sets of modes are of comparable intensity in the tunneling spectra. Furthermore, in the IR and Raman spectra the intensities of the bands characterized by different k-values decrease as k increases, while in the tunneling spectra all the bands due to a particular set of modes are of comparable intensity. This observation is explained by a theory of the tunneling spectra (*8*), which attributes the tunneling intensities to the interaction of the molecules with electrons which have short wavelengths, making the intensities almost independent of k.

The close correspondence between the frequencies of the strong features of the tunneling spectra and the frequencies of the IR and Raman spectra of the solid hexanoates shows that in much of the monolayer, the carbon chains of the hexanoate are in the extended all-trans conformation. Furthermore, some of the chain modes appear split in the tunneling spectra, for example, 945-963-990, 1218-1230, and 1325-1340 cm^{-1}, corresponding to doublets that appear in the IR and Raman spectra. These doublets are due to intermolecular interactions in the solid salts, and their appearance in the tunneling spectra suggests that the all-trans molecules occur in ordered regions with intermolecular interactions like those of the crystal. However, there are some bands in the tunneling spectra which cannot be assigned to the vibrations of the all-trans molecules. The bands at 300, 735, 775, 835, and 1174 cm^{-1}, for example, are not found in the IR or the Raman spectrum of potassium hexanoate and often differ among tunneling spectra for identically prepared samples, as indicated by the arrows in Fig. 1. These frequencies are not near those of the end groups and so are probably not due to the perturbation of the vibrational modes by the electrodes. They are frequencies at which molecules with gauche conformations should have modes (*9*), and therefore we conclude that the monolayers observed contain some molecules with gauche conformations and to this extent at least are disordered. The presence of molecules with different conformations may also account for some of the intensity differences between bands in the tunneling spectra and bands in the IR and Raman spectra of the solid salts.

Finally, one other feature of the tunneling spectra is that the low-frequency chain deformations appear at a lower frequency than those in potassium hexanoate. This shift is expected if the ends of the molecules in the tunneling junction are more constrained than they are in the crystal.

In summary, we have measured and assigned, to the best of our knowledge for the first time, the vibrational spectrum of monolayer chemisorbed fatty acids. The appearance of series of peaks associated with both gauche and trans conformations supports the conclusion that these films are disordered, although they may contain ordered regions of all-trans molecules interacting as in the solid. The different regions may arise as a result of the geometry of the bonding sites on the alumina support. In agreement with recent theoretical work (*8*), the various vibrational modes, such as the twists and wags, are all present with comparable intensity in the tunneling spectrum, regardless of their activity in optical vibrational spectra.

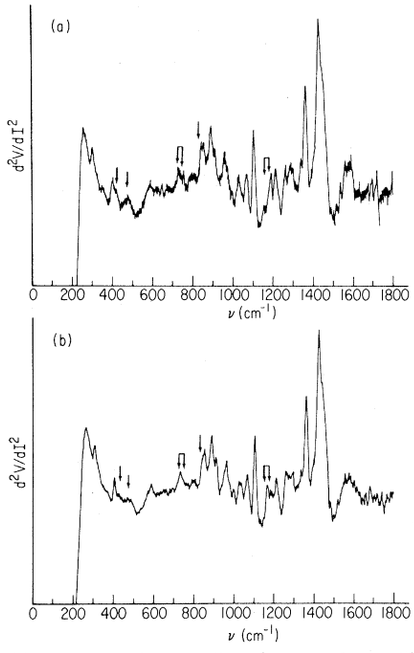

Fig. 1. Inelastic tunneling spectra of two different preparations of hexanoic acid. The spectra show d^2V/dI^2 plotted against voltage, with the voltage scale converted to energy units in wave numbers. Most of the features of the two spectra are identical; the arrows indicate regions where there are differences.

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Methylation of Selenium in the Aquatic Environment

Abstract. *Conversion of inorganic and organic selenium compounds to volatile selenium compounds (dimethyl selenide, dimethyl diselenide, and an unknown compound) by microorganisms in lake sediment has been observed. This conversion could also be effected by pure cultures of bacteria and fungi. Such transformations are significant in the transportation and cycling of elements in the environment.*

Selenium and its compounds have long been recognized as inorganic carcinogens of concern in the spectrum of identified environmental pollutants, based on observations of proved toxic effects and relative accessibility (*1, 2*). Movement of toxic elements through the geocycle and their biological methylation in the environment to volatile products of extreme toxicity further complicates the problem (*3, 4*). It is known that methylation of certain heavy metals, such as Hg (*4*), As (*5, 6*), Sn (*7*), and Pb (*8, 9*), can yield compounds that are more toxic to higher organisms. Apart from the observation that $(CH_3)_2Se$ was produced when sodium selenite was mixed with municipal sewage (*5*), sufficient information on the methylation of selenium in the aquatic environment is not available.

It is known that volatile selenium compounds are produced through methylation by various organisms. For instance, fungi of several genera produce $(CH_3)_2Se$ from inorganic selenium compounds (*10–12*). As well, rats fed with selenite and selenate exhaled a volatile selenium compound identified as $(CH_3)_2Se$ (*13, 14*). Other studies, conducted with higher plants, identify $(CH_3)_2Se$ as the single

product of a nonaccumulator species, cabbage (*15*), and $(CH_3)_2Se_2$ as the product of an accumulator species, *Astragalus* (*16*).

Very little, however, is known about methylation of selenium by bacteria. One coryneform bacterium was reported to convert inorganic selenium to $(CH_3)_2Se$ in vitro (*17*). In another study, a large number of selenium-reducing bacterial isolates were investigated for their capacity to produce $(CH_3)_2Se$. No isolate, however, was found to possess this characteristic (*12*).

We performed exploratory experiments, using 250-ml filter flasks containing 50 g of garden soil or lake sediment suspended in 150 ml of distilled water or lake water, respectively. Nutrient broth (0.5 percent) and glucose (0.1 percent) were added to stimulate microbial growth. After inorganic and organic selenium compounds (5 mg/liter) were added, the flasks were sealed and incubated at 20°C for 1 week. The headspace gases of the culture flasks were analyzed for volatile selenium compounds by a gas chromatography–atomic absorption spectroscopy method (*18*) with a detection limit of ~ 0.1 ng of selenium. The volatile selenium compounds were methylated derivatives, as confirmed by mass spectrometry. We observed the production of $(CH_3)_2Se$ and $(CH_3)_2Se_2$ from both soil and sediment samples enriched with the following selenium compounds: sodium selenite, sodium selenate, selenocystine, selenourea, and seleno-DL-methionine. In many cases, an unknown volatile selenium compound was also produced. Dimethyl selenide, reported as a volatile product, is evolved from soils enriched with sodium selenite (*17*). No volatile selenium was detected in either soil or sediment samples except when selenium compounds were added. The occurrence of selenium methylation in the sediment prompted us to use other sediments, which were collected from 12 lakes in the Sudbury area of Ontario, where metals are known to be abundant in the sediment. The sediment samples, suspended in their respective lake waters, with and without the addition of selenium compounds, were incubated in the manner described above. The results (Table 1) showed that almost all of these sediment samples, with the addition of selenite and selenate, produced volatile selenium. The only exception was the Windy Lake sample, where virtually no microbial growth was visually observed. In certain lakes, volatile selenium was produced even without the addition of selenium compounds. However, no relationship was observed between the selenium concentrations in the sediment and the amounts of volatile selenium produced. The chemical forms of the selenium in these sediment samples have not yet been determined.

Table 1. Methylation of selenium compounds in sediment samples from 12 lakes in the Sudbury area. In experiments where selenium compounds were added, the concentration of added selenium was 5 mg/liter; volatile selenium was measured in nanograms. Abbreviation: U, unknown compound.

Lake	Se in sediment (μg/g, dry wt.)	No addition			Sodium selenite			Sodium selenate		
		$(CH_3)_2$-Se (ng)	$(CH_3)_2$-Se_2 (ng)	U (ng)	$(CH_3)_2$-Se (ng)	$(CH_3)_2$-Se_2 (ng)	U (ng)	$(CH_3)_2$-Se (ng)	$(CH_3)_2$-Se_2 (ng)	U (ng)
Elbow	0.48	34	0	3.3	33.3	0	0	34	0	3.3
Ramsey	1.64	0	0	0	0	0	0	33.6	0	0
Kelley	20.48	2.7	3.3	2.3	14	0	0	20	3.3	5.3
Long	0.90	1.7	0	0	27.3	0	0	24.7	0	0
Simons	16.28	0	0	0	18.7	0	18	20	4.7	19.7
Vermillion	0.52	0	0	0	20.3	0	0	9.7	8	5.3
Windy	0.65	0	0	0	12.3	0	0	0	0	0
Moose	0.67	0	0	0	5	0	0	10.5	0	2
Kukagami	0.44	0	0	0	33.3	0	0	23.4	0	0
Nepewassi	0.53	0	0	0	25.3	0	0	28.7	0	0
Johnnie	0.55	6.3	0	3.7	43.3	16.5	0	94.6	19.4	54.8
George	0.67	0	0	0	56.8	0	8.7	94.7	11	7.3

In all the experiments, the production of volatile selenium compounds was observed to be associated with microbiological growth. While we have not attempted to identify the organism or organisms responsible for the methylation, three bacteria (*Aeromonas* sp., *Flavobacterium* sp., and one tentatively identified as *Pseudomonas* sp.) have been isolated, and an unidentified fungus was found in the lake sediment. These microorganisms, when grown in nutrient broth (Difco), were able to methylate sodium selenite to $(CH_3)_2Se$, $(CH_3)_2Se_2$, and the unknown volatile selenium. Methylation only occurred when there was growth of the microorganisms.

The production of volatile selenium is temperature-dependent. About 75 percent as much $(CH_3)_2Se$ was produced with incubation at 10°C as at 20°C. At 4°C the production was lowered to about 10 percent. For the conversion of selenate, the temperature effect is even more drastic. The production at 10°C was about 15 percent of that at 20°C; at 4°C no production was observed. The conversion is observed under both aerobic and anaerobic conditions.

Fine-structural examinations of bacteria, after growth in the presence of sodium selenite, revealed electron-opaque deposits of irregular shape, composed of smaller units, within the cytoplasm but not on the cell wall and cell membrane (*19*). The deposits were interpreted to contain selenium on the basis of energy dispersive x-ray microanalysis. Adaptation of microorganisms to a toxic chemical is not a novel occurrence. Evidently, bacteria have the ability to accommodate against toxic selenium by methylating selenium salts in the cytoplasm to volatile selenium compounds. The impact on the environment of such microbial methylation of selenium requires further investigation.

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