Fatty acids and sterols in sediments of Hooghly estuary, northeast coast of India

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Total fatty acid content of sediments varied from 7.7 to 25 % of total organic carbon. Chain lengths of fatty acids ranged from C₁₂ to C₂₀. Among the normal saturated fatty acids, palmitic acid (C₁₆:0) was the major component in almost all sediment samples. Unsaturated fatty acids like myristoleic (C₁₄:1), palmitoleic (C₁₆:1), oleic (C₁₈:1), linoleic (C₁₈:2) and linolenic (C₁₈:3) acids were also present. Total sterol varied from 0.12 to 6.64 % of total organic carbon. The sediments showed the presence of cholesterol, campesterol, stigmasterol and β-sitosterol. Distributions and abundances of cholesterol and β-sitosterol were inversely related to each other.

Fatty acid and sterol fraction of lipids are major components of most organisms¹. Many of them have the intrinsic chemical stability to persist for geologically long periods of time² and carry useful information about their transformations in the sediments³. Moreover, the structural specificity of organic compounds in various organisms has been used as molecular indicator of the biological sources of organic matter in the sediments⁴. Hence attention is being focussed on the composition of organic matter in the marine and estuarine sediments as a means of defining the biological sources of organic matter and the chemical reactions that are operating in these systems. The present study deals with the fatty acid and sterol composition of organic matter in the sediments of Hooghly estuary.

Materials and Methods

Surface sediment samples were collected monthly during November 1984 to October 1985 from 3 stations of Hooghly estuary (Fig. 1) in plastic containers and air dried. In order to understand the seasonal variations in the chemical parameters the calender year is divided into 3 seasons—premonsoon (March to June), monsoon (July to October) and postmonsoon (November to February). Sediment samples collected during 4 consecutive months comprising a season were pooled to get a composite sample and this represented the seasonal sample.

For extraction of lipids, sediment samples were refluxed¹ with KOH in methanol (95%), filtered and transferred quantitatively using methanol. After removal of methanol, the organic phase was dried over

Fig. 1—Location of sampling stations
anhydrous Na_2SO_4 and passed through Cu powder to remove elemental sulphur. The separated organic layer was diluted with water and acidified with H_2SO_4 (4 N). Fatty acids were extracted with diethyl ether. Brine was used to break the emulsion. The ethereal extracts of fatty acids were pooled and dried over anhydrous Na_2SO_4 and evaporated in a rotary film evaporator at room temperature. Fatty acids thus obtained were transferred quantitatively using minimum amount of chloroform into conical flasks. The chloroform was evaporated with nitrogen gas and the traces were removed at 40°C under reduced pressure.

Fatty acids were esterified by diazomethane generated from nitrosomethyl urea and were purified by preparative TLC using silica gel type 60 (E. Merck) and solvent system light petroleum ether (40°-60°C)-diethyl ether - acetic acid (90:10:1 v/v). The methyl ester band corresponding to standard methyl oleate (Sigma) was scraped and eluted with diethyl ether. Ether was evaporated with nitrogen and the purity of the methyl esters was further checked by IR spectrum (Shimadzu, model IR-408) in KBr.

The nonsaponifiable fraction of the organic matter was extracted with diethyl ether and the sterols were separated by preparative TLC using light petroleum ether (40°-60°C) - diethyl ether (1:1 v/v) as developing solvents. The sterol band was identified by comparing its Rf value with that of cholesterol. The bands were visualised by iodine vapour and scraped off the plate, leaving a little portion which gave positive Liebermann-Burchard test. The sterol was extracted with chloroform and analysed as steryl acetates for individual sterols using GLC.

The gas chromatographic instrument (Pye Unicam, model 104) was equipped with dual glass column and dual flame ionisation detector (FID). The column used for fatty acid was 10% DEGS (diethylene glycol succinate, polyester liquid phase) coated on chromosorb - W (HP) stationary phase (liquid and stationary phases were obtained from Pierce Chem. Co., Rokford, USA). The column material was packed into a coiled glass column (1.8 m x 3 mm internal diam.) and was activated under a slow stream of nitrogen gas (25 ml min^{-1}) at 195°C for 16 h. Gas chromatography of the fatty acid methyl esters was done with a column temperature of 180°C. The injection and detector temperatures were 230°C and 240°C respectively. Authentic standard methyl ester samples (Sigma) were also used under identical operational conditions. For sterols, the column used was 3% SE-30 glass column (1.2 m x 3 mm internal diam.) The column temperature was isothermal at 285°C. Detector and injection temperatures were 350°C and 335°C respectively. Nitrogen (60 ml min^{-1}) was the carrier gas in all cases. Identification of fatty acids and sterols was done by comparing retention times of the peaks of the samples with those of standards. The results presented were average of duplicate analyses.

Results and Discussion

Fatty acids—Total fatty acid content in the sediments varied between 100 and 720 ppm of dry weight and was 7.7 to 25% of total organic carbon. The chain lengths of normal even numbered saturated fatty acids were in the range 12-20 carbon atoms. Among the saturated fatty acids, palmitic acid (C_{16:0}) was the major component (11-33% w/w) in almost all sediments (Fig. 2) followed by stearic acid (C_{18:0}). Of the unsaturated fatty acids, myristoleic (C_{14:1}), palmitoleic (C_{16:1}, oleic (C_{18:1}), linoleic (C_{18:2}) and linolenic (C_{18:3}) acids were identified by comparing gas chromatographic retention time with authentic standards. A significant amount (20-39% w/w) of myristoleic acid (C_{14:1}) was found in sediments at st 2 and oleic acid (C_{18:1}) was the major unsaturated fatty acid. In general, most of the unsaturated fatty acids showed an increase in concentration in monsoon whereas the saturated fatty acids of carbon number C_{16} and C_{18} decreased. The ratios of unsaturated to saturated fatty acids in the sediments of all stations increased in monsoon season (Table 1), but to a larger extent in the case of C_{18:1}/C_{18:0} (n = 1 to 3), than the sediments in other seasons.

Distribution of organic components in the sediments is influenced mainly by relative contribution of autochthonous and allochthonous organic matter.
Rivers play the main role in the transport of allochthonous organic matter from the inland sources. Thus in the estuarine sediments the carbon number distribution of fatty acids is also correlated with trophic status, showing bimodal distribution with the predominance of low molecular weight fatty acids (< C_{18}) from autochthonous origin and larger fatty acids (> C_{22}) from terrigenous sources.

In the sedimentary environment decomposition under aerobic condition results in slow hydrolysis of wax esters, derived from terrestrial biota, producing acids in the range C_{22}-C_{34} and a more rapid hydrolysis of glycerides and esters of lower fatty acids with complete breakdown of the C_{16} and C_{18} saturated and unsaturated acids present therein, either before deposition or in the microbiologically active zone at the sediment-water interface. Thus free fatty acids in these sediments appear to be derived from complete breakdown of glycerides and from esters of lower fatty acids. The probable absence or very low concentration of higher molecular weight fatty acids (> C_{22}) in these sediments (Fig. 2) indicates that the possible principal sources of fatty acids are of autochthonous origin and the dominance of C_{16} and C_{18} free n-alkanoic acids in the present study (Fig. 3) can be associated with the rate of breakdown of lipids of fatty acids, rate of sedimentation and consequent rapid burial in sediments.

The fatty acid content in sediments shows some degree of seasonal variation but qualitatively they do not differ at all. Comparatively lower content of fatty acids of carbon number C_{16} and C_{18} (Fig. 3), which are the major component in plankton, in the sediments of monsoon samples can be due to less organic production in the water at the time of high freshwater discharge with intense water circulation that makes the water turbid.

The ratio of unsaturated to saturated fatty acids has been used as an important index of the rate of transformation of unsaturated fatty acids in the sediments during diagenesis. Oleic acid (C_{18:1}) can be converted to saturated fatty acid in only a few days, both by reduction of carbon skeleton and by degradation and resynthesis. Seasonal variation of ratios of unsaturated to saturated fatty acids in the sediments shows that the ratios of C_{14:1}/C_{14:0} and C_{16:1}/C_{16:0} do not vary profoundly. But a significant variation of the ratios of the unsaturated fatty acids of C_{18:n} (n = 1 to 3) to its saturated fatty acid (C_{18:0}) has been noted in monsoon, where the ratios increase in higher degree than other seasons. This can be attributed in terms of textural characteristics of the sediments, finer particles in sediments may play a role in protecting the double bonds of the unsaturated acids from bacterial attack. Wang et al. have suggested that the bonding in the form of pi electron complex between unsaturated fatty acids and the terrigenous carbon matter is a key to the preservation of unsaturated acids in the sediments.

<table>
<thead>
<tr>
<th>Fatty acids ratio</th>
<th>Premonsoon</th>
<th>Monsoon</th>
<th>Postmonsoon</th>
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<tr>
<td></td>
<td>St 1</td>
<td>St 2</td>
<td>St 3</td>
</tr>
<tr>
<td>C_{14:1}/C_{14:0}</td>
<td>0.27</td>
<td>2.10</td>
<td>2.00</td>
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<td>C_{16:1}/C_{16:0}</td>
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<td>0.39</td>
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<td>C_{18:1}/C_{18:0}</td>
<td>0.73</td>
<td>1.55</td>
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<td>C_{18:2}/C_{18:0}</td>
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<td>1.16</td>
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<tr>
<td>C_{18:3}/C_{18:0}</td>
<td>0.72</td>
<td>0.44</td>
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acids and the clay particles of soil is stronger and less labile to be broken. In the monsoon season, the fraction of finer particles\textsuperscript{19} in the sediments increases and may prevent the selective decomposition of the unsaturated fatty acids. This consequently, may relatively, favour the preservation of those acids in the sediments. Alternatively, in more stable conditions during premonsoon and postmonsoon seasons, the concept of possible conversion of unsaturated fatty acids to their corresponding hydrocarbons or any other related compounds, undergoing selective reaction processes at the sediment-water interface cannot also be ruled out.

Sterols—Concentrations of sterols varied from 5 to 95 ppm of dry weight of the sediments. The percentage of sterol to total organic carbon ranged between 0.12 and 6.64. The sediments of all stations exhibited higher percentage of sterol in postmonsoon season (Fig. 5) and it seems that the prevailing environmental conditions during this season at the sedimentary zone are, perhaps, quite favourable for the preservation of sterols in comparison to other seasons.

In the present investigation, sediments showed presence of altogether 4 different sterols (Fig. 4). Cholesterol and \( \beta \)-sitosterol were high in proportion while campesterol and stigmasterol were low. An interesting observation is that the concentration of cholesterol and \( \beta \)-sitosterol in the sediments varied inversely to each other in different seasons. The maximum values of cholesterol were always accompanied with minimum values of \( \beta \)-sitosterol as observed, especially in postmonsoon and premonsoon seasons (Fig. 5). Conversely, higher values of \( \beta \)-sitosterol along with lower values of cholesterol were encountered during monsoon in all sediments.

It has been suggested that the organic matter of marine sources and terrigenous sources can be differentiated by the content of the sterol components\textsuperscript{4}. Cholesterol is the abundant sterol in most marine organisms\textsuperscript{20} whereas \( \beta \)-sitosterol is the major sterol in terrigenous plants\textsuperscript{21}. Hence it can be presumed that higher values of cholesterol encountered during premonsoon and postmonsoon season, may be due to the contribution from autochthonous sources. Because these seasons are comparatively stable with minimum rate of riverflow and also favourable to good amount of organic production\textsuperscript{14} in the overlying water.

On the other hand, during monsoon months, the river runoff containing large amount of terrigenous organic matter from various sources, dominates the estuarine system and consequently also reflects the allochthonous impact on the sedimentary environments. This may be the reason for the higher values of \( \beta \)-sitosterol in the sediments. Moreover, it is also observed that \( \beta \)-sitosterol content decreases from freshwater (st 3) to marine (st 1) sediments which indicates the decrease of terrigenous influence in different locations in the same way.

Fig. 4—Gas chromatogram of sterols as acetates in sediments at st 1 during premonsoon season

Fig. 5—Distribution of sterols in sediments (1, cholesterol; 2, campesterol; 3, stigmasterol; and 4, \( \beta \)-sitosterol)
Acknowledgement

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