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Improved Erythrocyte Lysis Assay in Microtitre Plates for Sensitive Detection and Efficient Measurement of Haemolytic Compounds from Ichthyotoxic Algae†

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Key words: ichthyotoxic algae; erythrocyte lysis assay (ELA); photometric determination; haemolytic substances; carp erythrocytes.

Haemolytic substances produced by ichthyotoxic algae often are unknown in molecular structure or specific mechanism of toxicity. Detection and quantification of such substances are dependent on bioassays, using markers that are sensitive for haemolytic impairment and generation of a recordable response. The erythrocyte lysis assay (ELA) represents an advantageous bioassay in this respect, because the lytic response can be measured photometrically by the amount of released haemoglobin. The aim of the present study was to establish an improved assay based on the ELA principle, for sensitive determination of haemolytic substances of microalgae and for high sample throughput. For this purpose we adapted the ELA to a 96-well microtitre plate format, which significantly reduced the sample volumes and allowed rapid processing of samples. Further improvement was achieved by measuring absorption of lysed erythrocytes at 414 nm, which significantly increased the sensitivity of the ELA compared to the measurements at 540 nm that are usually applied in this type of assay. Using carp (*Cyprinus carpio*) erythrocytes it was possible to detect haemolysis induced by 4 μg ml^{-1} of saponin and as little as two haemolytic *Alexandrium tamarense* cells. It is suggested that this improved ELA in microtitre plates be used as a low-cost monitoring tool for detection and analysis of potential harmful algae. Furthermore, this ELA can be useful as a sensitive screening system for substances of pharmacological interest, e.g. selectively acting cytolytic antibiotics. Copyright © 2001 John Wiley & Sons, Ltd.

INTRODUCTION

Detection and quantification of ichthyotoxic substances produced by algae is an important aspect in marine biomonitoring. In many cases haemolytic activity of ichthyotoxic substances is part of the toxic mechanism.[1–3] The chemical nature of these substances and consequently the mode of causing haemolysis are different between species and often not known (e.g. ichthyotoxic substances of *Chrysochromulina* and *Prymnesium* species[4,5]).

With the erythrocyte lysis assay (ELA) it is possible to detect and quantify the haemolytic activity of chemically different (e.g. fatty acids, peptides, proteins) and even unknown substances, based on lysis of erythrocytes by these compounds and subsequent photometrical determination of the released haemoglobin.[6] This bioassay can be used to detect haemolytic compounds from different organisms such as bacteria,[7–10] marine microalgae,[1–3,11,12] marine invertebrates[13,14] and higher plants (e.g. saponin produced from the bark of *Quillaja saponaria* (soap-bark tree), used as a test substance in this study).

Our special interest is the detection of haemolytic compounds in ichthyotoxic marine microalgae. This group is quite heterogeneous and comprises species from different algal genera, such as *Prymnesium parvum*[1] and *Chrysochromulina polylepis*[2] of the genus Prymnesiophyta and *Gyrodinium aureolum*[3,12] of the genus Dinophyta. The aim of the study presented here was to modify the ELA for a more sensitive detection of haemolytic activity and for increased throughput capacity, allowing rapid processing of large numbers of samples.

We started with a protocol described by Edvardsen *et al.*,[2] which includes the following procedures: collection of erythrocytes from cod (*Gadus morhua*) by heart puncture; extraction of algal toxins using methanol and chloroform; incubation of relatively large sample volumes (3 ml) for the lysis assay; and reading the absorbance of supernatants at 540 nm in cuvettes.

The ELA presented here (patent pending) has been modified with regard to all of these aspects. Because fish are a main target of ichthyotoxic algae, we used erythrocytes from carp (*Cyprinus carpio*). However, blood was collected by caudal vein puncture as described by

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Stoskopf.[15] Thus, the fish were not killed and repeated bleeding without causing severe harm to the animals was possible. Crude algal extracts were prepared for toxin analysis without using hazardous organic solvents and the assay was adapted to a 96-well microtitre plate format to allow rapid processing of large numbers of samples and a significant reduction of sample volumes. Absorbance of lysed erythrocytes was read at 414 nm, which significantly increased the sensitivity of the ELA.

The results obtained with the modified ELA are promising with respect to potential application in, for example, harmful algal monitoring or screening for pharmacologically valuable substances.

MATERIAL AND METHODS

Algal culture

Algae were grown under white fluorescent light in batch cultures in seawater media. Table 1 shows the algal strains investigated and their respective culture conditions.

Preparation of algal extracts

Samples of algal cultures were centrifuged using 14-ml Sarstedt tubes in a swing-out rotor at 3200 *g* for 15 min at 4 °C. Algal pellets were resuspended in assay buffer (150 mM NaCl, 3.2 mM KCl, 1.25 mM $MgSO_4$, 3.75 mM $CaCl_2$ and 12.2 mM TRIS base; pH adjusted to 7.4 with HCl) to yield 10^6 and 10^5 cells ml^{-1} for prymnesiophytes and dinophytes, respectively. Cell suspensions were sonicated with a Sonoplus HD70 disintegrator equipped with a MS73 sonotrode (Bandelin Electronics, Berlin, Germany) using the following settings: 50% pulse cycle, 70% amplitude for 1 min on ice. During the whole preparation procedure, algal extracts were kept on ice in the dark. Besides using freshly prepared algal extracts, we tested algal pellets and extracts stored at −20 °C.

Preparation of saponin standard

Saponin (Sigma-Aldrich, Deisenhofen, Germany; preparation from the soap-bark tree *Quillaja saponaria*) stock solution, 2 mg ml^{-1} in assay buffer, was stored at −20 °C. Aliquots were thawed immediately prior to use and diluted with assay buffer to yield saponin concentrations of 1–20 μg ml^{-1} in the ELA experiments.

Fish husbandry

Carp (*Cyprinus carpio*) 4–5 years old and weighing 2000–3000 g were used for blood collection. The fish were maintained in groups of 7–10 in 450-l tanks at 17–20 °C in recirculated filtered tapwater under conditions free of specific pathogens. They were fed daily to satiation with pelleted dry food (Milkivit, Germany).

Blood collection, storage and preparation

For blood collection and storage, RPMI 1640 culture medium (Rosswell & Park Memorial Institute cell culture medium no. 1640; Biochrom, Berlin, Germany) without phenol red was diluted with 10% (v/v) distilled water to adjust its osmotic pressure according to carp serum osmolarity.[16] Blood collection was performed with 10-ml syringes with a Pravaz No. 1 needle. Syringes were prefilled with 5 ml of diluted RPMI 1640 culture medium supplemented with 50 IU ml^{-1} of sodium heparin (Sigma-Aldrich, Steinheim, Germany) and stored on ice until

Table 1—Strains and sources of the investigated algal species and their respective culture conditions

Species	Strain	Source	Culture medium	Temperature (°C)
Chrysochromulina polylepis	B1511	University of Oslo	IMR (Refs 32 and 33)	15
C. ericina	CCMP 281	Provasoli-Guillard National Centre for Culture of Marine Phytoplankton	IMR	15
C. simplex	—	University of Oslo	IMR	15
C. throndsenii	—	University of Oslo	IMR	15
Prymnesium parvum	RL10	University of Bergen	K (Ref 34)	15
P. nemamathecum	—	Isolate from South Africa	K	15
P. patelliferum	Ply527	Plymouth Marine Laboratory	DREBES (Ref 35)	15
Gyrodinium aureolum	K-0303	University of Copenhagen	IMR	15
G. instriatrium	LME176a1310	Isolate from Portugal	DREBES	20
Gymnodinium chlorophorum	BAHME100	Biological Institute on Helgoland	IMR	15
G. varians	CCMP421	Provasoli-Guillard National Centre for Culture of Marine Phytoplankton	IMR	20
Alexandrium fundyense	CCMP1719	Provasoli-Guillard National Centre for Culture of Marine Phytoplankton	DREBES	15
A. tamarense	CCMP115	Provasoli-Guillard National Centre for Culture of Marine Phytoplankton	DREBES	15
A. lusitanicum	—	Isolate from Portugal	DREBES	15
A. ostenfeldii	BAHME136	Biological Institute on Helgoland	DREBES	15

use. For blood sampling, the carp were anaesthetized in a solution of aminobenzoic acid ethyl ester (Tricaine™, Sigma, St. Louis, USA, 0.02% (w/w) in water from the respective fish tank). Anaesthetized fish were placed on wet tissues and 5 ml of blood per fish was sampled by caudal vein puncture in the ventral approach.[15] The fish were allowed to wake up from the anaesthesia in a separate tank before they were removed to their respective tank. Repeated bleeding of individual fish was performed with a minimum interval of 4 weeks. The filled syringes were kept on ice. Individual blood samples were transferred into sterile 15-ml centrifuge tubes. Blood samples were forwarded overnight on wet ice. Whole fish blood finally was diluted 1:10 with diluted RPMI 1640 culture medium containing 22.5 IU ml^{-1} of sodium heparin. Cultures were stored at 4 °C and cells were resuspended every 2–3 days on a Belly Dancer shaker (Stovall Life Science Inc., Greensboro, NC, USA). For the lysis assay, cell numbers were determined with a Fuchs-Rosenthal chamber. The desired amount of erythrocytes was washed twice with assay buffer (same as for algal extracts) in a 1.5-ml reaction tube by vortexing and centrifugation at 2000 *g* for 5 min at 4 °C. Cell suspensions were adjusted to 10^7 cells ml^{-1} and stored at 4 °C until use.

Erythrocyte lysis assay

The assay was performed in 96-well microtitre plates with V-shaped wells (Nunc, Wiesbaden, Germany) using 120 µl of erythrocyte suspension (10^7 cells ml^{-1}) and 120 µl of algal extract (10^6 and 10^5 cells ml^{-1} of prymnesiophytes and dinophytes, respectively). Erythrocytes incubated in assay buffer alone served as negative controls. For positive controls—representing 100% lysis—parallel sets of samples were sonicated for 30 s on ice at the same settings as for algal extracts. Each set of samples was pipetted in triplicate. Microtitre plates were placed on the ice-cold surface of a metal block. All pipetting steps were performed under dimmed light conditions. Finally, microtitre plates were taped with sealing foil (Nunc, Wiesbaden, Germany) and incubated at 15 °C in the dark for various periods of time. Following incubation, microtitre plates were centrifuged for 5 min at 2000 *g* at room temperature and 200 µl of each supernatant was transferred to a 96-well microtitre plate with U-shaped wells (Nunc, Wiesbaden, Germany). Absorption was read at 414 nm with a Spectra Max 190 plate reader and Soft Max Pro 3.0 Software (Molecular Devices Corporation, Sunnyvale, CA). Absorption at 620 nm served as a reference and was subtracted from the absorption values obtained at 414 nm.

Scanning experiments

Photometric scans were performed with completely lysed carp erythrocytes (5×10^6 cells ml^{-1}; preparation as for positive controls described in the experimental set-up of the ELA) and crude algal extracts of *C. polylepis* (5×10^5 cells/ml; produced as described for the preparation of algal extracts). Absorption was scanned from 350 to 700 nm with an Ultrospec III UV/Visible photometer using Wavescan Application Software (Pharmacia LKB Biotechnology, Uppsala, Sweden) in a standard cuvette with an effective light path of 10 mm.

RESULTS

Treatment of fish and erythrocytes

None of the carp maintained for blood collection died during the sampling period. The day after the bleeding procedure, fish were feeding normally. In the case of repeated bleeding of individual fish, scar formation occurred in the area of puncture. Carp whole blood contained, on average, 2×10^9 erythrocytes ml^{-1}. Thus, ca. 100 µl of carp whole blood yielded a sufficient amount for one microtitre plate. Carp erythrocytes were stored for at least 3 months at 4 °C in diluted RPMI culture medium, exhibiting no considerable cell loss or changes in lysis behaviour. Carp erythrocytes showed a strict requirement for Ca^{2+} because they lysed in assay buffer without this cation. (This is in contrast to the protocol by Edvardsen *et al.*[2] that used a buffer without Ca^{2+} for washing of cod blood.)

Treatment of algal cells

Storage of algal pellets and extracts for up to 3 months at −20 °C did not reveal a significant loss in lytic activity in the ELA as compared to fresh extracts. Frozen pellets proved to be slightly superior in lytic activity over frozen extracts (data not shown).

Photometric determination of erythrocyte lysis

Scanning of completely lysed carp erythrocytes (5×10^6 cells ml^{-1} = standard concentration for ELA experiments) from 350 to 700 nm revealed peaks of absorption at 414, 540 and 575 nm, respectively (Fig. 1). Absorption at 414 nm was ca. 10-fold higher than at 540 or 575 nm. In Fig. 1, additionally, a scan of a crude extract of *C. polylepis* is shown (5×10^5 cells ml^{-1} = maximum concentration in ELA experiments). Absorbance of this extract at 414 nm did not interfere with the absorbance of lysed erythrocytes at the same wavelength. Higher absorption at 414 nm was observed for other extracts (e.g. from/of *G. aureolum*), which were subtracted using appropriate blanks. Parallel measurements of increasing concentrations of completely lysed carp erythrocytes at 414 and 540 nm (Fig. 2) showed, for both wavelengths, a linear correlation of absorption and cell concentration up to the maximum of 5×10^6 cells ml^{-1}. Absorptions measured at 414 nm were 10-fold higher than the respective absorptions measured at 540 nm. Absorption at 620 nm can be used as a reference to eliminate non-specific absorption of microtitre plates, because neither lysed erythrocytes nor algal compounds showed considerable absorption at this wavelength (Fig. 1).

Erythrocyte lysis induced by saponin

Saponin was used to test the ELA in microtitre plates with a chemically defined haemolytic agent. Haemolytic activity was detected at a saponin concentration of ≥4 µg ml^{-1}. Complete erythrocyte lysis was observed with 10 µg ml^{-1} of saponin (Fig. 3). Incubation of carp erythrocytes with saponin (1–20 µg ml^{-1}) at 15 °C for 1–20 h revealed identical kinetics of cell lysis (data not shown).

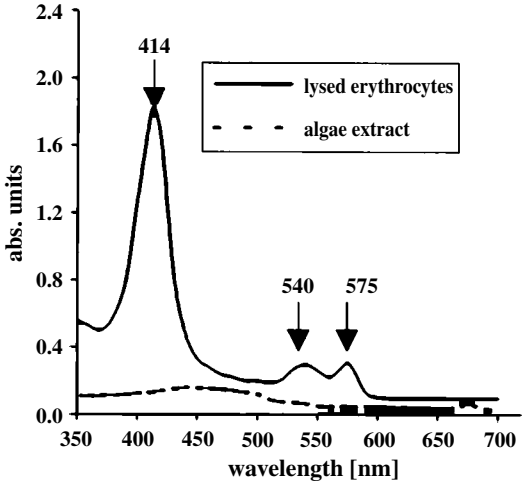

Figure 1. Photometric scan of completely lysed carp erythrocytes (5×10^6 cells ml^{-1}) and an algal extract of *Chrysochromulina polylepis* (5×10^5 cells ml^{-1}). Absorption (*y*-axis) was scanned from 350 to 700 nm (*x*-axis). Arrows point out the maxima of absorption of lysed erythrocytes.

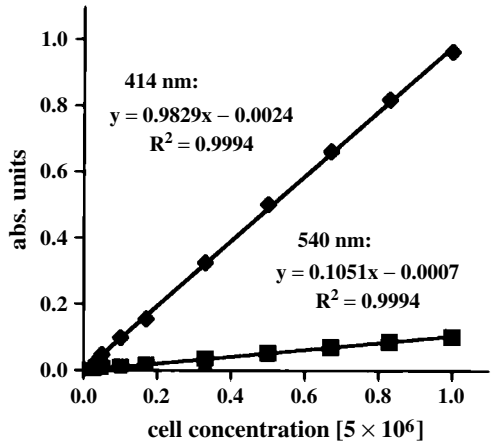

Figure 2. Comparative measurement of light absorption (*y*-axis) of different concentrations of lysed erythrocytes (*x*-axis) at 540 and 414 nm. Formulae indicate respective equations and coefficients of correlation (R^2 values) for the resulting curves.

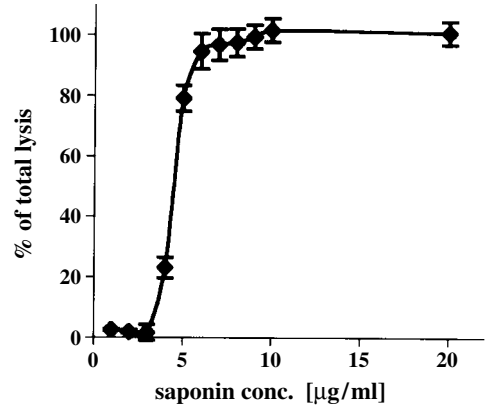

Figure 3. Lysis (*y*-axis) of erythrocyte suspensions (5×10^5 cells ml^{-1}) obtained with different concentrations of saponin (*x*-axis). Erythrocytes were incubated in microtitre plates for 1 h at 15 °C. Lysis values represent the average of five measurements ± standard deviation.

Haemolytic activity of different algal species

Crude algal extracts of seven prymnesiophyte and eight dinophyte species using 5×10^5 and 5×10^4 cells ml^{-1}, respectively, were tested with the ELA in microtitre plates for their haemolytic activity (Fig. 4). Both groups contained known ichthyotoxic species. The group of prymnesiophytes comprised four *Chrysochromulina* and three *Prymnesium* species. Among the *Chrysochromulina* species only *C. polylepis* (C1 in Fig. 4)—a species known to be ichthyotoxic—exhibited haemolytic activity leading to 90% lysis after 20 h. For *C. ericina, C. simplex and C. throndsenii* (C2–C4) no haemolytic activity was found after 20 h of incubation. All *Prymnesium* species tested here are known to be ichthyotoxic. However, only *P. parvum* (P1) and *P. nemamathecum* (P2) showed haemolytic activity, with total lysis of erythrocytes reached already after 4 h of incubation, whereas *P. patelliferum* (P3) exhibited no haemolytic activity even after 20 h of incubation.

The dinophyte group comprised two *Gyrodinium*, two *Gymnodinium* and four *Alexandrium* species. *Gyrodinium aureolum* (G1) did not show any haemolytic activity, *G. instriatrium* (G2) revealed minor haemolytic activity of ca. 40% after 20 h of incubation and the two *Gymnodinium* species (G3 and G4) did not show any haemolytic activity. All *Alexandrium* strains exhibited haemolytic activity, which was most prominent in *A. tamarense* (A2, total lysis after 4 h), followed by *A. fundyense* (A1, total

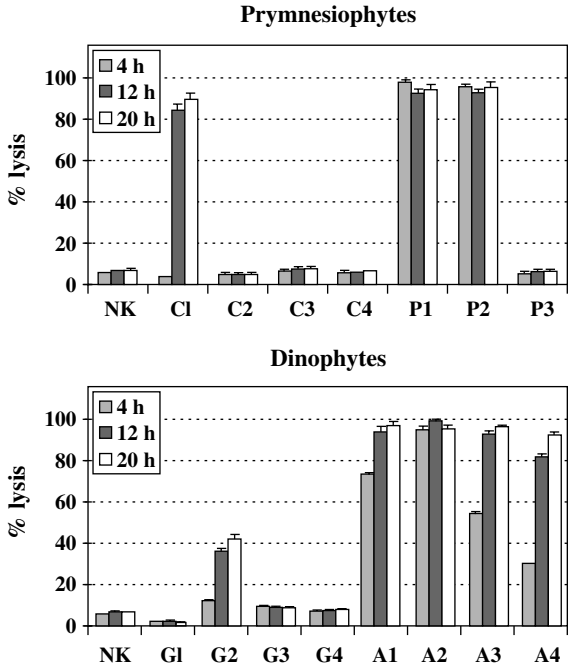

Figure 4. Haemolytic activity (*y*-axis) of crude extracts prepared from different prymnesiophyte and dinophyte algae (*x*-axis: C1 = *Chrysochromulina polylepis* B1511, C2 = *C. ericina* CCMP 281, C3 = *C. simplex*, C4 = *C. throndsenii*, P1 = *Prymnesium parvum* RL10, P2 = *P. nemamathecum*, P3 = *P. patelliferum* Ply527, G1 = *Gyrodinium aureolum* K-0303, G2 = *G. instriatrium* LME176a1310, G3 = *Gymnodinium chlorophorum* BAHME100, G4 = *G. varians* CCMP421, A1 = *Alexandrium fundyense* CCMP1719, A2 = *A. tamarense* CCMP115, A3 = *A. lusitanicum*, A4 = *A. ostenfeldii* BAHME136). All experiments were carried out in microtitre plates in triplicate at 15 °C for different periods of time (legend). Each column represents the average of three assays ± standard deviation.

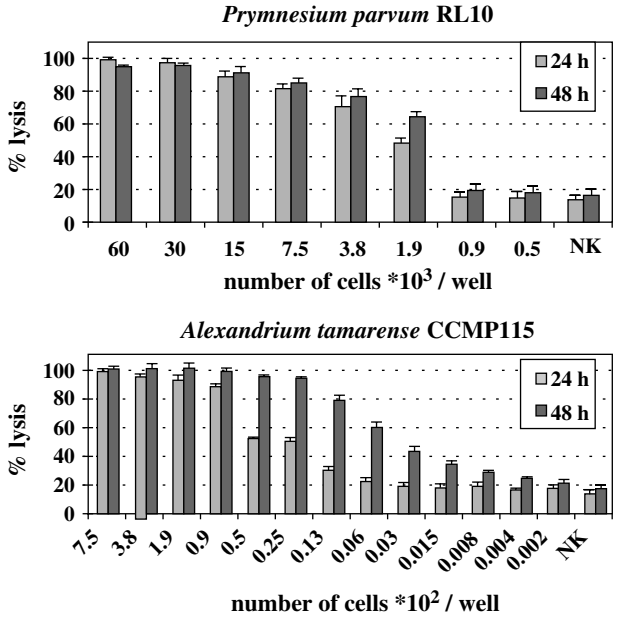

Figure 5. Limits of detection for haemolytic algae using the ELA in microtitre plates. Decreasing cell numbers (*x*-axis) of *Prymnesium parvum* RL10 and *Alexandrium tamarense* CCMP115 as representatives of the prymnesiophytes and dinophytes, respectively, were tested for their haemolytic activity (*y*-axis). Erythrocytes were incubated for 24 and 48 h (legend) at 15 °C. Each column represents the average of three assays ± standard deviation, NK = negative control.

lysis after 12 h), followed by *A. lusitanicum* (A3, total lysis after 20 h). *Alexandrium ostenfeldii* (A4) compared to the other *Alexandrium* species showed the slowest haemolytic reaction but also reached almost 100% lysis after 20 h of incubation.

The limit of detection of haemolytic activity with the ELA in microtitre plates was tested with extracts from *P. parvum* (P1 in Fig. 4) and *A. tamarense* (A2 in Fig. 4) as representatives from the two algal genera tested (Fig. 5). Compared with negative controls, *P. parvum* extracts showed distinct haemolysis down to a dilution of 1900 algal cells well^{-1} after 24 h of incubation. *Alexandrium tamarense* extracts, after 48 h of incubation, exhibited detectable haemolytic activity down to a dilution of 2 algal cells well^{-1}.

DISCUSSION

Detection and quantification of toxic substances of unknown molecular structure depend on bioassays. For haemolytic substances the ELA frequently is applied.[6] In previous investigations haemolytic activity was analysed photometrically by measuring the amount of released haemoglobin at a wavelength of 540 nm.[2,4,5,17] In the present study, scanning of the spectrum of absorption of completely lysed carp erythrocytes from 350 to 700 nm revealed a maximum at 414 nm, being 10-fold larger compared with absorption at 540 nm. This is not a peculiarity of fish erythrocytes, because the absorption spectrum taken for comparison from human erythrocytes showed identical absorption characteristics (data not shown).

Because absorption at 414 nm is correlated in a linear proportional manner to the lysis of erythrocytes across the whole effective range, and because absorption of algal extracts did not interfere at 414 nm, quantification of haemolytic activity can be analysed by measurement of absorption at this wavelength. The major advantage of measuring absorbance at 414 nm in comparison to 540 nm is the increased sensitivity of this bioassay by a factor of 10. With the use of microtitre plates for the ELA this is an advantage because the distance that the light has to pass through in the sample is considerably shorter than in a cuvette (ca. 5 mm in a 200-µl volume compared with the standard in cuvettes of 10 mm) and therefore absorption by the sample is less.

The main reason for developing an ELA in microtitre plates has been to simplify and speed up the assay, enabling large numbers of samples to be managed rapidly. Moreover, the analysis of algae for haemolytic potential was quickened by using crude algal extracts in the ELA. Time-consuming extractions with hazardous organic solvents, such as methanol and chloroform, are completely avoided using one aqueous buffer containing calcium as an ingredient essential for carp erythrocytes. This assay buffer is used for algal preparation as well as for the washing and resuspension steps of carp erythrocytes, providing maximum compatibility of all ELA components.

A further advantage of an ELA in microtitre plates is the reduction of sample volumes by a factor of 12 compared with the protocol that we started with.[2] This is of relevance for the preparation of algal extracts if there is only a limited amount of field sample available or if, in laboratory experiments, frequent sampling from a restricted volume of algal culture is necessary. Also, small volumes of blood are required (ca. 100 µl of carp whole blood per microtitre plate). Thus the ELA presented here is a considerate method concerning the requirement of donor animals. Additionally, by applying the method described by Stoskopf[15] the fish were not killed for blood collection and storing carp erythrocytes for at least 3 months in a refrigerator allowed several assays to be performed over prolonged periods of time.

Checking the ELA in microtitre plates with saponin as a chemically defined haemolytic substance showed that the assay sensitively detects erythrocyte lysis. Saponin has been used by others to express lysis as 'haemolytic units'[17] or as 'saponin equivalents'[4] and may be employed as an external standard in the ELA if, in the case of chemically unknown substances, authentic standards are not available.

In order to apply the ELA in microtitre plates for the investigation of haemolytic activity of marine microalgae, we first had to select a suitable incubation temperature of erythrocytes with algal extracts. In the literature one is confronted with a wide variability in this respect, reaching 8 °C for cod (*Gadus morhua*)[2] and 35 °C for human erythrocytes.[5] We have chosen an incubation temperature of 15 °C for all algal extracts with carp erythrocytes, because it coincides with the temperature used for the culturing of algae (except for *Gyrodinium instriatrium* and *Gymnodinium varians*, grown at 20 °C) and therefore was considered a suitable temperature for measuring full haemolytic activity in algal extracts. An impairment of carp erythrocytes at 15 °C was not expected because the temperature of maintenance (17–20 °C) of the donor carp was not exceeded.

Because Simonsen *et al.*[4] reported a reduction in the haemolytic activity of algal extracts after exposure to

light, preparation of all algal extracts was performed under dimmed light conditions. Incubation of algal extracts with erythrocytes was performed in complete darkness for the same reason.

By applying these conditions to the experimental set-up of the ELA in microtitre plates, haemolytic and non-haemolytic algal species were clearly distinguished. Erythrocyte lysis caused by prymnesiophyte species, known to be harmless for fish, did not exceed the range of negative controls. Prymnesiophytes, known to be ichthyotoxic, expressed haemolytic activity, with the notable exception of *Prymnesium patelliferum*,[5,18,19] a close relative of *P. parvum*. A similar finding was obtained within the dinophyte group. Extracts of *Gyrodinium aureolum*, a species definitely known to be ichthyotoxic and haemolytic,[3,12] did not show any lytic activity in the ELA, like the harmless species *Gymnodinium varians* and *G. chlorophorum. Gyrodinium instriatrium*, another harmless dinophyte, was identified as a species with an intermediate haemolytic phenotype.

A possible explanation for this phenomenon could be that culture strains of *P. patelliferum* and *G. aureolum* have lost their haemolytic ability after a long time in culture, or that a certain stimulus (e.g. of fish origin) is lacking for an increased expression of haemolytic products by these algal species. Parallel analysis of 'wild type' extracts will be necessary for further evidence. If, however, the phenotype of these algal strains should prove to be stable, these non-haemolytic variants represent helpful tools in experiments designed for the cloning of genes involved in algal toxin biosynthesis.[20,21]

Another interesting result obtained from the dinophyte group is that all *Alexandrium* species tested here were identified to be strongly haemolytic. These algae are producing saxitoxins,[22,23] a class of algal toxins causing paralytic shellfish poisoning syndrome in humans. Simonsen *et al.*,[11] who also described a strain of *A. tamarense* with strong haemolytic activity, showed that haemolysis is not caused by simultaneously produced saxitoxins. Thus, the causative haemolytic agent in these algae has not been identified but, noteworthy, all are known to produce polyunsaturated fatty acids, of which any one of them could be haemolytic.[24,25]

Sensitivity of the ELA presented here is demonstrated by significant lysis of carp erythrocytes by 1900 *Prymnesium parvum* and as few as two *Alexandrium tamarense* cells per well of a microtitre plate. This ELA currently is used in our laboratory, to determine the toxin production of *Chrysochromulina polylepis* throughout the different phases of its cell cycle. Preliminary results on synchronized cultures confirmed the sensitivity and efficiency of this assay.[26]

The ELA in microtitre plates has proved to be a rapid and sensitive tool to detect and quantify haemolytic substances. We therefore suggest the consideration of this assay as a low-cost monitoring tool for the detection of algal species with haemolytic activity, which will include many ichthyotoxic species. To identify clearly known toxic algae, the ELA can be accompanied by specific identification assays, e.g. *in situ* hybridization with rRNA probes.[27,28]

The ELA may serve, additionally, for investigation of the influence of environmental factors (e.g., human-induced changes in the amount and composition of nutrients in coastal waters) on the toxicity of harmful algal species.

Currently, marine research is focusing strongly on pharmacologically active compounds obtained from natural sources. Among such natural products is a new class of antibiotics with selective cytolytic action.[29–31] Because the ELA has the ability to detect cytolytic events independent of the chemical nature of the causative agent, this assay may become a screening tool for the identification of pharmacological substances urgently needed for human healthcare.

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