Marine Biology 115, 187–193 (1993)

Marine Biology
International Journal on Life in Oceans and Coastal Waters

A comparison of Lowry, Bradford and Smith protein assays using different protein standards and protein isolated from the marine diatom *Thalassiosira pseudonana*

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Received: 13 August 1992 / Accepted: 9 September 1992

Abstract. Two spectrophotometric assays for protein commonly used in marine research (Coomassie stain, "Bradford"; alkaline copper, "Lowry") and a more recent assay which has not been applied in this field (bicinchoninic acid, "Smith") were compared for homogenates of the marine diatom *Thalassiosira pseudonona* using bovine serum albumin (BSA) as a standard. When homogenates were prepared by precipitating protein with trichloroacetic acid (TCA) and redissolving in 1 N NaOH, the protein content estimated by the Lowry and Smith assays agreed closely, but was consistently 20% higher than that indicated by the Bradford assay. To determine if this difference was due to the choice of a protein standard, protein from *T. pseudonana* was purified and compared to BSA, bovine gamma-globulin (BGG), and casein. The reactivity of the purified protein (expressed as the slope of the absorbance vs protein concentration curve) did not differ between cultures grown at high or low irradiance. For the Smith and Bradford assays the reactivity of BSA was not significantly different from algal protein, but for the Lowry assay, algal protein was significantly higher in reactivity than BSA. BGG was not significantly different in reactivity from algal protein for the Lowry and Smith assays, but BGG gave significantly lower absorbances than algal protein in the Bradford assay. These results suggest that BSA is a suitable standard for algal protein in the Bradford assays, while BGG is preferable for the Lowry assay. Either protein standard could be used for the Smith assay. Differences in purified algal protein reactivity compared to BSA could not account for the differences among the assays, nor could interference by chlorophyll *a*. Precipitating protein with TCA prior to analyses gave lower protein than direct analyses of homogenates for the Lowry and Smith assays, but no differences were found for the Bradford assay. As a result, the Lowry and Smith assays indicated up to 60% greater protein than the Bradford if TCA precipitation was not performed. This may be due to removal of free amino acids and small peptides which are less reactive in the Bradford assay. The 20% higher protein found in the Lowry or Smith vs Bradford assays may be due to different assay sensitivity to small peptides or other compounds which are precipitated along with proteins by TCA. Although the Smith assay is substantially simpler to perform than the Lowry, there appear to be no quantitative differences in the results. It remains unclear which spectrophotometric assay is most accurate, but the Bradford assay is faster and simpler, and is less likely to be affected by non-protein compounds found in marine phytoplankton.

Introduction

In marine phytoplankton ecology and physiology it is often useful to use protein as an index of biomass and as a scaling factor for variables such as enzyme activity (e.g. Dortch et al. 1985). Although absolute quantification of protein requries fractionation followed by Kjeldahl nitrogen determination (e.g. Whyte 1987), the difficulties with obtaining sufficient material, and the time-consuming nature of this method have lead researchers to use spectrophotometric analyses. Commonly used assays include the alkaline copper assay ("Lowry" assay, Lowry et al. 1951) and the Coomassie dye binding assay ("Bradford" assay, Bradford 1976). A substantially simpler modification of the Lowry assay, using bicinchoninic acid in place of the Folin-Ciocalteu reagent, has been proposed by Smith et al. (1985; "Smith" assay).

It is recognized that spectrophotometric methods are, at best, relative measurements because it is necessary to use a purified protein standard, most commonly bovine serum albumin (BSA). However, it is a frequent practise to infer absolute protein content from such data, despite suggested precautions (e.g. Zamer et al. 1989). In such cases, it is necessary to have an indication of the reactivity of the protein standard relative to the protein being assayed. It is also important to be able to compare the results of different assays. A growing body of evidence suggests that the Lowry and Bradford assays give different measures of protein relative to BSA for marine inver-

tebrates (Zamer et al. 1989), higher plants (Eze and Dumbroff 1982) and marine phytoplankton (Clayton et al. 1988). Differences between the assays might be expected because the Lowry and Bradford procedures use distinctly different principles. The Lowry assay detects protein through a copper-catalysed reduction of Folin phenol reagent. This reaction will detect peptide bonds, but it is also highly sensitive to specific amino acids such as tyrosine and tryptophan (Legler et al. 1985). In the Bradford assay, Coomassie Brilliant Blue dye is bound by protein, primarily by arginine residues, but also to a lesser degree by histidine, lysine, tyrosine, tryptophan and phenylalanine (Compton and Jones 1985). As a result, the reactivity of either of the assays to a particular protein will be a function of that protein's composition as well as any other compound which might oxidize the Folin phenol reagent, or bind the Coomassie dye (Stoscheck 1990). In fact, disagreements between the Lowry and Bradford assays have been ascribed to different reactivities of the assays to the specific protein being measured, and to interferences by compounds such as chlorophyll *a* (Eze and Dumbroff 1982). The Smith assay has seldom been compared to the other assays and has not to our knowledge been applied to marine phytoplankton. If different protein reactivity relative to a standard is responsible for the discrepancy, it is also plausible that protein reactivity may vary in a single species under different growth conditions. This possibility has never been examined. There are also many minor variations on methods. For example, some researchers have chosen to precipitate proteins with TCA in order to remove free amino acids (e.g. Clayton et al. 1988), while others (e.g. Mayzaud and Martin 1975) have not.

The objectives of this study were to: (1) compare Lowry, Bradford and Smith protein assays using a typical marine diatom, *Thalassiosira pseudonana* (2) to purify proteins from *T. pseudonana* grown under either high or low light conditions and compare their reactivity to BSA and other common protein standards, (3) to assess the potential for chlorophyll *a* to interfere with each assay, and (4) to test whether precipitation of protein using TCA affects estimated protein content or the relationship between the results of the different assays.

Materials and methods

Culture conditions

The marine diatom *Thalassiosira pseudonana* (Hustedt) Hasle and Heimdal (3H clone) was obtained from the Northeast Pacific Culture Collection, Department of Oceanography, University of British Columbia. Cultures were grown in enriched artificial seawater (ESAW) based on the recipe by Harrison et al. (1980), with sodium glycerophosphate replaced with an equimolar concentration of sodium phosphate, ferrous ammonium sulphate with an equimolar concentration of ferric chloride and additions of selenite, nickel and molybdate to achieve 1 n*M* final concentration. Temperature was maintained at either 17.5 ± 0.5 °C using a circulating water bath, or at 22 ± 1 °C in a cold room. Cultures were grown in glass flasks ranging from 1 to 12 litres, stirred at 60 rpm with teflon-coated stir bars and bubbled with air filtered through a 0.22 µm membrane filter. Continuous illumination was provided by Vitalite™ fluorescent tubes and attenuated by distance or neutral density screening to give a range of irradiances from 6 to 120 µmol quanta $m^{-2} s^{-1}$. Growth rates were followed by in vivo fluorescence, measured twice daily using a Turner Designs™ Model 10 fluorometer and cell counts using a Coulter Counter™ model TAII equipped with a population accessory.

Protein assay comparisons

Samples for protein determination were collected from 35 *Thalassiosira pseudonana* cultures grown as described above and harvested in a range of growth phases. Homogenates were prepared as described by Dortch et al. (1984), grinding with 3% TCA and solublizing in 1 *N* NaOH.

Lowry assays were performed as modified by Dortch et al. (1984). Bradford assays were performed using the micro-assay procedure of the Bio-Rad Protein Assay kit (Bio-Rad Laboratories, 500-0001). The Smith assay was performed using a Sigma Protein Assay kit (Sigma Chemical Co., BCA-1). Protein was calculated on a per cell basis and Bradford and Smith results were compared to those of the Lowry assay using Student *t*-test comparisons at the 95% confidence level.

Protein reactivity comparisons

Three separate experiments were performed, the first utilizing two 6 litre cultures, one grown at high light (120 µmol quanta $m^{-2} s^{-1}$), the other at low light (15 µmol quanta $m^{-2} s^{-1}$). The second and third experiments used four 12 litre cultures, two grown at high light, two at low light. For each experiment cultures were inoculated from a common high-light-grown culture at a density which allowed eight generations before harvest. Mean growth rates were 0.32 (± 0.06) and 2.04 (± 0.08) d^{-1} for low and high light cultures, respectively.

Cultures were harvested in late log phase, approximately one generation before stationary phase. Cells were collected by filtration onto 47 mm pre-combusted 934 AH glass fibre filters and frozen at −20 °C until protein purification could be performed.

Protein purification followed the method of Zamer et al. (1989). Each filter was ground for 2 min in ca. 5 ml of 1 *N* NaOH in a 15 ml glass/teflon tissue homogenizer. Homogenates were pooled for each culture, allowed to extract for 30 min and centrifuged for 15 min at high speed in a clinical centrifuge to remove filter fibres. The supernatant was decanted and its volume measured. Proteins were precipitated by adding 25% trichloroacetic acid (2.5:1.0 v/v, TCA: homogenate). The mixture was centrifuged for 15 min and the pellet was washed with 10% TCA. The pellet was resuspended in 5% TCA (5:1 v/v, TCA:homogenate) and heated to 90 °C for 15 min in a water bath to remove nucleic acids. The cooled supernatant was centrifuged and washed with 5% TCA. To remove pigments associated with proteins, the pellet was resuspended in 90% acetone (5:1 v/v, acetone:pellet) and centrifuged for 15 min. This was repeated until the supernatant retained no visible color. Lipids were extracted by the method of Bligh and Dyer (1959). The water-methanol fraction was collected and placed in a 50 ml beaker. This was dried in a desiccator for several days.

Purity of the protein extract was assessed by stoichiometric analysis. Ash-free dry weight was determined by placing known quantities of purified protein into pre-weighed aluminum cups. These were re-weighed after ashing in a muffle furnace at 550 °C for 6 h. Samples were analysed for carbon and nitrogen content using a Carlo-Erba CNS analyser with sulfanilamide as a standard. Purity was assessed assuming a nitrogen-protein conversion factor of 6.0. Although a ratio of 6.25 is generally used, and Gnaiger and Bitterlich (1984) suggest that 5.8 may be better for a variety of aquatic organisms, a review by Laws (1991) found 6.0 to be the best value for diatoms.

Standards for protein assays consisted of bovine serum albumin (BSA, Sigma Chemical Co. A 7638), bovine gamma-globulin

(BGG, Sigma G 7516) and casein (Sigma C 6905). Replicate samples and standards were individually weighed and dissolved in 1.0 *N* NaOH to a concentration of ca. 1 mg ml^{-1}. For Bradford and Smith assays, both samples and standards were diluted with distilled water to appropriate final concentrations. For each purified sample and separately weighed standard, a linear regression was performed. In no case was the intercept significantly greater than zero. Therefore the mean of these samples was taken as the best estimate of the slope of the absorbance vs protein curve. These slopes were compared within each assay using a one-way analysis of variance (ANOVA) followed by Tukey's multiple range test to investigate differences (Steel and Torrie 1980).

Chlorophyll interference experiments

To assess chlorophyll interference with assays, two approaches were used. The first involved preparation of two BSA standards (1 mg ml^{-1}, 4 ml total volume) with and without addition of chlorophyll *a*. Parsons et al. (1961) reported protein:chlorophyll *a* ratios between 23 and 75 for diatom species. We selected a ratio of 40:1 for these experiments. One standard received 0.1 mg of chlorophyll, dissolved in 100 µl of 90% acetone, while the second standard received only 100 µl of 90% acetone. Each assay was performed and the slopes of absorbance-concentration curves were compared by regression analysis. The second approach used two sets of subsamples ($n=6$) taken from a single culture. The first set was homogenized as described previously. The second set was extracted twice with 5 ml aliquots of 90% acetone before being centrifuged and solubilized in 1 N NaOH. Protein contents were compared within each assay using Student *t*-tests.

TCA precipitation experiment

As in the chlorophyll interference experiment, two sets of subsamples ($n=5$) were taken from a single culture. The first set was homogenized as previously described. The second set was homogenized directly in 1 *N* NaOH without TCA precipitation. Results were compared for each assay using Student *t*-tests.

Results and discussion

Assay comparisons of *Thalassiosira pseudonana* homogenates

For 35 different *T. pseudonana* cultures, a ratio of 1.23 (± 0.04) was found between the Lowry and Bradford assays, indicating significantly higher protein determined by the Lowry assay ($P<0.001$). The Smith assay was not significantly different from the Lowry; the ratio of Lowry : Smith was 0.97 (± 0.05) and not significantly different from 1.0 ($P>0.5$). There is general agreement that the Bradford assay gives lower protein values in a variety of organisms. Chiapelli et al. (1979) and Manahan and Nourizadeh (1990) determined a ratio of 1.6 for rat liver preparations and a range of marine invertebrates, respectively. Using data from Eze and Dumbroff (1982), we calculated a ratio of 1.4 for kidney bean leaves. Clayton et al. (1988) compared the Bradford and Lowry assays for *T. pseudonana* and reported the ratio ranged from 1.8 to 2.0. Alternatively, Setchell (1981) found no difference between protein content determined with Lowry and Bradford assays in either *T. pseudonana* or *Skeletonema costatum*. Unlike other researchers, however, Setchell (1981) used BGG as a standard. The reasons for differences in the ratio of protein measured by Bradford and Lowry assays are unclear. Part of the explanation may involve minor variations in the Lowry procedure, including use of detergents (Clayton et al. 1988) and sample treatment with TCA (Eze and Dumbroff 1982). As well, there are differences in the Bradford assays. In the present study we used a micro-volume version of the Bradford assay which has a higher dye:sample ratio than the assay used in many other studies (Bradford 1976).

The Smith and Lowry assays gave identical results. Relatively little is known about the comparability of these assays. Smith et al. (1985) noted that most proteins show similar reactivities using the two assays, but there were exceptions (e.g. avidin). Brown et al. (1989) found good agreement between Lowry and Smith assays. Because the two reactions have a common first step, this similarity is not unexpected. The Smith assay uses only one reagent and does not require precise timing, and may therefore be preferable.

Protein assays and cell composition

Since there are differences between the Bradford and the other two assays, one must resolve which assay is more accurate. In order to assess this, we measured cell nitrogen content. For 16 different cultures, protein measured by the Bradford assay accounted for $47 \pm 3\%$ (standard error) of cellular nitrogen, while the Lowry and Smith assays account for $58 \pm 3\%$. Both these values appear low compared to values in the algal literature. Using acid hydrolysis and amino acid detection, protein values in the range of 75 to 85% of cell nitrogen have been found for *Chlorella* spp. (Fowden 1952), and various marine phytoplankton species (Parsons et al. 1961, Cowey and Corner 1966, Mayzaud and Martin 1975, Laws 1991). It is important to recognize that hydrolysis techniques without fractionation cannot distinguish between protein, free amino acids and small peptides. It is unlikely that the cells in this study were abnormal in composition, since carbon:nitrogen ratios were 6.78 ± 0.14 ($n=16$), which is very close to the average value for phytoplankton of 6.5 found by Laws (1991).

Alternatively, other data suggest that percentages of protein nitrogen may be lower. Mayer et al. (1986) found cell protein accounted for 71% of the nitrogen in a mixed culture of *Thalassiosira pseudonana* and *Dunaliella tertiolecta*. Using a fractionation and a micro-Kjeldahl technique, Whyte (1987) calculated that protein in *T. pseudonana* accounted for 75% of the nitrogen in early logarithmic growth phase, but 53% of the nitrogen in stationary phase. In *T. pseudonana*, Dortch et al. (1984) reported $67 \pm 15\%$ of the cell nitrogen in protein. The values obtained in the present study fall into the lower end of this range, but experiments of Dortch et al. were conducted under blue light, which Kowallik (1978) predicts will increase protein content. Conover (1975) measured protein in the co-generic *T. fluviatilis* and calculated that 55.7% of the cell nitrogen was in protein in log phase, while only 27% was accounted for in senescence. Both Conover

(1975) and Whyte (1987) point out that *Thalassiosira* species have a high chitan content, which may account for substantial portions of non-protein nitrogen. As well, Lui and Roels (1972) showed that the TCA-soluble nitrogen pool in the diatom *Biddulphia aurita* accounted for 18 to 33% of the cell nitrogen, and that up to 58% of it was free or combined amino acids. Thus, on the basis of cell nitrogen content, we are unable to suggest which method is more accurate.

Relative protein reactivity

One explanation for differences among the Bradford, Smith and Lowry assays is that these assays may show different reactivity for algal protein relative to BSA. To assess this, we purified and assayed algal protein. In all cases, CNS analyses (following Zamer et al. 1989) indicated that >90% of the ash-free dry weight was protein. Fig. 1 shows the relationship between algal protein and other pure protein standards for each assay. Statistical comparisons are summarized in Table 1. The reactivity of algal protein is identical whether cells are grown under high or low light for every assay. BSA appears to be a suitable standard for Bradford and Smith assays because its reactivity is not significantly different from algal protein, but significant differences were detected between BSA and algal protein using the Lowry method. The differences were greatest at high concentrations of protein (Fig. 1 B) and suggest that the Lowry assay might overestimate algal protein relative to BSA. The fact that the Lowry estimate of *Thalassiosira pseudonana* protein agrees with the Smith assay (where there are no differences between algal protein and BSA) may be explained by the fact that Lowry assays of *T. pseudonana* cultures typically gave absorbance values <0.20, where the curves of algal protein and BSA fall relatively close together. Selecting a suitable standard protein is critical to the accuracy of these assays. Stoscheck (1990) points out that BSA has a high reactivity with the Bradford assay, and suggests that BGG gives a more "normal" response. In addition, Mayer et al. (1986) suggest that BGG is more representative in studies of mixtures of proteins. Setchell (1981) used BGG as a standard and found no differences between protein content of *T. pseudonana* using Bradford and Lowry assays. Our results indicate that the use of BGG as a standard will give inflated values for Bradford assays because BGG gives significantly lower absorbances than algal protein. This may explain why Setchell (1981) found no differences between assays when he used BGG as a standard, while researchers previously cited have noted differences. Dortch et al. (1984) also recommended caution when using BSA as a standard. Alternatively, data from Dorsey et al. (1978) show similar reactivity between protein from *T. pseudonana* and a BSA standard. As well, Dorsey et al. (1977) compared "purified protein" (details were not given in their paper) from *Chaetoceros curvisettus* and noted that the characteristics of its reactivity in a modified biuret assay were quite similar to BSA. Lowry et al. (1951) pointed out that mixtures of protein were less variable than individual

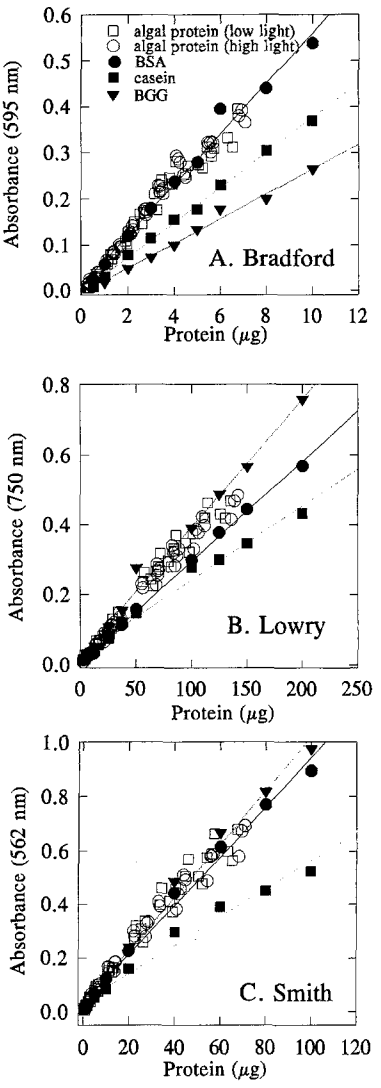

Fig. 1. Absorbance vs protein content for different pure proteins and purified algal protein from *Thalassiosira pseudonana* for (A) Bradford, (B) Lowry, and (C) Smith protein assays. For clarity, only one out of six sets of bovine serum albumin (BSA), bovine gamma globulin (BGG) and casein data are shown. Lines represent least squares regression fits to pooled data. Fits to algal protein data for high-light-grown and low-light-grown cultures are not shown. Note scale changes for protein

purified proteins. Our results suggest that BSA is not an unreasonable standard for *T. pseudonana* but they contrast with those of Zamer et al. (1989), who showed that purified protein from sea anemones was much lower in reactivity than BSA. For the Lowry assay there is the potential to overestimate phytoplankton protein at high concentrations. In this case, BGG may be a more accurate standard.

Chlorophyll interference

Eze and Dumbroff (1982) showed that chlorophyll in kidney bean leaves could increase absorbances in protein determination by up to 400%, particularly for the Lowry assay. They found that TCA precipitation was able to remove the interference, probably by denaturing the

Table 1. Comparison of absorbance vs protein content slopes for bovine serum albumin (BSA), bovine gamma-globulin (BGG), alpha-casein, and protein purified from *Thalassiosira pseudonana* cultures grown under either high or low light. Values represent mean (SE in parentheses) of five different determinations from separately prepared standards. Summaries of statistical comparisons (one-way ANOVA, followed by Tukey's multiple range test) are provided below the table. Lines join proteins which are not significantly different from each other at the 95% confidence interval

Assay	Slope				
	BSA	BGG	Casein	High light	Low light
Bradford	0.0570 (0.0014)	0.0284 (0.0010)	0.0379 (0.0003)	0.0561 (0.0016)	0.0567 (0.0019)
Lowry	0.0026 (0.0001)	0.0037 (0.0001)	0.0021 (0.0001)	0.0035 (0.0001)	0.0036 (0.0002)
Smith	0.0092 (0.0001)	0.0097 (0.0002)	0.0052 (0.0003)	0.0102 (0.0011)	0.0102 (0.0010)

Bradford:	BSA	High light	Low light	Casein	BGG
Lowry:	BGG	High light	Low light	BSA	Casein
Smith:	BGG	High light	Low light	BSA	Casein

chlorophyll. In our work, we noted that TCA-precipitated homogenates still remained green. Fig. 2 demonstrates that the addition of chlorophyll to a BSA standard did not result in a significantly different slope for any assay ($P > 0.20$ in all cases). There is apparently some deviation from linearity at high concentrations for the Lowry assay, perhaps due to the addition of acetone. Some differences appear at high concentrations in the Lowry assay, but because the Lowry assay agrees with the values for the Smith (Fig. 2), and the Smith assay shows no evidence of interference with chlorophyll, it seems unlikely that chlorophyll represents a real interference under these assay conditions despite earlier warnings by Eze and Dumbroff (1982). Fig. 3 summarizes comparisons of TCA-precipitated homogenates with those which have been extracted with acetone. Acetone removed all trace of color in the protein pellet, and gave a clear green supernatant. No differences were found for Bradford and Smith assays ($P > 0.2$ in both cases), but the Lowry assay gave a significantly *higher* protein content when acetone extraction was used ($P < 0.001$). This is contrary to the expected results if chlorophyll *a* itself caused an increased absorbance.

Other potential interfering compounds such as amino acids (Cowey and Corner 1966), lipid (Hopkins et al. 1984) and phenols and phenolases (Mattoo et al. 1987) have been identified, but in each case the interference can be corrected by TCA precipitation. These compounds might be expected to increase absorbance, but they could also decrease absorbances by inhibiting the reactions with the reagents.

TCA precipitation

Results of TCA precipitation experiments are summarized in Fig. 4. For the Bradford assay, no differences were found ($P > 0.4$), but both the Lowry and Smith assays gave up to 36% higher values for non-precipitated homogenates ($P < 0.001$ and $P < 0.002$, respectively). As a result, Lowry and Smith assays gave protein contents that were 50 to 60% higher than Bradford assays, if TCA precipitation was omitted. It is possible that precipitation removes compounds that are detected selectively by the Lowry/Smith assays. Chlorophyll does not seem likely to interfere, based on previous evidence, nor do phenols and phenolases that would also affect the Bradford assay (Mattoo et al. 1987). However, other groups of compounds known to be reactive with protein assays include amino acids and peptides. The literature suggests that microalgae have quantities of these compounds, particularly in stationary phase. Dortch et al. (1984) observed large TCA-soluble pools of nitrogen, and Whyte (1987) has shown that non-protein nitrogen is principally amino acids, peptides and amines in *Thalassiosira pseudonana*. Lui and Roels (1972) showed that 30% of soluble nitrogen was free amino acids, and an additional 30% was combined amino acids. The Bradford assay is known to be less sensitive than the Lowry to small peptides and amino acids. Mayer et al. (1986) found that, of the peptides tested, none smaller than 6 amino acids gave a reaction, and little sensitivity was noted in peptides smaller than 10 to 25 amino acids. Chiapelli et al. (1979) previously tested the hypothesis that Bradford-Lowry differences could be accounted for in terms of a class of small peptides. Most of his data, drawn from rat liver samples, indicate no significant differences. In at least one case, however, the ratio of TCA to normal homogenates is markedly lower for the Lowry (79.2%) than for the Bradford assay (91.8%). There are also reports suggesting that TCA precipitation may leave some protein in solution. Beckman et al. (1943) reported that 4 to 20% of total protein in human urine samples was not precipitated in

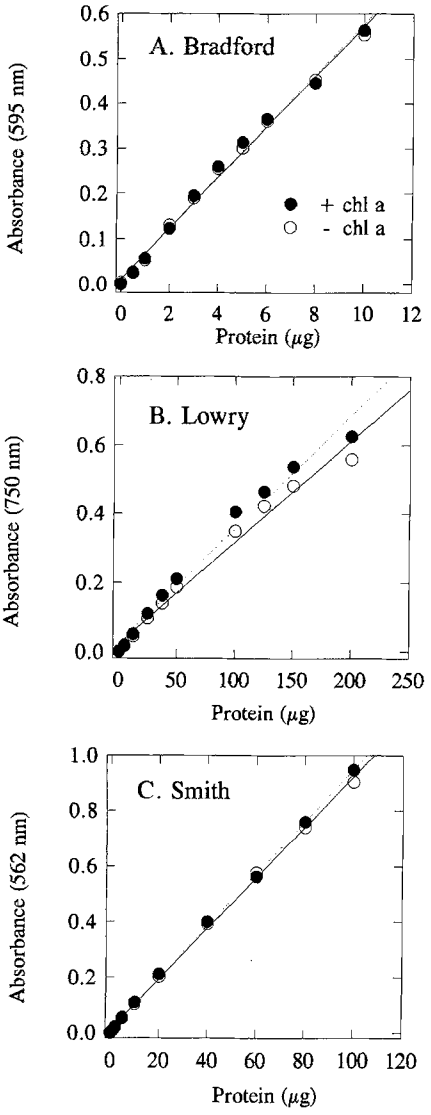

Fig. 2. Comparison of absorbance vs protein content curves for (A) Bradford, (B) Lowry, and (C) Smith protein assays for BSA samples with and without additions of 0.1 mg chlorophyll *a* in 90% acetone. Points represent means of two separately prepared standards. In all cases, associated error bars are smaller than the symbols. Lines represent least squares regression fits to the data

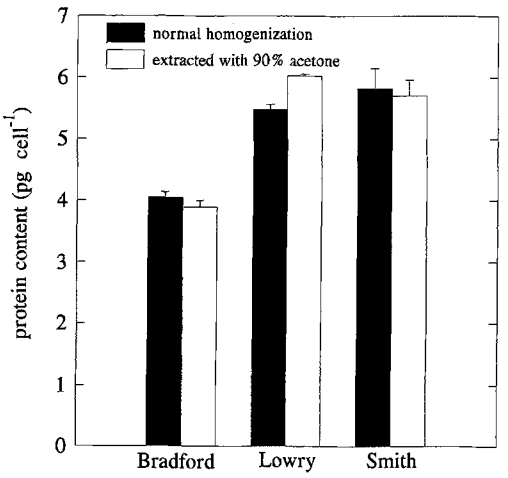

Fig. 3. Comparison of protein content (expressed as pg cell^{-1}) for acetone-extracted vs non-acetone-extracted homogenates of *Thalassiosira pseudonana* ($n=6$ for each treatment). Error bars represent +1 standard error of mean protein content

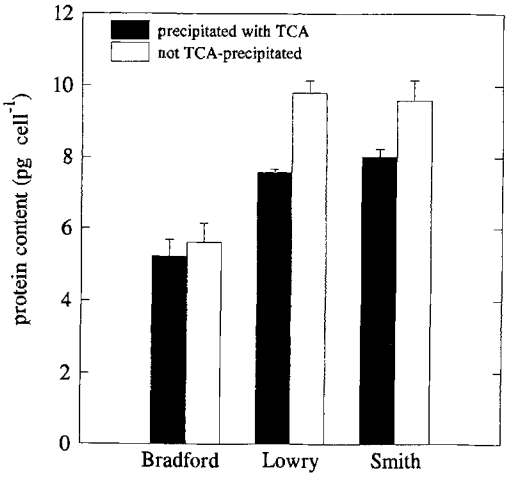

Fig. 4. Comparison of protein content (expressed as pg cell^{-1}) for TCA-precipitated vs non-TCA-precipitated homogenates of *Thalassiosira pseudonana* ($n=5$ for each treatment). Error bars represent +1 standard error of mean protein content

4% TCA. Such TCA-soluble proteins may react differently in Bradford, Lowry and Smith assays.

Extending this argument, if TCA precipitates a class of peptides to which the Lowry and Smith are sensitive, but the Bradford is not, this could explain the differences reported here in estimated protein, despite the similarity in pure algal protein reactivity. If this is true, the size of intracellular amino acid and peptide pools might also constitute a significant source of variability in measurements of algal protein based on spectrophotometric assays. We have attempted to address this question by comparing Lowry, Bradford and Smith assays against BSA standards in the presence and absence of the amino acids, proline, glycine and betaine, which Dickson and Kirst (1987) have found in marine diatom species. Preliminary results indicate that even at concentrations as high as 100 m*M*, these compounds do not show interference with any of the assays.

In conclusion, although selection of a protein standard may be seen as unimportant if only a relative measure of protein content is required, such data are often misleading because they are presented as absolute protein values. Despite criticism in the literature, BSA appears to be a suitable standard for algal protein measured in the Bradford or Smith assays. There is evidence that BGG is preferable as a standard for the Lowry, but the error which results from the use of BSA is probably small. Researchers must be aware that the Bradford assay gives different results from the Smith or Lowry, but such differences are not due to different reactivities of algal protein relative to a standard. In this study, we have been unable to resolve which assay is most accurate; however, Manahan and Nourizadeh (1990) have suggested that the Lowry assay (and thus the Smith assay) tend to overestimate protein. On the basis of speed, simplicity and relative insensitivity to interfering compounds, we recom-

mend use of the Bradford assay. However, because of the volume of research previously performed using the Lowry assay, it is both useful and possible to calibrate the assays for the specific system being investigated. Researchers using spectrophotometric protein assays must make every effort to give precise details of methodology (e.g. TCA precipitation) and indicate the protein standard being used.

Acknowledgements. This work was supported by a Natural Sciences and Engineering Research Council Canada Operating Grant to P.J.H. an NSERC Post-graduate Fellowship to J.A.B. and an NSERC Undergraduate Research Fellowship to A.E.F. We thank Drs. J. N. C. Whyte, P. A. Thompson and R. E. Brownsey for comments and discussion.

Literature cited

Beckman, W. W., Hiller, A., Shedlovsky, T., Archibald, R. M. (1943). The occurrence in urine of a protein soluble in trichloroacetic acid. J. biol. Chem. 148: 247–248

Bligh, E. G., Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911–917

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt. Biochem. 72: 248–254

Brown, R. E., Jarvis, K. L., Hyland, K. J. (1989). Protein measurement using bicinchoninic acid: elimination of interfering substances. Analyt. Biochem. 180: 136–139

Chiapelli, F., Vasil, A., Haggerty, D. F. (1979). The protein concentration of crude cell and tissue extracts as estimated by the method of dye binding: comparison with the Lowry method. Analyt. Biochem. 94: 160–165

Clayton J. R., Jr., Dortch, Q., Thoresen, S. S., Ahmed, S. I. (1988). Evaluation of methods for the separation and analysis of proteins and free amino acids in phytoplankton samples. J. Plankton Res. 10: 341–358

Compton, S. J., Jones, C. G. (1985). Mechanisms of dye response and interference in the Bradford protein assay. Analyt. Biochem. 151: 369–374

Conover, S. A. M. (1975). Partitioning of nitrogen and carbon in cultures of the marine diatom *Thalassiosira fluviatilis* supplied with nitrate, ammonium, or urea. Mar. Biol. 32: 231–246

Cowey, C. B., Corner, E. D. S. (1966). The amino-acid composition of certain unicellular algae, and of the faecal pellets produced by *Calanus finmarchicus* when feeding on them. In: Barnes, H. (ed.) Some contemporary studies in marine science. Allen and Unwin, London, p. 225–231

Dickson, D. M. J., Kirst, G. O. (1987). Osmotic adjustment in marine eukaryotic algae: the role of inorganic ions, quanternary ammonium, tertiary sulphonium and carbohydrate solutes. I. Diatoms and a rhodophyte. New Phytol. 106: 645–655

Dorsey, T. E., McDonald, P., Roels, O. A. (1977). A heated biuret-folin protein assay which gives equal absorbance with different proteins. Analyt. Biochem. 78: 156–164

Dorsey, T. E., McDonald, P., Roels, O. A. (1978). Measurements of phytoplankton protein content with the heated biuret-folin assay. J. Phycol. 14: 167–171

Dortch, Q., Clayton J. R., Jr., Thoresen, S. S., Ahmed, S. I. (1984). Species differences in accumulation of nitrogen pools in phytoplankton. Mar. Biol. 81: 237–250

Dortch, Q., Clayton J. R., Jr., Thoresen, S. S., Cleveland, J. S., Bressler, S. L., Ahmed, S. I. (1985). Nitrogen storage and use of biochemical indices to assess nitrogen deficiency and growth rate in natural phytoplankton populations. J. mar. Res. 43: 437–464

Eze, J. M., Dumbroff, E. B. (1982). A comparison of the Bradford and Lowry methods for the analysis of the protein in chlorophyllous tissue. Can. J. Bot. 60: 1046–1049

Fowden, L. (1952). The composition of the bulk proteins of *Chlorella*. Biochem. J. 50: 355–358

Gnaiger, E., Bitterlich, G. (1984). Proximate biochemical composition and caloric content calculated from elemental CHN analysis: a stoichiometric concept. Oecologia 62: 289–298

Harrison, P. J., Waters, R. E., Taylor, F. J. R. (1980). A broad spectrum artificial seawater medium for coastal and open ocean phytoplankton. J. Phycol. 16: 28–35

Hopkins, C. C. E., Seiring, J. V., Nyholmen, O., Hermannsen, A. (1984). Ecological energetics from total lipid and total protein: fact and artifact using a gravimetric method for lipid and a biuret method for protein. Oceanogr. mar. Biol. A. Rev. 22: 211–261

Kowallik, W. (1978). Blue light effects on carbohydrate and protein metabolism. In: Senger, H. (ed.) Blue light responses: phenomena and occurrence in plants, Vol. 1. CRC Press, Boca Raton, p. 8–13

Laws, E. A. (1991). Photosynthetic quotients, new production and net community production in the open ocean. Deep-Sea Res. 38: 143–167

Legler, G., Muller-Plutz, C. G., Mentges-Hettkamp, M., Pflieger, G., Julich, E. (1985). On the chemical basis of the Lowry protein determination. Analyt. Biochem. 150: 278–287

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951). Protein measurement with Folin-phenol reagent. J. biol. Chem. 193: 265–275

Lui, N. S. T., Roels, O. A. (1972). Nitrogen metabolism of aquatic organisms. II. The assimilation of nitrate, nitrite, and ammonium by *Biddulphia aurita*. J. Phycol. 8: 259–264

Manahan, D. T., Nourizadeh, S. (1990). Comparisons of spectrophotometric and chromatographic techniques for the absolute determination of protein content in marine invertebrate eggs and larvae: implications for studies of growth and energetics. Eos 71(2): 12 (abstract)

Mattoo, R. L., Ishaq, M., Saleemuddin M. (1987). Protein assay by Coomassie Brilliant Blue G-250-binding method is unsuitable for plant tissues rich in phenols and phenolases. Analyt. Biochem. 163: 376–384

Mayer, L. M., Schick, L. L., Setchell, F. W. (1986). Measurement of protein in nearshore marine sediments. Mar. Ecol. Prog. Ser. 30: 159–165

Mayzaud, P., Martin, J. L. M. (1975). Some aspects of the biochemical and mineral composition of marine plankton. J. exp. mar. Biol. Ecol. 17: 297–310

Parsons, T. R., Stephens, K., Strickland, J. D. H. (1961). On the chemical composition of eleven species of marine phytoplankters. J. Fish. Res. Bd Canada 18: 1001–1016

Setchell, F. W. (1981). Particulate protein measurement in oceanographic samples by dye binding. Mar. Chem. 10: 301–313

Smith, P. K., Krohn, R. I., Hermanson, T. I., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., Klenck, D. C. (1985). Measurement of protein using bicinchoninic acid. Analyt. Biochem. 150: 76–85

Steel, R. G. D., Torrie, J. H. (1980). Principles and procedures of statistics, 2nd edn. McGraw-Hill. New York

Stoscheck, C. M. (1990). Quantitation of protein. Meth. Enzym. 182: 50–69

Whyte, J. N. C. (1987). Biochemical composition and energy content of six species of phytoplankton used in marineculture of bivalves. Aquaculture 60: 231–241

Zamer, W. E., Shick, J. M., Tapley, D. W. (1989). Protein measurements and energetic considerations: comparisons of biochemical and stoichiometric methods using bovine serum albumin and protein isolated from sea anemones. Limnol. Oceanogr. 34: 256–263

Communicated by R. Thompson, St. John's