the sulfolipid ; the diatom *Skeletonema costatum* for its highly unsaturated sulfolipid (69%). The combination of the sulfolipid polar head with a selected fatty acid composition, especially an enrichment in very long chain and unsaturated fatty acids (AA, EPA or DHA) which are abundant in some red and brown algae, is presently under evaluation in order to determine whether or not it gives an improvement of the

antiviral effect.

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Presented at the International Symposium "Prospects of Marine Enrironments and Marine Bioresources in the 21st Century," October 6, 2000, Yeungnam University, Kyongsan, Korea.