

DETERMINATION AND REACTIVITY OF FOLIC ACID IN NATURAL WATERS

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Abstract

Folic acid (FA) was determined in natural waters (Adriatic Sea water and Rogoznica Lake water) and in algal cultures by cathodic stripping voltammetry. The concentration of FA in seawater varies between 0.03 and 4.3 nM L⁻¹. In Rogoznica Lake water (S = 30-38) the concentration of FA varies between 0.18 and 4 nM L⁻¹. High concentration of FA (36 nM L⁻¹) was determined in the Rogoznica Lake at the depth below 8 m during the period of anoxia. The influence of different types of organic matter (humic and fulvic acid, polysaccharide and protein), and sulfide ions on the determination of FA in natural waters was studied. The interfering effect of humic acid could be eliminated by passing the sample through Sep-Pak-C-18 cartridges. The experiments with algal cultures (*Dunaliella*, *Emiliania* and *Phaeodactylum*) have shown that folic acid could be a tentative nutrient.

Key-words: nutrients, organic matter, Adriatic sea

Introduction

Folic acid is the most common name, among several synonyms (vitamin Bc, vitamin M, biotin, folacin, foliamin, folsan, folvit), for a compound N-4-(2-amino-1,4-dihydro-4-oxo-6 pteridiny) methyl aminobenzoyl L-glutamic acid. Folic acid is a vitamin that is required in the transfer of single carbon moieties, thus playing an important role in several metabolic pathways in mammals (1). FA is also produced by plants (green leaves, algae) and by microorganisms. Little is known about folic acid occurrence and distribution in natural waters, although its occurrence as a result of algal exudations or involuntary releases has been reported (2). Recently, a very sensitive electrochemical method of cathodic stripping voltammetry has been developed (3), which has enabled us to determine the relatively low concentrations of folic acid in natural waters (3, 4, 5). In this paper we report on the reactivity of FA in natural waters as well as the influence of several organic compounds and sulfide on its determination. The possible importance of FA as a nutrient for algae was evaluated from uptake experiments with cultured marine algae.

Experimental

Measurements of FA in seawater and lake water have been done on a PAR 174 A polarograph connected to a Metrohm HMDE (hanging mercury drop electrode) and μ -Autolab (ECO-CHEM) connected to a Metrohm VA 663. For capacitive current measurements a Metrohm E-506 instrument was used. Folic acid in natural water samples was determined according to a previously described procedure (3). Milli Q water (Millipore water purification system, USA) was used throughout the experiments. Monocultures of *Emiliania huxleyi*, *Dunaliella minuta* and *Phaeodactylum tricornutum* were grown in 250 F/2 medium in synthetic seawater to which 1 ml of algal culture was added, giving a starting concentration of 3×10^3 cells mL⁻¹. Incubation was carried out in a temperature controlled incubator (Mercia scientific) with 24 h illumination at 15°C. Samples were filtered off through a GF/F glass fibre filters of 0.7 μ m pore size.

Results and discussion

Voltammetric analyses of seawater samples from different parts of the Adriatic Sea revealed the presence of FA, variable with depth, throughout the year. The highest FA concentration was found in a surface seawater sample in May (Fig.1A) which could be a result of increased biological activity at that time. In summer months, the concentration of folic acid in surface waters decreased due to the sensitivity of folic acid to photo-decomposition. In deeper waters of the Adriatic sea a certain increase in FA concentration was observed. In the case of Rogoznica Lake (~14 m deep, salinity 30-38, anoxia below 8 m in certain periods of year) near Sibenik, which is connected with the Adriatic Sea, the concentration of FA in surface waters was relatively low (few nmoles L⁻¹) (Fig.1B). In deeper waters, especially in the anoxic water, the concentration of folic acid increased with its maximum at 11 m in April 1995 and at 12 m in May 1997. The maximum at 11 m depth in April 1995 coincided with the chlorophyll *a* maximum, and the combined concentration of sulfur and sulfide (6) as well as with the iodide maximum (7). The high FA concentrations in anoxic waters are probably derived from sinking, decomposing algae, which are not broken down by oxidative bacterial activity in the prevailing anoxic conditions. In Fig. 3 the cathodic stripping scans for water samples from Rogoznica Lake are presented. The presence of sulfur species did not mask the FA voltammetric peak in the sample. The

interference of some organic compounds (humic acid, fulvic acid, Dextran T-500 and protein - egg albumin) with the FA determination was investigated (Fig. 3). Egg-albumin does not influence the determination of FA significantly. Dextran-T-500 influenced the determination of FA in a concentration range 2 mg L⁻¹. Humic and fulvic acids influenced the determination of FA even at low concentration ranges (1 mg L⁻¹ of fulvic or humic acids). The influence of humic acid on the FA peak can be minimized by passing the seawater through a SEP-PAK-C-18 column at high flow rate (15 ml min⁻¹) and pH = 1.8. The determination of FA in the samples has been done with a standard addition method and it was estimated that there was no complexation reaction between HA and FA, only the sensitivity of the determination was changed (5).

Variation of the FA concentrations in the water column in the Atlantic Ocean (4) suggests localized FA production and uptake by microorganisms. The uptake of FA was studied in algal cultures of *Dunaliella*, *Emiliania* and *Phaeodactylum* to see if they derive an advantage from this uptake. Preliminary experiments showed that FA

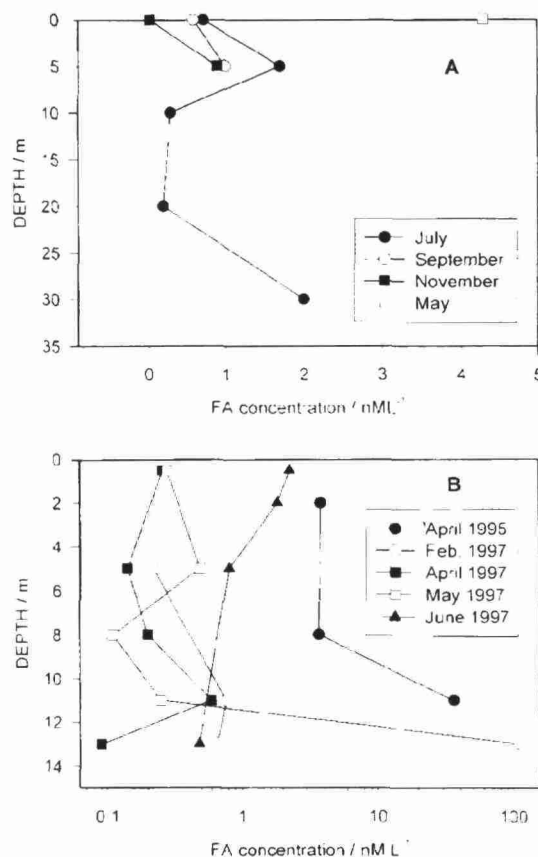


Fig. 1. Vertical profiles of FA for North Adriatic seawater samples for different months in 1994 (A) and for Rogoznica Lake water column (B).