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SYNTHETIC DETERGENTS: THEIR INFLUENCE UPON IRON BINDING COMPLEXES OF NATURAL WATERS[1]

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[1]Contribution from Dingell-Johnson Project F-27-R, Michigan

Abstract

Organic compounds extracted from Michigan lakes and streams and added to algae cultures increase the growth rate of the green alga (Chlamydomonas Reinhardi) when iron is present. Two dimensional paper chromatography has shown that the iron is complexed by organic fractions containing an amine group. When isolated from natural waters containing a concentration over 0.3 ppm alkyl benzene sulfonate (ABS), these compounds do not show an iron binding capacity. Separation of the ABS from the amine complexes restores the iron binding capability. These findings suggest that detergents may influence the mobility of iron by reducing the number of binding sites and in this way may have an important secondary effect upon the primary production of lakes and streams.

The binding of iron by organic compounds has been shown to have an important influence upon cycling of iron in lake ecosystems (1) and secondarily upon the utilization of iron in photosynthesis (2). Amines isolated from lake waters increase the growth rate of algae (3) and do so by forming iron complexes (2). We now wish to report upon the influence of synthetic detergents upon the iron binding capability of naturally occurring amines and amino acids.

A large series of samples of naturally occurring organic compounds were collected from Michigan lakes and streams during the past 3 years by filtering 3000 to 3800 liters of water through a portable underwater filtering system using activated cocoanut charcoal (6-14 mesh) as an absorbent. We eluted organic compounds from the charcoal using a modified Soxhlet apparatus and employing the solubility differentials of petroleum ether, methanol and chloroform. Solvents were concentrated by rotary vacuum evaporation and a Kuderna-Danish evaporative concentrator. We combined the residues and extracted with ether. We extracted the ether solution with dilute HCl (5%) and made the HCl extract strongly basic with NaOH. This basic solution was then extracted with ether to remove amines. We removed water soluble amino acids from the basic solution by acidifying with HCl to a pH of 1.5 and extracting with n-butanol.

We identified amines by paper chromatography using a ninhydrin spray with a solvent system of n-butanol, glacial acetic acid, and water (4:1:4). To test for iron binding we subjected amine residues to two

dimensional chromatography. The above solvent system and ninhydrin were used for the first phase. For the second phase a solvent system consisting of t-amyl alcohol, water, formic acid, and ethyl acetate (30:20:30:20) was used. An iron fraction separated from the amine fraction during the second phase of chromatography. Iron was identified by spraying with a solution of 10 mg of diphenylthiocarbazone in 50 mg of chloroform.

Identification of organic compounds was achieved by the use of infrared and combustion analysis. For infrared analysis we used a Perkin Elmer Model 237B double beam spectrophotometer employing both a NaCl cell with Nujol and a compressed potassium bromide crystal. Bands between 1700 and 1600 cm^{-1} were interpreted as indicating NH deformation, and carboxylate ion was indicated by bands between 1600 and 1500 cm^{-1}. Most significant, however, was absorption between 2800 and 2300 cm^{-1} which is indicative of an acid or protonated amine (4). Combustion analysis was undertaken with a F & M Model 180 carbon, hydrogen, nitrogen (CHN) analyzer. A column temperature of 137° C and furnace temperature of 760° C were used. Helium was used as a carrier gas at a flow rate of 9 cc/sec.

We prepared extracts of amines from 14 Michigan lakes and streams in the above manner. All extracts except those from marl lakes have exhibited iron binding capability and have brought about a significant increase in growth rate of Chlamydomonas Reinhardi when

added to laboratory cultures of this species. Four ninhydrin positive areas have consistently appeared in paper chromatography of these extracts. Mobility data have indicated histidine, tyrosine, aspartic and glutamic acids. We confirmed the identity of the latter two compounds which were most abundant in these extracts by infrared and combustion analysis.

Samples collected from the Huron River, Washtenaw County, Michigan, on April 19, 1965, failed to show the iron binding and growth response characteristics although we noted these properties in samples collected earlier from this location. Analyses of river water on April 19 indicated that anionic detergents were present at a concentration of 0.54 mg per liter (5). Since binding of anionic surfactants to protein molecules has been noted previously (6), we then investigated the possibility that detergents were bound to these amino acids.

We analyzed fractions that failed to complex iron by infrared spectrophotometry. These analyses indicated that alkyl benzene sulfonates were bound to the amino acids (7). We separated the ABS group from the amino acids by chloroform extraction. A binding capability was established when we reintroduced these amino acids into media containing iron (Table 1). Further analysis of Huron River water indicated that detergent concentration varied considerably and that iron binding did not occur in either culture media containing algae or the media alone when the detergent concentration was greater than 0.31 ppm (Table 1). Although removal of the ABS group from the amino acids restored the ability to complex iron, this removal did not restore the amino acid and iron stain intensity

to the level found in waters with low concentrations of ABS (Table 1). This may have been due to incomplete recovery of amino acids or incomplete ABS removal. Slight alteration of the amino acid by the ABS may also have been possible. Evidence that iron binding decreases at ABS levels above 0.3 mg per liter but is not affected in a concentration below 0.1 mg per liter suggests that ABS competes with iron for binding sites.

The addition of various levels of ABS to laboratory cultures containing amino acids from the Huron River confirmed the finding that 0.3 mg per liter is a critical concentration as regards iron binding. Iron staining intensity dropped from 69% at ABS concentrations of 0.2 mg per liter to 12.3% at 0.3 mg per liter. We could not detect iron binding at 0.4 mg per liter ABS.

ABS concentrations frequently exceed 0.3 mg per liter in lakes and streams receiving domestic drainage and sewage effluents. The above findings suggest that primary production of these waters may be reduced by the interference of detergents with transport of iron and other metals required in photosynthesis.

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References and notes

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Table 1. The effect of the alkyl benzene sulfonate (ABS) concentration of Huron River water upon the iron binding capability of amino acids extracted from river water and added to (1) algal culture media, and (2) to culture media containing Chlamydomonas Reinhardi. Data given are means and their 95% confidence intervals. Numbers in parentheses indicate number of flasks read for percent transmittance values.

Percent transmittance of media without algae was set at 100%.

Percent transmittance of cultures without iron or amino acid was 91.1 ± 3.6%.

ABS concentration	(1) Culture media with iron		(2) Media and Chlamydomonas with iron	
	Iron stain intensity[1]	Amino acid staining intensity[2]	Percent transmittance of culture after 10 days incubation	
			With amino acid extracts	Without amino acid extracts
0.08	73.6 ± 2.6 (3)	82.2 ± 3.6 (3)	...*	...*
0.16	71.4 ± 7.6 (3)	78.6 ± 1.8 (3)	65.6 ± 7.8 (6)	79.3 ± 2.2 (6)
0.31	21.6 ± 5.0 (3)	34.2 ± 4.2 (3)	...*	...*
0.41	0.0 (3)	29.4 ± 4.8 (3)	81.3 ± 1.2 (6)	82.6 ± 3.1 (6)
		After ABS removal		
0.31	37.0 ± 3.8 (3)	51.1 ± 6.5 (3)	...*	...*
0.41	35.2 ± 6.1 (3)	43.3 ± 5.3 (3)	71.1 ± 3.1 (6)	81.3 ± 1.9 (6)

[1] Percentage of light adsorbed by photometer scan after two dimensional chromatography and staining with diphenylthiocarbazone.

[2] Percentage of light adsorbed by photometer scan after one dimensional chromatography and staining with ninhydrin spray.

* Not tested.