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ULTRAVIOLET RADIATION INDUCED CHANGES IN THE PRODUCTION OF ORGANIC COMPOUNDS IN ANTARCTIC MARINE PHYTOPLANKTON

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Abstract: Experiments were conducted during January 1995 in the Southern Ocean to examine the impact of enhanced solar ultraviolet–B radiation (UVBR) on the biosynthesis and composition of organic compounds in Antarctic marine phytoplankton. Our results revealed distinct changes in the biochemical composition of phytoplankton attributable to UVBR exposure. Fatty acid concentrations increased in the presence of UVBR, mainly on account of a large increase in the content of saturated fatty acids within the cells. On the other hand, polyunsaturated fatty acids declined in cells exposed to UVBR. Amino acid concentrations were higher in the UVBR exposed samples, attributable largely to a UVBR induced increase in cellular concentrations of glutamic' acid (glutamic acid+glutamine) and aspartic acid. Monosaccharide constituents of cellular storage and structural carbohydrates, however, showed a decline in the cells exposed to UVBR. Except for the decline in structural monosaccharides, these changes in the patterns of organic compounds observed in Antarctic phytoplankton were remarkably similar but, greater in magnitude in comparison to those observed in temperate phytoplankton exposed to UVBR.

1. Introduction

Every year, a large increase in UVBR accompanies the annual cycle of stratospheric ozone depletion over the Antarctic and its surrounding seas (SMITH *et al.*, 1992; WEILER and PENHALE, 1994). This depletion of ozone has continued unabated over the last few years, resulting in increasing UVBR (STOLARSKI *et al.*, 1992; KERR and MCELROY, 1993; JONES and SHANKLIN, 1995). Since UVBR is biologically harmful, its increase has led to concern about its impact on the flora and fauna of this region (WEILER and PENHALE, 1994; PREZELIN *et al.*, 1994). Furthermore, since UVBR can penetrate to biologically significant depths (20–60 m) into the water column (SMITH *et al.*, 1992), its enhancement it is believed, could be detrimental to life in the seas around Antarctica. Marine phytoplankton appear to be particularly vulnerable to UVBR as they depend on solar energy for photosynthesis and growth (SMITH *et al.*, 1992; HOLM-HANSEN and LUBIN, 1994), and, although it has been shown that UVBR is suppressing phytoplankton productivity in the Antarctic Ocean

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(Holm-Hansen *et al.*, 1993; Prezelin *et al.*, 1994), lack of information in several key areas of phytoplankton photophysiology and photobiochemistry has precluded an assessment of its consequences on the marine food web and biogeochemical cycles in these waters (Häder, 1993; Holm-Hansen and Lubin, 1994).

During a cruise of the R.V. Hakuho Maru to the Southern Ocean in January 1995, we examined the effects of UVBR on the biochemical composition of Antarctic marine phytoplankton. The stimulus for this research was the belief that such data would help us recognize the broader implications of enhanced solar UVBR on the marine food web and biogeochemical cycles in the Southern Ocean.

2. Materials and Methods

Experiments were undertaken on the 21 and 22 of January 1995 (both cloud free days), at two locations approximately 65°30′S, 140°E. Sea water samples were collected from a depth of about 5 m by means of a submersible pumping system and concentrated onto a 20 μm mesh net. After careful removal of the larger zooplankton, phytoplankton, microzooplankton and protozooplankton retained on the net were resuspended in 4.8 l of filtered (Whatman® GF/F filters) sea water. Aliquots of the suspension were immediately filtered (GF/F filters pre-combusted at 450°C for 4h) for natural ^{13}C abundance and pre-incubation values of fatty acids, amino acids and monosaccharide constituents of structural and storage carbohydrates (Kouchi, 1982). Aliquots were also preserved with 1% glutaraldehyde for identification and cell counts of phytoplankton and other organisms. Exposure to UVBR (+UVBR) was achieved by incubating the experimental phytoplankton in duplicate sets of 200 ml quartz bottles. Glass bottles (200 ml, duplicate sets) opaque to UVBR (−UVBR) but, transparent to photosynthetically active radiation (PAR) served as controls. The transmission characteristics of the quartz and glass bottles have been reported earlier in Goes *et al.* (1995a). During the incubations (6 h), performed on the deck in running surface sea water (−0.2 to −0.6°C), noon time UVR levels (as measured with a Philips UVX digital radiometer, Model UVX–36, peak detection at 365 nm) exceeded 1 mW cm^{-2}. Phytoplankton photosynthesis was estimated using ^{13}C as a tracer, and in conjunction with GC and GC–MS techniques (Kouchi, 1982; Hama *et al.*, 1993) was used to examine the impact of UVBR on the synthesis of fatty acids, amino acids and monosaccharide constituents of structural and storage carbohydrates (see Goes *et al.*, 1994, 1995a, b, 1996, for details of the analytical procedures). All filter samples after incubation were stored at −85°C prior to analysis. Owing to hydrolysis and derivatization of the samples during the analytical process for amino acids, it was difficult to obtain separate estimates of glutamic acid and glutamine. In this study, glutamic' acid refers to estimates of these two compounds in combination.

3. Results and Discussion

Nutrient concentrations in these waters were extremely high (NO_3+NO_2=~27, PO_4=~2 and SiO_4=~43 μg at l^{-1}). These concentrations are much in excess of the amount required for sustaining photosynthesis in Antarctic phytoplankton (Holm-

HANSEN *et al.*, 1977). Microscopic examination of the organisms preserved with 1% glutaraldehyde revealed the predominance of diatoms. Differences in the species composition of phytoplankton collected on the two days were not large, with *Rhizosolenia* spp., *Nitzschia* spp. and *Corethron* spp. being the dominant forms. Dinoflagellates and other phytoplankton groups were present but, in small numbers. A complete list of phytoplankton identified in the samples is being presented elsewhere (GOES *et al.*, in preparation). Microzooplankton and protozooplankton were also poorly represented in the samples.

At the end of the incubation period, there were striking differences in the composition of organic compounds in the −UVBR and the +UVBR samples. Since phytoplankton were a major constituent of the samples, we believe that the differences in the biochemical composition of the samples observed at the end of the experiments was largely a reflection of the changes within phytoplankton rather than other organisms present in the samples. This view is also supported by the appearance of the ^{13}C tracer within the different organic compounds under scrutiny (GOES *et al.*, in preparation). If we assume thus, that the organic material within the samples was largely of phytoplankton origin, our results suggest that biochemical processes within Antarctic phytoplankton undergo significant alterations as a result of exposure to UVBR. The consistency in the trend of changes observed for the two sets of experiments performed on separate days and separate populations of Antarctic phytoplankton exposed to UVBR, reinforces the view of BASSHAM (1971), that phytoplankton, as part of their adaptive response for growth optimization and survival under stress, respond preferably by reorganizing their cellular metabolic processes.

3.1. Effect of UVBR on the composition of fatty acids

At the end of the incubation, absolute concentrations of fatty acids were markedly higher in the +UVBR samples as compared to the −UVBR samples. As can be seen in Fig. 1A and B, this increase was largely on account of the extremely high increase in the concentrations of the saturated fatty acids, 16:0, 14:0 and other saturated fatty acids in phytoplankton exposed to UVBR. Concentrations of polyunsaturated fatty acids on the other hand, were lower in the +UVBR samples. When expressed as a percentage of the total fatty acids within the cells, values of polyunsaturated fatty acids were lower in phytoplankton exposed to UVBR. The polyunsaturated fatty acids, 20:5 and 22:6 in particular, were much higher in samples shielded from UVBR. In many respects, these changes in the composition of fatty acids in the presence of UVBR observed in phytoplankton from the Southern Ocean are remarkably similar to our results obtained with temperate marine phytoplankton (GOES *et al.*, 1995a, b, 1996; GOES, 1996).

In order to illustrate the possible effects of UVBR on the biosynthesis of fatty acids based on our findings, a schematic diagram showing the major pathways of fatty acid synthesis in marine phytoplankton is shown in Fig. 2. It can be seen that the basic process of fatty acid synthesis consists of a fatty acid desaturation process which is superimposed on a carbon chain elongation process, that leads to the formation of saturated fatty acids (OPUTE, 1974). POHL and WAGNER (1972) and OPUTE (1974) have

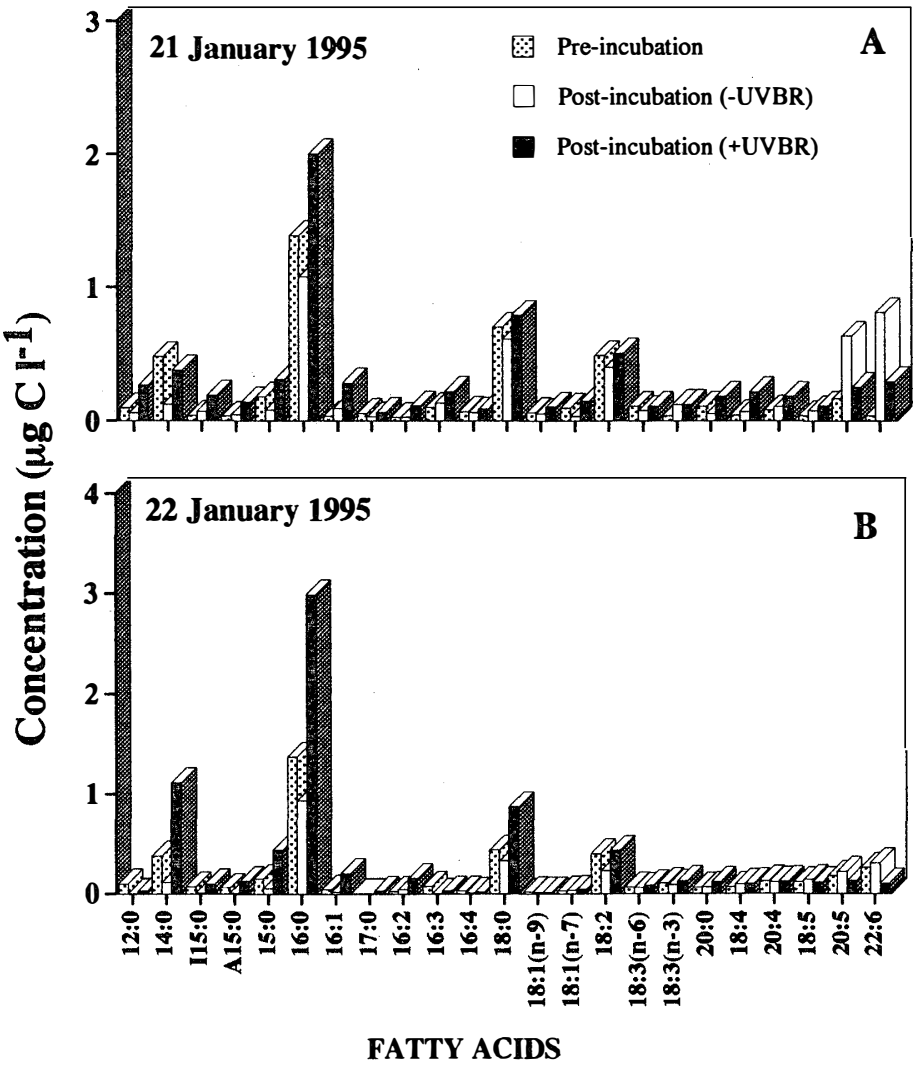

Fig. 1. Changes in the absolute concentrations of component fatty acids in the post-incubation (control) and (UVBR exposed) samples of phytoplankton on the (A) 21 January 1995 and (B) 22 January 1995.

reported that the chain elongation process and the increase in the concentration of saturated fatty acids, basically, represents an energy storage mechanism linked to photosynthesis under unfavorable growth conditions. Our data reveal that the chain elongation process was unaffected by UVBR. Furthermore, the increase in saturated fatty acids in the presence of UVBR indicates that growth under this condition was not favorable.

The process of desaturation of fatty acids on the other hand, is dependent on an abundant supply of ATP (THOMPSON *et al.*, 1990) and inorganic nitrogen (OPUTE, 1974; POHL, 1974). Evidence is available to show that both, the production of ATP (VOSJAN *et al.*, 1990), as well as assimilation of nitrogen (DÖHLER, 1985, 1990, 1992) are markedly reduced in the presence of UVBR. It is possible therefore, that on account of the high incident levels of UVBR in the Antarctic, the reduction in the supply of both ATP and inorganic nitrogen could have retarded the desaturation

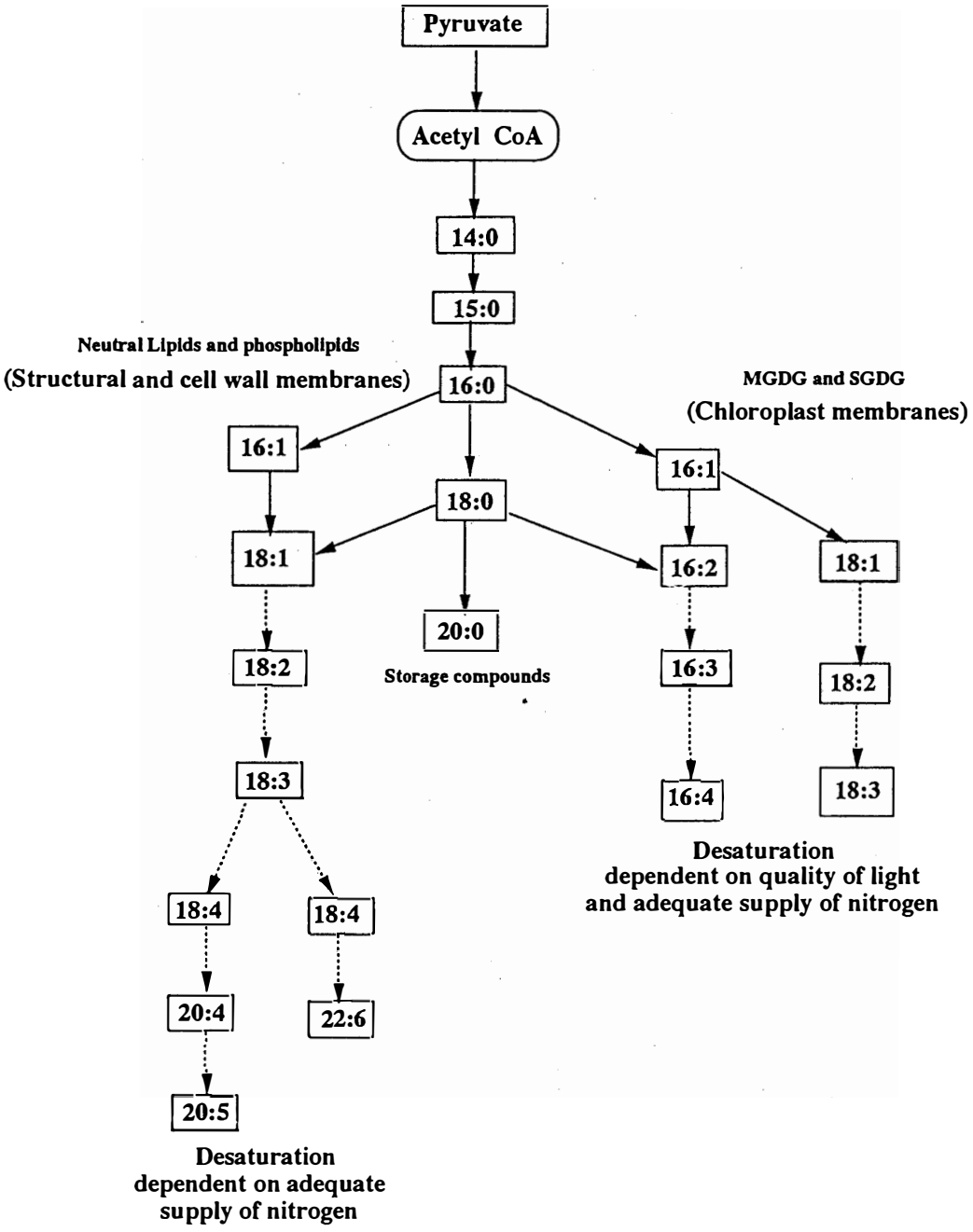

Fig. 2. Schematic diagram showing the major pathways of fatty acid synthesis in marine phytoplankton. Pathways possibly affected by UVBR are depicted by broken lines. MGDG: monogalactosyl diglyceride, SGDG: sulphoquinovosyl diglyceride.

process leading to a decline in cellular levels of polyunsaturated fatty acids. Although polyunsaturated fatty acids make up a small fraction of the total amount of fatty acids within the lipid pool of phytoplankton cells, they are important constituents of the cell (HAMA, 1988). For example polyunsaturated fatty acids are vital constituents of the cell wall as well as the membranes of the chloroplast (POHL, 1974). It has been shown that a decrease in the proportion of polyunsaturated fatty acids within the cell wall

membranes of phytoplankton can reduce membrane permeability and therefore the ability of phytoplankton to assimilate nutrients and grow (CLAUSTRE *et al.*, 1989; GOES *et al.*, 1994). This effect, in combination with the direct effect of UVBR on certain enzymes involved in nutrient assimilation (DÖHLER *et al.*, 1991), could, in nutrient rich regions like the Southern Ocean, be an important determining factor for the formation and growth of phytoplankton blooms. It is possible that UVBR at the present levels in the Southern Ocean is a significant factor retarding the formation and growth of phytoplankton blooms (GOES *et al.*, 1994).

3.2. *Effect of UVBR on the composition of amino acids*

In both sets of experiments, concentrations of amino acids were higher in the samples exposed to UVBR. Glutamic' acid (glutamic acid+glutamine), aspartic acid, lysine and phenylalanine were the major constituents of the amino acid pool and their extremely high increase in the +UVBR samples marked the most profound differences from the −UVBR samples (Fig. 3A and B). Alanine and valine, which are known to represent a sizeable fraction of the amino acid pool in temperate phytoplankton (BROWN and JEFFREY, 1992) were extremely low in the phytoplankton

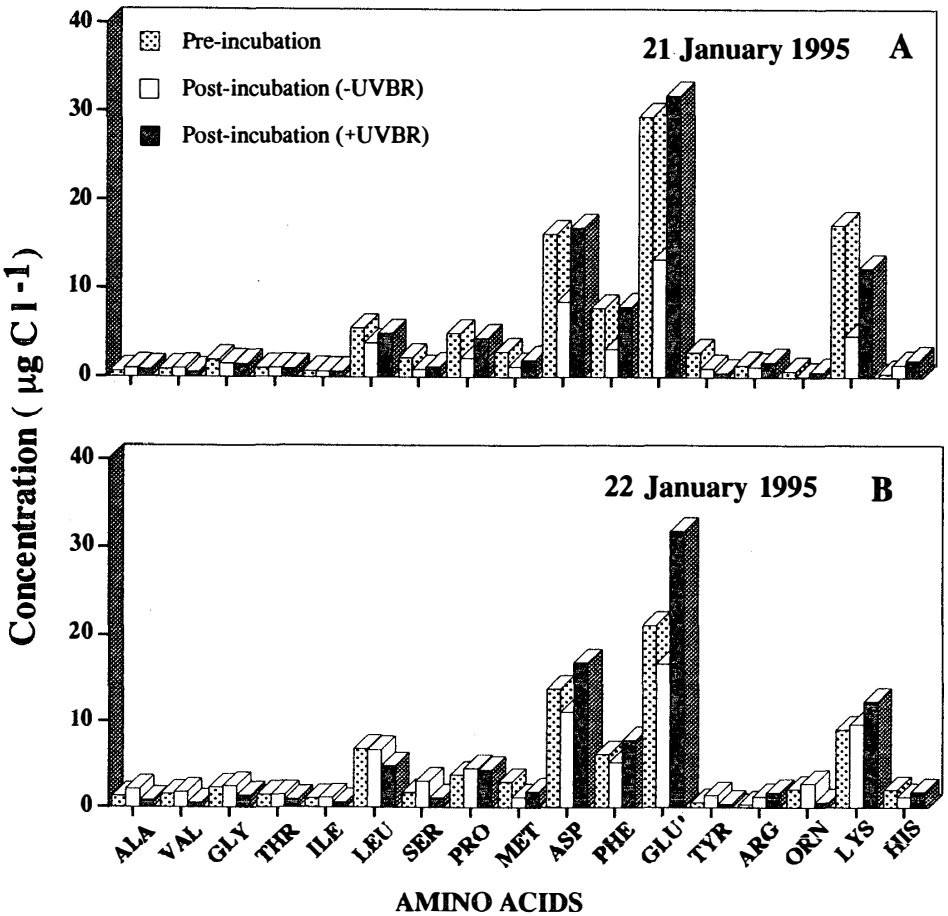

Fig. 3. Changes in the absolute concentrations of total cellular amino acids in the post-incubation (control) and (UVBR exposed) samples of phytoplankton on (A) 21 January 1995 and (B) 22 January 1995. GLU': glutamic' acid (glutamic acid+glutamine).

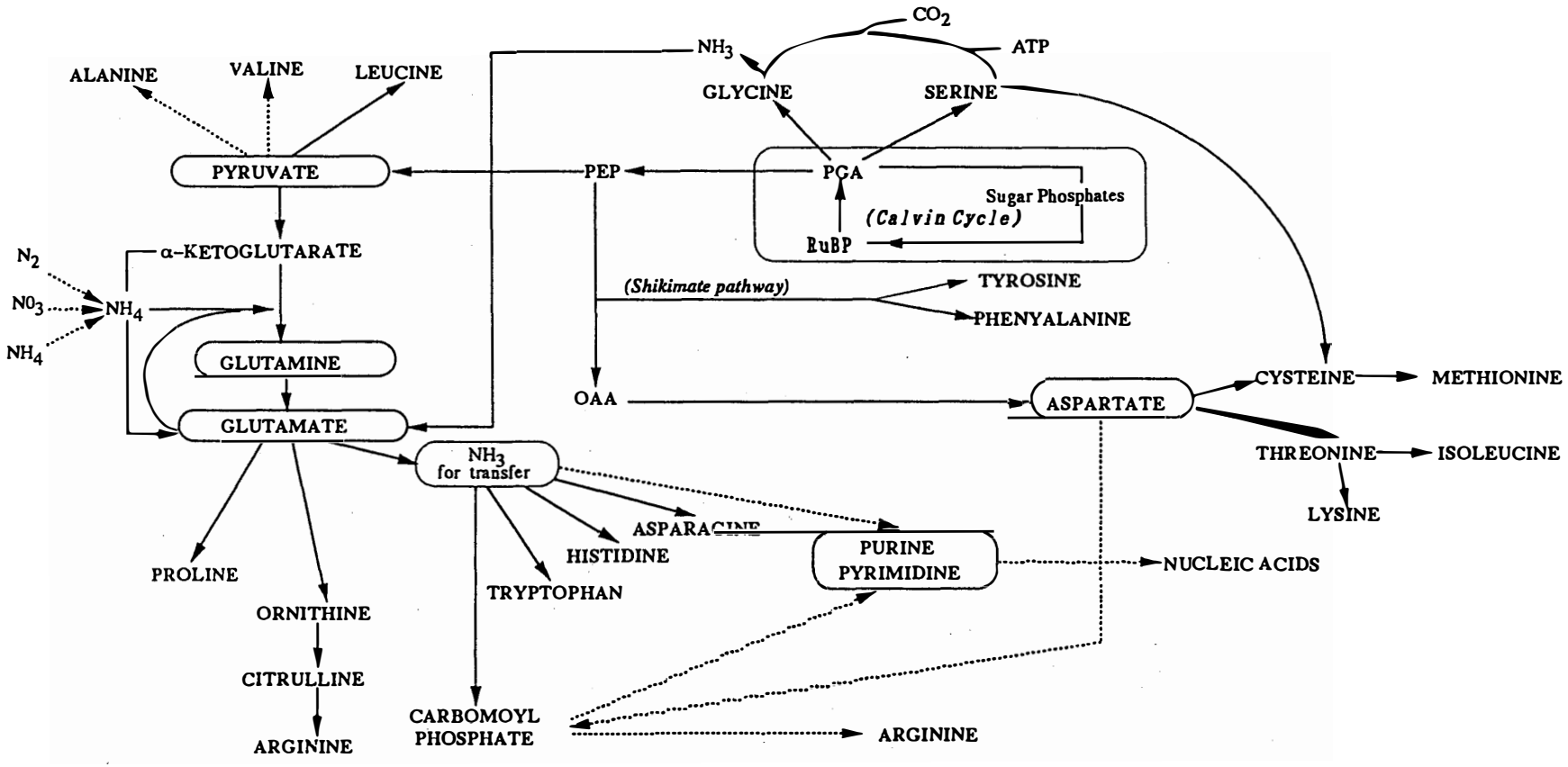

Fig. 4. Schematic diagram showing the major pathways of amino acid synthesis in phytoplankton. Unbroken lines indicate pathways that are probably unaffected by UVBR whereas broken lines indicate pathways probably affected by UVBR.

from the Southern Ocean. These changes in the amino acid pool within Antarctic phytoplankton exposed to UVBR are remarkably similar to our previous studies with temperate phytoplankton (GOES *et al.*, 1995a), but differ only in magnitude, being higher in phytoplankton from the Southern Ocean. In order to get an overall view of the changes in the amino acid composition of phytoplankton that could be induced by UVBR, a schematic diagram which highlights the major pathways of amino acid biosynthesis has been presented (Fig. 4). According to this figure, it is apparent that if there is a reduction in the supply of nitrogen, or if the activities of certain amino transferases are suppressed, both glutamine as well as glutamic acid, cannot be utilized for further transamination/amination reactions leading to their eventual increase in the cell. The accumulation of large quantities of glutamic' acid is consistent with the observations of DÖHLER (1985) and GOES *et al.* (1995a, b) who attributed this to suppression of inorganic nitrogen assimilation by UVBR. STEWART and RHODES (1977) reported that large quantities of glutamic acid within the amino acid pool could by itself inversely regulate nitrogen assimilation by phytoplankton, leading to extreme nitrogen depletion within the cells.

Aspartic acid which showed an increase in the presence of UVBR, is formed primarily from phosphenol pyruvate (PEP) via oxaloacetic acid (OAA). Whilst it is immediately evident that this pathway is not affected by UVBR, the accumulation of this compound in cells exposed to UVBR could have resulted primarily from its non-utilization in the synthesis of nucleic acids as a result of a decrease in the supply of carbomoyl phosphate formed from glutamate. As is evident from Fig. 4, the increase in lysine that we observed in the present study could represent an overflow mechanism associated with the UVBR induced increase in aspartic acid (GOES *et al.*, 1995a, b).

3.3. *Effect of UVBR on the composition of monosaccharides within the storage and structural carbohydrate pool*

The absolute concentrations of monosaccharides within storage and structural carbohydrates accounted for less than 20% of the total carbon content of Antarctic phytoplankton. These values are unusually low for marine phytoplankton. The accumulation of carbohydrates in the storage carbohydrate pool of phytoplankton has been dealt with in previous studies (HANDA, 1969; MORRIS, 1981; HAMA, 1988, 1992). In general, when nutrients are not limiting and photosynthetic rates are in excess of metabolic requirements, storage carbohydrates accumulate. The present observations thus provide clear evidence that during exposure to UVBR, these optimal conditions are not fulfilled, and storage carbohydrates do not accumulate. The composition of monosaccharide components within the storage carbohydrate pool was also unusual in that, it was characterized by greatly reduced concentrations of hexose sugars such as glucose, galactose and mannose known to be the major monosaccharides of the storage carbohydrate pool (Fig. 5A and B). On the contrary, Antarctic phytoplankton were characterized by higher concentrations of ribose. Exposure to UVBR resulted in a significant reduction in the absolute concentrations of ribose and all other storage sugars.

In the structural carbohydrate pool, absolute concentrations of almost all

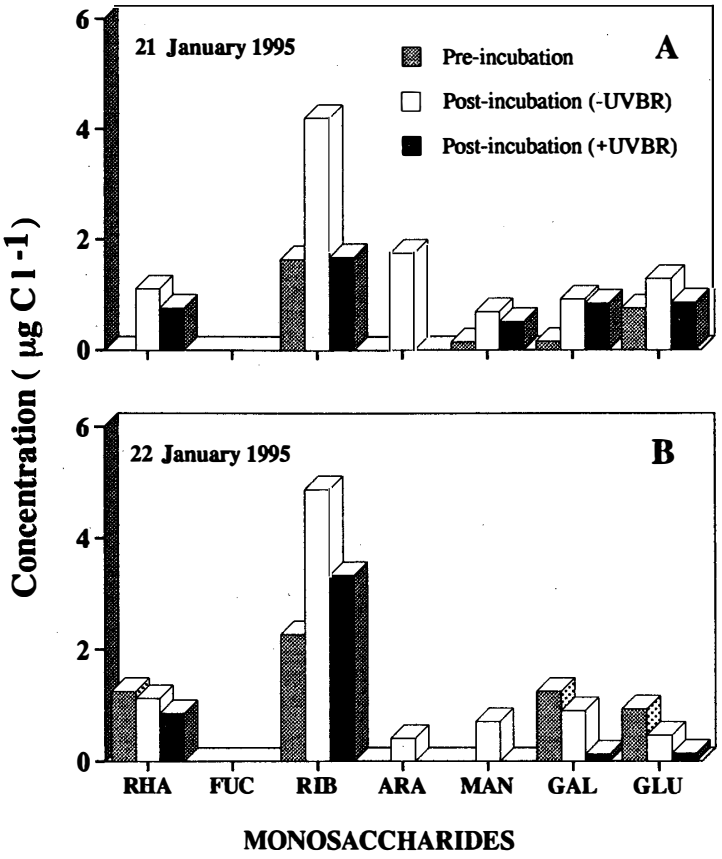

Fig. 5. Changes in the absolute concentrations of neutral monosaccharide components within the storage carbohydrate pool in the post-incubation (control) and (UVBR exposed) samples of phytoplankton on (A) 21 January 1995 and (B) 22 January 1995.

monosaccharides, except for arabinose were lower in cells exposed to UVBR (Fig. 6A and B). This reduction in the pool sizes of storage and structural carbohydrates clearly bears on the reduced capacity of Antarctic phytoplankton to photosynthesize on account of UVBR.

Based on the results of the present study and those reported earlier (GOES *et al.*, 1994, 1995a, b, 1996), we have attempted to illustrate the impact of UVBR on the major metabolic biosynthetic pathways in phytoplankton in Fig. 7. The reduction in the rates of CO_2 assimilation which we observed in the presence of UVBR has generally been attributed to a reduction in the activity of ribulose 1–5, bisphosphate carboxylase, the carbon dioxide fixing enzyme (DÖHLER *et al.*, 1987) and sometimes to a reduction in cellular levels of ATP and other reducing compounds (VOSJAN, 1990). While the reduction in the rates of CO_2 assimilation in the presence of UVBR appears to have led to a decline in the overall rates of production and pool sizes of storage carbohydrates, we believe that on account of a continuous demand for storage carbohydrates for protein synthesis, which, in the presence of UVBR was in excess of their rates of production, most of the newly synthesized storage carbohydrates especially glucose, were being rapidly utilized to sustain a steady rate of protein synthesis. This notion is based on earlier views that the accumulation of storage

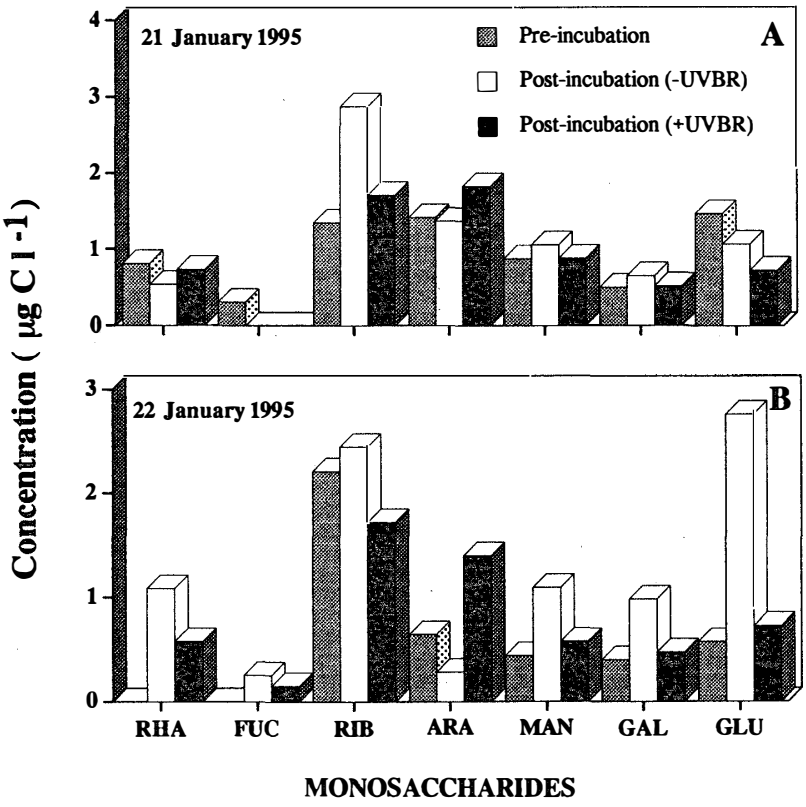

Fig. 6. Changes in the absolute concentrations of neutral monosaccharide components within the structural carbohydrate pool in the post-incubation (control) and (UVBR exposed) samples of phytoplankton on (A) 21 January 1995 and (B) 22 January 1995.

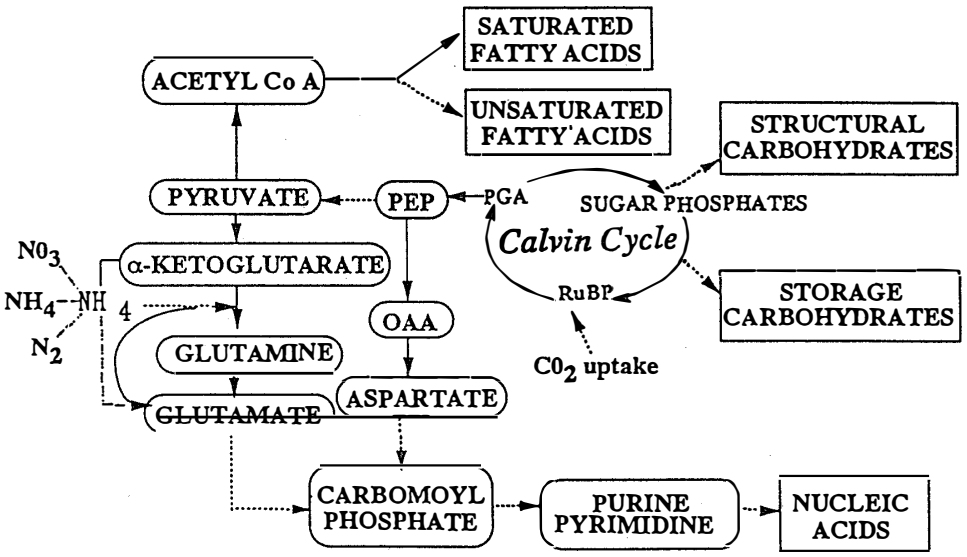

Fig. 7. Schematic diagram showing the major pathways of synthesis of carbohydrates, fatty acids and amino acids in phytoplankton. Unbroken lines indicate pathways that are probably unaffected by UVBR whereas broken lines indicate pathways probably affected by UVBR.

carbohydrates takes place only when the production of newly fixed photosynthetic energy is over and above that required for maintaining optimal rates of cellular metabolic activities and growth (STEER, 1974; MACKIE and PRESTON, 1974; BEARDALL *et al.*, 1976; MORRIS, 1981). This hypothesis explaining the reduction in the pool sizes of storage carbohydrates in the presence of UVBR is also in accordance with that of SHUTER (1975), who concluded that the overall strategy of phytoplankton cells even under unfavorable conditions is to maximize protein synthesis and growth which they achieve by regulating cellular metabolism of storage compounds especially, carbohydrates.

3.4. Impact of UVBR on phytoplankton growth rates and its implications for the Antarctic marine food web and biogeochemical cycles

Growth of phytoplankton is largely determined by the ability of the cells to synthesize proteins which is in turn dependent on the availability of nitrogen and an adequate supply of carbon skeletons and energy that are largely available from storage carbohydrates (RIGANO *et al.*, 1991). In the presence of UVBR, a reduction in storage carbohydrates in combination with an inadequate supply of nitrogen required for protein synthesis would have contributed greatly to the reduced rates of phytoplankton growth.

Over the last few years, the requirement for studying compounds of phytoplankton photosynthetic metabolism, such as amino acids and fatty acids has assumed new importance (WATANABE *et al.*, 1983) following the discovery that a dietary deficiency of these compounds could severely limit growth in certain zooplankton and other juveniles of higher trophic animals. A dietary deficiency of polyunsaturated fatty acids, for example, can severely restrict the growth of several herbivores which are unable to synthesize these compounds by themselves (VOLKMAN *et al.*, 1989). In sinking particles also, amino acids of phytoplankton origin play an important role in sustaining the growth of benthic organisms (ITTEKOT *et al.*, 1984). Similarly carbohydrates in sinking particles, especially storage monosaccharides, are preferentially and rapidly utilized by microheterophs highlighting their role as a major energy source for microrganisms living in deeper waters (HANDA and TOMINAGA, 1969; HANDA, 1969).

UVBR induced changes in composition of organic compounds within Antarctic marine phytoplankton resemble remarkably the results of similar studies undertaken on temperate marine phytoplankton except that the changes recorded within Antarctic phytoplankton were of a greater magnitude. We suspect that the greater degree of changes observed in Antarctic phytoplankton in comparison to our observations in temperate phytoplankton are related to the higher levels of UVBR Antarctic phytoplankton were exposed to (GOES *et al.*, 1995a, b, 1996; HELBLING *et al.*, 1990). The similarity in the trends of change in the biochemical composition of phytoplankton in the presence of UVBR, irrespective of the phytoplankton species present indicates that phytoplankton in general possess a definite biochemical response to UVBR. These results also suggest that careful studies under more controlled conditions could present changes in the ratios of various biochemical compounds as excellent indicators of physiological stress due to UVBR. Additionally,

the usefulness of these ratios could be significantly enhanced if UVBR measurements are weighted with an appropriate biological weighting factor (CALDWELL *et al.*, 1986; CULLEN *et al.*, 1992).

The present results refer to phytoplankton in incubation bottles. In the natural environment, an assessment of the true significance of the changes induced by UVBR will require that we study them in conjunction with mixing processes and residence times of phytoplankton in the water column. This is essential because recent evidence shows that phytoplankton have a remarkable capacity for recovery when transferred to non UVBR conditions or very low UVBR but sufficiently high PAR conditions (LESSER *et al.*, 1994).

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