form. It would be interesting to study the NMR spectrum as a function of temperature.

A referee has called our attention to the structures of two cyclic hexapeptides, *cyclo*-(D-Phe-Pro-Val)$_2$[18] and *cyclo*-(Gly-D-Leu-L-Leu)$_2$,[19] neither of which contains a transannular hydrogen bond. He also has pointed out a recent compilation[20] of bond lengths and angles in peptide units.

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Supplementary Material Available: Temperature factors (2 pages). Ordering information is given on any current masthead page.

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Selenium-77 Relaxation Time Studies on Compounds of Biological Importance: Dialkyl Selenides, Dialkyl Diselenides, Selenols, Selenonium Compounds, and Seleno Oxyacids

J. D. Odom,* W. H. Dawson, and P. D. Ellis*†

Contribution from the Department of Chemistry, University of South Carolina, Columbia, South Carolina 29208. *Received December 26, 1978*

Abstract: Spin–lattice relaxation times have been determined for several classes of selenium compounds that are biologically important. The classes of compounds include dialkyl selenides, dialkyl and diaryl diselenides, selenols, selenonium compounds, and seleno oxyacids and their salts. The relaxation times were measured under a variety of conditions, including aqueous and nonaqueous solutions, variable temperature, variable concentration, and variable pH. For the molecules studied, the spin-rotation and chemical-shift anisotropy mechanisms were found to be the most important means of spin–lattice relaxation for the ^{77}Se nucleus. The dipole–dipole mechanism was totally absent in all compounds studied. For selenium-containing biopolymers, the spin-rotation mechanism is not likely to contribute to spin–lattice relaxation. However, it has been shown that chemical-shift anisotropy and dipole–dipole mechanisms will most likely be effective mechanisms in larger molecules and that ^{77}Se Fourier transform (FT) NMR of these large molecules will not be encumbered by exceptionally long recycle times.

Introduction

While Fourier transform nuclear magnetic resonance (FT NMR) spectroscopy has been used to great advantage to study the chemistry of many elements of the periodic table, those of group 6A have received little attention. For oxygen and sulfur, the two lightest and most chemically prolific members of this group, the only NMR active isotopes (^{17}O and ^{33}S) are quadrupolar nuclei that suffer from very low natural abundance and relatively low sensitivity. Selenium and tellurium, on the other hand, both have spin-$\frac{1}{2}$ isotopes (^{77}Se, ^{123}Te, ^{125}Te) with sufficient sensitivity to make their study readily accessible by Fourier transform (FT) NMR. For the purposes of this investigation, our interest in selenium stems from the active role it plays in many biological systems, not to mention its increasing involvement in organic synthesis and an extensive inorganic chemistry.[1] Selenium-77 NMR has great potential as a means of exploring the chemistry of this interesting element and, as part of our continuing interest in the applications of multinuclear NMR to biological systems,[2] we have initiated a program aimed in this direction.

Early continuous wave (CW) NMR studied by Birchall et al.[3] followed by the INDOR studies of McFarlane and Wood[4] demonstrated the large chemical-shift range of ^{77}Se and the stereospecificity of its coupling constants. To date there have been only a few reports concerned with the direct observation of ^{77}Se by FT NMR.[2c,5-9] The nuclear spin–lattice relaxation time, T_1, is a critical parameter in determining the recycle time of FT NMR experiments. More importantly, it can often be used as a powerful, diagnostic tool for the determination of molecular structure, conformation, and composition.[10] It can also be employed as a probe to investigate molecular motions and interactions. Dawson and Odom,[2c] Pan and Fackler,[6] Gansow et al.,[7] and Koch et al.[8] have briefly examined spin–lattice relaxation times for ^{77}Se in a variety of chemical envi-

† Alfred P. Sloan Foundation Fellow, 1977–1979.

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ronments. The results of these studies indicated the importance of spin-rotation (SR) and, to a lesser extent, chemical-shift anisotropy (CSA) mechanisms for spin–lattice relaxation.

Relaxation considerations assume an added significance when dealing with large biological macromolecules that contain selenium. Those seleno systems that are involved in biochemical reactions will eventually be the focus of greatest concern in our NMR studies. However, their high molecular weights impart a motional sluggishness, which, from the viewpoint of NMR spectroscopy, requires operating near the limits of the so-called "region of extreme narrowing" ($\omega\tau \ll 1$) and complicates the task of extracting useful physicochemical information.[11] It is advantageous to be forearmed with a knowledge of the mechanisms governing T_1, and we have, therefore, commenced our biological studies using ^{77}Se NMR with an examination of the spin–lattice relaxation of this nucleus as it appears in a number of functional forms and in both aqueous and nonaqueous solvents.

Experimental Section

Spin–lattice relaxation times were determined on a modified Varian XL-100-15 NMR spectrometer. Field-frequency stabilization was achieved by locking to the deuterium resonance of the solvent (either $CDCl_3$ or D_2O). Selenium-77 resonances were initially detected at 19.1 MHz using the Gyrocode Observe accessory with the deuterium lock frequency at 15.400 960 MHz. Under these conditions, however, it was not possible to simultaneously proton decouple the sample at 100 MHz using either coherent or modulated (noise or squarewave) irradiation without introducing a prohibitive amount of noise into the receiver. This feature is apparently common to all XL-100 spectrometers.[6,12] A solution to this difficulty was achieved by lowering the field slightly, such that ^{77}Se resonates at 18.90 MHz with proton decoupling at 99.41 MHz. The deuterium lock frequency at this new field strength is exactly 15.30 MHz. During the early stages of this work this latter frequency was obtained by replacing the 15.400 960-MHz master crystal by 15.3 MHz from a frequency synthesizer. Since the Gyrocode Observe reference frequencies are ultimately derived from this master frequency, the outputs of this unit using the new frequency were automatically scaled to the required 99.41-MHz decoupling and 18.90 observe frequencies when the "normal" 15.4-MHz Gyrocode settings were used. In the latter stages of this study, the XL-100 was converted to a synthesizer-based instrument, wherein the observe frequencies were derived from a General Radio 1061 frequency synthesizer. The 15.3-MHz master frequency was generated by a home-built frequency synthesizer that was phase locked to the GR 1061 synthesizer. The standard components of the Varian 4412 probe were retunable from 15.4 to 15.3 MHz and 19.1 to 18.9 MHz without any perceptible performance losses.

The inversion–recovery pulse sequence, $(180°-\tau-90°-T)_n$, was used to obtain the relaxation times (T_1) when the magnitude of this parameter was on the order of 5 s or less.[13] The 90 and 180° pulse widths were determined in the usual manner with a 90° pulse being on the order of 65 μs. The waiting time between pulses (T) was always set to be greater than $5T_1$s. When T_1s longer than 5 s were encountered, the homospoil saturation–recovery sequence, $(PD-HS-90°-HS-\tau-AT)_n$, was employed, where PD, HS, and AT are the pulse delay (nominally 0.1 s), the homospoil (Z-gradient) pulse, and the acquisition time, respectively.[14] In the case of either pulse sequence, at least one spectrum was obtained with the value of τ exceeding five times T_1.[15] The value of T_1 was extracted from the experimental data using a nonlinear least-squares routine in the usual manner. The uncertainty in the T_1 values determined in this way is estimated to be within 10%, although repetition of the measurements for selected samples used in this study indicated that the values are reproducible to well within these limits.

Nuclear Overhauser effect enhancement factors (η) were determined as the difference in integrated intensity between ^1H-decoupled and ^1H-coupled spectra and are reported as NOE = $1 + \eta$.[16] The measurements were taken from two spectra, one in which the ^1H-decoupling frequency was set exactly on resonance and the second with the decoupling frequency offset at least 10 kHz from this resonance frequency, with the modulation removed. The recycle time between pulses in these experiments was at least seven times the value of T_1.[17]

All spectra were obtained using 250- or 500-Hz spectral widths and transformed using 8K data points (zero-filling as required). Chemical shifts are reported relative to the resonance of dimethyl selenide in $CDCl_3$ (~1.0 M), in the sense that a positive chemical shift denotes a resonance to lower shielding.[18]

Temperature control was achieved using standard Varian accessories and temperatures were measured with a copper-constantan thermocouple unit, the "hot" end of which was inserted into an open sample tube containing the appropriate solvent and held at a depth coincident with the receiver coils. The temperatures reported in this work are estimated to be accurate to ±1 °C.

All samples were degassed by several freeze–pump–thaw cycles and sealed under dynamic vacuum in 12-mm NMR tubes. The solvents ($CDCl_3$ and D_2O) were treated with dithizone prior to being distilled in an all-glass apparatus. Paramagnetic materials were removed from NMR tubes by allowing the tubes to stand in a nitric acid bath for several days and rinsing them several times with deionized water.

The following materials were obtained from commercial sources: selenium, selenium dioxide, and sodium selenate (Alfa-Ventron); D,L-selenomethionine and D,L-selenocystine (Sigma Chemical Co.). Anhydrous hydrogen selenide was a gift from A. J. Zozulin of this department, and *N,N*-dimethylselenourea was a gift from Dr. R. A. Zingaro (Texas A&M University). Dimethyl selenide (bp 54–57 °C; lit.[19] 57 °C) was prepared from the reaction of CH_3I and Na_2Se in aqueous base.[20] Di-*n*-butyl selenide (bp 196–197 °C; lit.[19] 82–83 °C (13 Torr)), di-*n*-octyl selenide[21] (bp 157–158 °C (0.1 Torr)) and diisopropyl selenide (bp 136–137 °C; lit.[22] 135 °C) were obtained from the reaction of the corresponding alkyl bromides with Na_2Se in liquid NH_3.[22] Methaneselenol (bp 23–25 °C; lit.[23] 25.5 °C) was prepared from the reduction of dimethyl diselenide with hypophosphorous acid.[24] Ethaneselenol (bp 50–53 °C; lit.[22] 52 °C) and decaneselenol (bp 125–130 °C (18 Torr); lit.[25] 128–129 °C (13 Torr)) were synthesized by the reduction of the corresponding dialkyl diselenides with elemental sodium in liquid NH_3.[22] Dimethyl diselenide was obtained as an orange oil (bp 156–158 °C; lit.[26] 155–157 °C) from the reaction of CH_3I and Na_2Se_2 in aqueous base.[20] Dibenzyl diselenide (mp 92–93 °C; lit.[27] 92–93 °C) was prepared from benzyl chloride and Na_2Se in ethanol.[28] Diphenyl diselenide (mp 63–64 °C; lit.[19] 61–63 °C) was synthesized by the air oxidation of a solution of benzeneselenol.[29] Didecyl diselenide (mp 12–16 °C; lit.[30] 13–14 °C) was obtained as an orange oil from the reaction of decyl bromide with Na_2Se_2 in liquid NH_3.[22] Trimethylselenonium iodide (mp 150–152 °C; lit.[31] 150–152 °C) and dimethylselentine bromide (mp 89–92 °C; lit.[20] 90 °C) were prepared by mixing cold diethyl ether solutions of dimethyl selenide (20% molar excess) and the corresponding alkyl halide and subsequently removing the volatile materials. Dimethyl selenoxide (mp 91–93 °C; lit.[32] 94 °C) was prepared from both the ozonolysis of dimethyl selenide in $CHCl_3$[19] and the reaction of silver oxide with dimethylselenium dibromide in methanol.[20] Selenocysteamine hydrochloride (mp 108–110 °C; lit.[33] 108–110 °C) was synthesized by the method of Klayman[33] and methylselenocysteamine hydrochloride (mp 149–151 °C; lit.[34] 149–151 °C) was prepared according to the method of Tanaka et al.[34] In addition to the determination of boiling points or melting points, the purity of all compounds was carefully checked by ^1H and ^{77}Se NMR.

Results and Discussion

Dialkyl Selenides. The data for this group of compounds are collected in Table I. In each of these molecules, scalar spin–spin coupling of the ^{77}Se nucleus to nearby alkyl protons is observed. Proton decoupling reduces the resonances to single sharp lines, *but in no case was a ^1H–^{77}Se NOE observed.* Thus, although the presence of these nearby spin-½ nuclei must certainly give rise to a ^1H–^{77}Se dipole–dipole relaxation, it is apparently very inefficient and its contribution to the observed T_1 is minor relative to the other mechanisms. An earlier study[7] reported an NOE for $(CH_3)_2Se$ of 1.04. One can eliminate in principle a contribution from scalar coupling relaxation, since there is no means by which it could be operative in these systems.

In order to distinguish which of the remaining two mechanisms is operative (chemical-shift anisotropy (CSA) or spin rotation (SR)), the T_1 was determined as a function of temperature and the data plotted as a semilog plot of $R_1 \equiv T_1^{-1}$ vs. the reciprocal temperature (Figure 1). The temperature

Table I. Selenium-77 Chemical Shifts and Spin-Lattice Relaxation Times

compd	chem shift,[a] ppm	T_1, s	temp, °C	conditions[c]
$(CH_3)_2Se$	0[b]	5.2	55	$CDCl_3$
		7.5	32	
		8.6	12	
		24.4	−60	
	3	4.3	40	acetone
		9.8	−30	
$(n\text{-}C_4H_9)_2Se$	167	19.1	40	$CDCl_3$
		23.1	0	
		16.5	−40	
$(n\text{-}C_8H_{17})_2Se$	168	10.4	41	$CDCl_3$
		14.8	0	
		6.0	−42	
$(i\text{-}C_3H_7)_2Se$	432	8.7	41	$CDCl_3$
		13.6	0	
		20.4	−42	
D,L-$CH_3SeCH_2CH_2CH(NH_2)COOH$	75	13.5	34	0.1 M, D_2O, pD 4
$CH_3SeCH_2CH_2NH_2$	44	8.4	43	0.5 M, $CDCl_3$
		15.9	0	
	50	24	43	0.5 M, D_2O, pD 4
		28	10	
$(CH_3Se)_2$	281	9	45	0.5 M, $CDCl_3$
		13	0	
$(C_6H_5CH_2Se)_2$	412	27	55	0.5 M, $CDCl_3$
		31	18	
$(C_6H_5Se)_2$	481	20	45	0.5 M, $CDCl_3$
		31	0	
$(n\text{-}C_{10}H_{21}Se)_2$	316	21	43	0.5 M, $CDCl_3$
		14	0	
CH_3SeH	−130	1.3	40	acetone-d_6
		3.3	−30	
		3.7	−45	
C_2H_5SeH	39	1.7	40	$CDCl_3$
		4.8	−30	
		9.5	−60	
	41	1.5	40	acetone-d_6
		3.7	−30	
		7.7	−83	
$n\text{-}C_{10}H_{21}SeH$	−7	1.9	42	$CDCl_3$
		3.4	0	
$HSeCH_2CH_2NH_2$	−212	7.1	32	0.5 M, D_2O, pD 8.3
D,L-$HSeCH_2CH(NH_2)COOH$	−141			0.1 M, D_2O, pD 5
H_2Se	−288	0.7	34	1.0 M, D_2O[d]
		1.0	10	
$(NH_4)_2Se$	−511			0.5 M, D_2O[d]
$NaSeCH_3$	−330	16.3	43	1.0 M, D_2O[d]
$(CH_3)_3SeI$	258	13.7	32	1.0 M, D_2O, pD 7
$(CH_3)_2Se(Br)CH_2CO_2C_2H_5$	140	1.8	32	0.5 M, $CDCl_3$
		1.7	−1	
		0.7	−30	
	298	25	65	0.5 M, D_2O, pD 7
		30	35	
		21	8	
H_2SeO_3	1292	3.1	63	1.0 M, D_2O, pH 9.6
		2.1	12	
	1307	2.8	63	1.0 M, D_2O, pH 1.5
		8.5	10	
	1281	3.6	67	0.5 M, D_2O, pH 9.8
		2.8	10	
	1307	10.0	63	0.5 M, D_2O, pH 1.5
		12.5	10	
Na_2SeO_4	1051	6.5	61	0.5 M, D_2O, pH 6.6
		16.8	15	
$(CH_3)_2SeO$	819	4.4	65	0.5 M, D_2O, pH 7
		8.9	30	
$(CH_3)_2NC(Se)NH_2$	147	8.6	55	0.5 M, D_2O, pD 4
		10.0	32	

[a] Relative to dimethyl selenide in $CDCl_3$. The chemical shifts are all temperature dependent and those given are for temperatures between 32 and 42 °C. [b] The exact Larmor frequency for $(CH_3)_2Se$ in $CDCl_3$ is 18.957 787 MHz with the ^2H lock frequency (internal $CDCl_3$) at 15.300 000 MHz. [c] Unless otherwise specified, the samples were 20% (v/v) in the specified solvent. [d] See text.

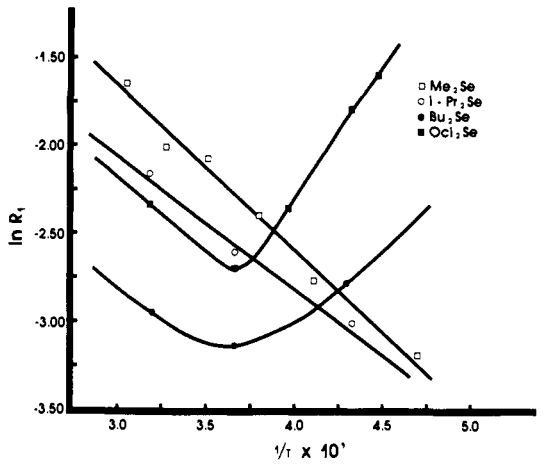

Figure 1. Arrhenius plots of the temperature dependence of the observed relaxation rates ($R \equiv 1/T_1$) of dialkyl selenides.

dependencies of the relaxation processes dictate that in such a plot the SR mechanism will give a negative slope, while the CSA mechanism will yield a positive slope, and under the assumption (valid for these small molecules at the Larmor frequency employed) that the extreme narrowing condition applies.[35] As may be seen from Figure 1, the negative slope and strict linearity of the plot for dimethyl selenide point to the fact that the spin-lattice relaxation of ^{77}Se in this compound is dominated by T_1 (SR) in the temperature range studied. This result is in accordance with theoretical and practical expectations, since the SR mechanism requires a coupling of the nucleus to the valence electrons in a manner similar to that which gives rise to the so-called "paramagnetic shielding" term of the chemical shift.[36] As a consequence, heavy, magnetically active nuclei that possess a large chemical-shift range will, in small molecules, usually, display significant SR relaxation. This fact has been demonstrated experimentally for a number of heavy nuclei, e.g., ^{113}Cd,[2a] ^{199}Hg,[2b] and ^{205}Tl.[37] The dioctyl selenide molecule should, by virtue of its larger size, tumble less rapidly in solution than dimethyl selenide and, hence, SR should be less important in this case. In fact, as is seen in Figure 1, only at the higher temperatures does SR become important, and at low temperatures the steep positive slope and lack of an NOE imply a CSA mechanism. The increased importance of the SR mechanism at higher temperatures is undoubtedly due to the increased overall motion of the molecule coupled with increased segmental motion of the selenium atom within the chain. The quantitative separation of these motions is beyond the scope of this report.

The slower tumbling experienced by selenium in dioctyl selenide at −50 °C contributes, in this case, to a diminution of the SR mechanism and an enhancement of CSA relaxation. This aspect is of considerable interest to the study of macromolecules containing an RSeR′ moiety because of the implication that the CSA mechanism may be important in these systems. If one assumes that the motion of the ^{77}Se nucleus can be described by a single correlation time, τ_c, then the relaxation rate for CSA processes can be expressed[11a] as:

$$T_1^{-1}(\mathrm{CSA}) = \frac{2}{15}\gamma_{Se}^2 H_0^2 (\Delta\sigma)^2 \frac{\tau_c}{1+\omega_{Se}^2\tau_c^2} \quad (1)$$

Here, γ_{Se} is the magnetogyric ratio for selenium, H_0 is the applied magnetic field strength, $\Delta\sigma$ is the chemical-shift anisotropy, and ω_{Se} is the resonance frequency for selenium in radians per second. If one assumes a value of 10^{-10} s/rad for a correlation time[38] for the ^{77}Se nucleus in dioctyl selenide at −50 °C, then from Figure 1 and eq 1, one can estimate a value of $\Delta\sigma$ for this system to be 971 ppm. This value is in accord with the value of $\Delta\sigma$ = 1260 ppm determined for a single crystal of elemental selenium.[39] Hence, for a protein with a correlation time in the range of 10 to 100 ns, one would expect T_1s in the range of 0.14 to 0.80 s, respectively. Chemical-shift anisotropy contributions to the line width for this hypothetical system can be calculated from eq 2:

$$T_2^{-1}(\mathrm{CSA}) = \frac{1}{90}\gamma_{Se}^2 H_0^2 (\Delta\sigma)^2 \left[8\tau_c + \frac{6\tau_c}{1+\omega_{Se}^2\tau_c^2}\right] \quad (2)$$

The expected line width would be between 5 and 38 Hz for correlation times between 10 and 100 ns/rad, respectively. If the value of 971 ppm is representative of the CSA for the ^{77}Se nucleus in RSeR′ compounds, one can expect that CSA relaxation mechanisms will dominate for molecules undergoing slow molecular motion.

The molecules diisopropyl selenide and dibutyl selenide have sizes intermediate between those of dimethyl selenide and dioctyl selenide and their T_1s reflect this transition. The plot for diisopropyl selenide (Figure 1) indicates that SR is the dominant mechanism, with the T_1s at equivalent temperatures being marginally longer than for the smaller dimethyl selenide. For dibutyl selenide the plot displays a pronounced curvature similar to that for dioctyl selenide, but the T_1s are longer at all temperatures. Clearly, the SR and CSA mechanisms, both of which are contributing here, are less efficient in this compound, a situation that arises because the molecular tumbling is too slow to provide an effective SR relaxation and too fast for an effective CSA relaxation.

For completeness, one must also consider the possibility that the changeover in spin-lattice relaxation mechanisms between the dimethyl and dioctyl selenides may be due to a difference in either the anisotropy of the chemical shift or the spin rotation coupling constant.[11a,36] However, these parameters should not be radically different for homologous compounds like these dialkyl selenides. The similar T_1s for dimethyl and diisopropyl selenide, whose resonances are separated by a chemical-shift difference of 340 ppm and for which one might expect the differences in these parameters to be the greatest, support the contention that motional arguments provide the most consistent rationale of the data and probable cause of the difference.

The amino acid D,L-selenomethionine, $CH_3SeCH_2CH_2CH(NH_2)COOH$, was studied as a 0.1 M solution in D_2O (pD ~2). The T_1 value of 13 ± 1 s at 34 °C falls between that for dimethyl and dibutyl selenide, which is in accord with the above data as regards its size relative to the simple dialkyl selenides. The fact that the amino acid will be protonated at pD 2 may increase its effective size as a result of solvation. To study this particular aspect as it relates to the T_1s of dialkyl selenides in aqueous and nonaqueous solvents, we measured the T_1 of a similar compound methylselenocysteamine, $CH_3SeCH_2CH_2NH_2$, in both $CDCl_3$ and D_2O (pD ~2). The magnitude of the T_1 value in $CDCl_3$ is similar to that of $(CH_3)_2Se$ (Table I), and the temperature dependence of this value implies that SR is the dominant mechanism. The compound appears to be much more mobile in $CDCl_3$ than in D_2O, as evidenced by the much longer T_1s in acidic aqueous solution (Table I). Thus, although the SR mechanism again dominates T_1, solvation in aqueous solution has increased its size and slowed the tumbling of the molecule. The chemical shift of this compound does not reflect this change to the same extent—the resonance in D_2O is very similar to that of the amino acid selenomethionine and shifts by only 6 ppm on changing the solvent to $CDCl_3$.

Dialkyl Diselenides. The spin-lattice relaxation of ^{77}Se in the diselenides (Table I) closely resembles that of the dialkyl selenides in that spin rotation is the dominant mechanism for the smaller diselenides, but CSA relaxation becomes important when the size of the molecule becomes very large, as in didecyl diselenide. These conclusions are based on the temperature

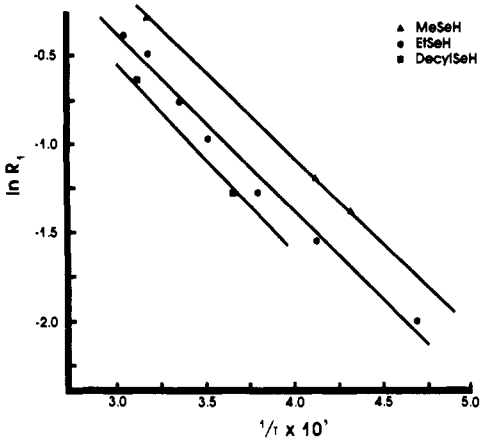

Figure 2. Arrhenius plots of the temperature dependence of the observed relaxation rates ($R \equiv 1/T_1$) of alkane selenols.

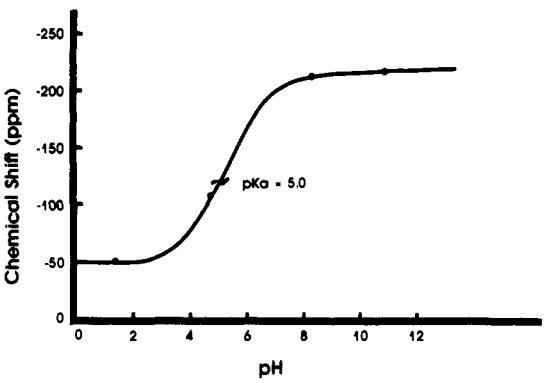

Figure 3. pH profile of the chemical shift of a 0.5 M solution of selenocysteamine in D_2O. The experimentally determined[25] pK_a is indicated on the graph.

dependencies of the T_1s and the lack of a measurable $^1H-^{77}Se$ NOE for any of the compounds. The T_1s for these compounds are of the same order of magnitude as for dialkyl selenides of comparable size. A value of 16 s for the T_1 of diphenyl diselenide has been previously reported.[7] This is shorter than any value observed in this study; however, without a knowledge of how the samples were prepared for the NMR experiments in the previous study, a meaningful comparison cannot be made.

Selenols. Spin rotation accounts for the relaxation in the alkane selenols (Table I and Figure 2). The T_1s are small relative to the dialkyl selenides and are largely independent of the length of the alkyl chain, suggesting that in the long-chain compounds there is enough segmental motion at the end of the chain to provide an efficient SR relaxation in spite of the molecules' long overall correlation times. The absence of a measurable NOE from the directly bound hydrogen in these compounds is in sharp contrast with what is commonly experienced in ^{13}C NMR, where the $^1H-^{13}C$ DD relaxation is a most important mechanism.[40] This situation reflects not only the much greater efficiency of SR relaxation for ^{77}Se as compared with ^{13}C, but also the inefficiency of $^1H-^{77}Se$ DD relaxation. If, under conditions of extreme narrowing, the ^{77}Se was relaxed exclusively by the DD process, the maximum NOE enhancement of 2.61 (total intensity 3.61) would be observed.[41] The $^1H-^{77}Se$ DD relaxation is diminished primarily because of the long Se-H bond distances (1.44 Å in ethaneselenol[42] compared to 1.09 Å for alkyl C-H bonds) and the inverse sixth power dependence of the DD process on this distance (eq 3):[40]

$$T_1^{-1}(\mathrm{DD}) \propto \frac{\gamma_{Se}^2 \gamma_H^2}{r_{SeH}^6} \qquad (3)$$

The lower gyromagnetic ratio for ^{77}Se is also a factor and one may readily calculate from eq 3 that, for a single bound hydrogen, the ratio of T_1(DD) for ^{77}Se to T_1(DD) for ^{13}C will be 9.4. Low molecular weight solutes have reorientational correlation times typically in the range of 10^{-11} to 10^{-13} s/rad,[38] and for these conditions a single hydrogen 1.44 Å away will give rise to a ^{77}Se T_1(DD) between 325 and 4270 s. Thus, because the T_1(DD) in these compounds is on the order of hundreds of seconds, it does not compete favorably with the SR mechanism. The correlation times for proteins are much longer[43] (ca. 10^{-8} to 10^{-7} s/rad), in which case the ^{77}Se T_1(DD) will lie in the range 0.3 to 2.0 s. Under these conditions the T_1(DD) should have a greater influence on the T_1(obsd).

The use of $CDCl_3$ as solvent in these studies reduced the tendency of the Se-H protons to undergo intermolecular exchange. Evidence in support of this comes from the highly shielded chemical shift of the selenol protons (δ = 0.6 ppm in $CDCl_3$ relative to Me_4Si) and the sharpness of the lines, as well as the retention of the spin-spin coupling in both the ^{77}Se spectrum and the ^1H spectrum. Ionization of this sort may be expected to take place in aqueous solution, however, and considering that water is the most common biological environment, it was deemed of interest to examine some selenols in aqueous solution to observe the effect on T_1.

The simple alkane selenols are insoluble in water but dissolve in basic solution. The T_1 for sodium methylselenolate (1.0 M in D_2O, pD ~10) was found to be 16.3 s at 43 °C. The resonance is shifted considerably to higher shielding compared to methaneselenol (−330 ppm relative to dimethyl selenide) and is split into a well-resolved quartet due to coupling with the methyl hydrogens (J_{SeH} = 6.6 Hz). The compound is apparently completely ionized at pD 10 with very little chemical exchange, as evidenced by the sharpness of the lines and the shielded chemical shift.

A slightly more complicated situation was encountered with the amino acid selenocysteine, $HSeCH_2CH(NH_2)COOH$, which was prepared from the reduction of selenocystine. A 0.5 M solution of this compound (pH ~5, 50% H_2O/D_2O) exhibited a broad resonance ($\nu_{1/2}$ = 150 Hz) centered at −141 ppm relative to dimethyl selenide. Proton decoupling had no effect on the line width, which appears to be governed entirely by exchange effects. Unfortunately, the low signal-to-noise ratio and sample limitations prevented an examination of this compound in greater detail.

Instead, we investigated an analogous water-soluble selenol, selenocysteamine, $HSeCH_2CH_2NH_2$. The resonance for this compound is very pH dependent. Its pK_a has been determined to be 5.01,[34] and the graph of chemical shift vs. pH has the form of a typical weak acid titration plot (Figure 3). At low pH the compound exists in the ammonium form **1**, while at higher

$$\underset{\mathbf{1}}{HSeCH_2CH_2\overset{+}{N}H_3} \rightleftharpoons \underset{\mathbf{2}}{\overset{-}{S}eCH_2CH_2\overset{+}{N}H_3}$$

pH it is predominantly the zwitterionic form **2**.[34] The T_1 for this compound (0.5 M in either H_2O or D_2O) was determined to be 7.0 s at pH 1.4 and 7.1 s at pH 8.3, which would indicate that the T_1s are the same for both forms **1** and **2**. The presence of chemical exchange is again evidenced by the breadth of the lines ($\nu_{1/2}$ = 13 Hz). From the graph it is apparent that the resonance for the RSeH species lies approximately 150 ppm to lower shielding of the RSe^- form, and dynamic exchange between these sites could account for the observed decrease in T_2, which broadens the lines to the extent that any $^1H-^{77}Se$ spin coupling is obscured. The ionization process could also

lead to an effect on T_2 through scalar coupling relaxation, but this mechanism is unlikely to affect T_1 since it may be easily shown that, for the anticipated $^1H-^{77}Se$ one-bond coupling of 40–60 Hz, $T_1(SC)$ will be negligible at the Larmor frequencies of the experiment. Furthermore, the same T_1s in both H_2O and D_2O solutions serve to eliminate the possibility that modulation of the scalar coupling interaction affects T_1. Finally, it may be noted that the T_1 for this compound demonstrated a marked sensitivity to dissolved oxygen. Prolonged exposure of an aqueous solution to the atmosphere converts the compound to the diselenide, selenocystamine, although the reaction requires several hours to reach completion.[34] It was found, however, that for solutions in the pH range 4–8, by simply opening the NMR tube to the atmosphere for 2 or 3 min, the small amount of oxygen so introduced resulted in a change of T_1 from 7 to 0.1–0.3 s.

In conjunction with this study we also prepared and investigated aqueous solutions of hydrogen and ammonium selenide. The ammonium selenide solutions were prepared on a vacuum line by codissolving hydrogen selenide and a 300% molar excess of anhydrous ammonia in D_2O. The resonance for this sample, at both 0.3 and 1.0 M, occurs at −511 ppm and is the most shielded selenium resonance yet reported. The line width is broad ($\nu_{1/2}$ = 37 Hz), no doubt due to an exchange between the species HSe^- and Se^{2-}, although as the shift would imply the predominant form is probably solvated Se^{2-}. Once again the breadth of the resonance led to such low signal-to-noise that an accurate T_1 could not be determined. A crude inversion recovery experiment indicated that the T_1 is relatively long and certainly greater than 5 s. Attempts to observe a resonance for sodium selenide were fraught with difficulties as this compound is almost totally insoluble in water.

Hydrogen selenide in water is primarily H_2Se and small amounts of HSe^-. The sample exhibits a resonance at −288 ppm, which may be compared with that reported for neat H_2Se, −226 ppm.[3,4] The T_1s for this sample were found to be 0.9 and 0.7 s at 10 and 34 °C, respectively. These values are similar to those for the alkaneselenols, and we tentatively attribute the slight decrease in T_1 with an increase in temperature to a SR mechanism, although the temperature dependence is not large and SR and CSA may well both be contributing mechanisms.

Selenonium Compounds. Biologically important, naturally occurring forms of selenium in aqueous solution are selenonium ions, R_3Se^+. Trimethylselenonium ion, for example, is the normal excretory product arising from the ingestion and metabolism of many forms of selenium: it constitutes about 20–50% of the selenium excreted in the urine of rats fed a selenium diet.[44] The resonance for a 1.0 M solution of trimethylselenonium iodide at 32 °C appears at 258 ppm, and the T_1 was found to be 13.7 s. The material is insoluble in nonaqueous solvents and so to aid the interpretation of the T_1 mechanisms, a more detailed analysis of the selentine, $(CH_3)_2Se(Br)CH_2CO_2C_2H_5$, was undertaken. The T_1s for a 0.5 M solution of this compound in $CDCl_3$ are given in Table I. The values of T_1 are relatively small and increase only slightly over the 60 °C temperature range of the experiment. This behavior, along with the absence of a measurable NOE, can be taken to indicate that CSA is an important mechanism, although the temperature invariance suggests that CSA and SR may both be contributing mechanisms for spin–lattice relaxation. The magnitudes of the T_1s in aqueous solution are much greater than in the organic solvent, and the increase in T_1 up to 35 °C followed by a decrease suggests again that both CSA and SR mechanisms are operative here. The greater rate of relaxation in $CDCl_3$ could reflect a lesser degree of mobility in the aqueous solution. However, the resonance position shifts from 140 ppm in $CDCl_3$ to 298 ppm in D_2O, which indicates very different environments for the selenium in these two solvents. The selenonium compounds are believed to be completely ionized in aqueous solution,[46] but some ion pairing or even coordination of the bromide is likely to occur in the organic solvent. In the latter case it is, therefore, quite possible that the selenium is relaxing via a SC interaction with the bromine nucleus. There are two magnetic nuclei of bromine, ^{79}Br (I = 3/2, 50.5% natural abundance) and ^{81}Br (I = 3/2, 49.4% natural abundance), and, at the magnetic field strength of these experiments, their Larmor frequencies are 24.89 and 26.83 MHz, respectively. Both of these nuclei have large electric quadrupole moments, and, in an asymmetrical environment such as in the selentine, their relaxation times, which are dominated by quadrupolar relaxation, will be on the order of microseconds or less. For a selenium–bromine SC interaction, the ^{77}Se T_1 is given by the expression:[11a]

$$\frac{1}{T_1(SC)} = \frac{8\pi^2 J^2}{3} S(S+1) \frac{T_2}{1 + (\Delta\omega)^2 T_2^2} \qquad (4)$$

where J is the selenium–bromine scalar spin coupling constant, S is the bromine nuclear magnetic quantum number, T_2 is the spin–spin relaxation time of the bromine nucleus, and $\Delta\omega$ is the difference in Larmor frequencies for selenium and bromine. Since the value of $(\Delta\omega T_2)^2$ may conceivably approach unity in the case of the selentine, one can see that for reasonable estimates of J the ^{77}Se $T_1(SC)$ will be a few seconds or less. By analogy with the coupling of ^{77}Se to nuclei such as ^{19}F, ^{31}P, and ^{195}Pt, wherein spin–spin coupling constants between 500 and 1600 Hz have been observed,[3,46] one can anticipate a large scalar coupling to ^{79}Br and ^{81}Br. In fact, by the judicious choice of J and T_2 one can reproduce on the basis of a SC relaxation alone the observed ^{77}Se T_1 and T_2 of the selentine in $CDCl_3$. Further experimentation is required to substantiate this argument, but at this stage it should be pointed out that SC relaxation is the most plausible explanation for the T_1s, which on any other terms are unusually short by comparison with the T_1s of the dialkyl selenides.

Oxyacids. Inorganic salts of the oxyacids H_2SeO_3 and H_2SeO_4 are common mineral forms of this element. They are readily absorbed and metabolized by a number of organisms, particularly the selenium accumulator plants, and are likely to be present in natural biological extracts.[47] In addition, selenite ion is the most prevalent means by which selenium is administered in animal tests. Thus, an examination of some ^{77}Se T_1s for this class of compounds seemed warranted.

It is apparent at the outset, given the polyprotic nature of these compounds, that their solutions are unlikely to consist of only one discrete species. Differing degrees of ionization are inevitable and for a given compound the composition of the solution will depend on pH, concentration, and temperature. Indeed, a recent study[9] of the pH dependence of the chemical shift of ^{77}Se in aqueous H_2SeO_3 demonstrated the selenium resonance was sensitive to various equilibria and stepwise protonation or deprotonation. In our study, when selenous acid was examined as a function of pH, concentration, and temperature, the T_1 was observed to vary sixfold over the range of conditions employed (Table I). The data thus serve to indicate that the T_1 is sensitive to the changes in the average environment of the selenous ion, a behavior that parallels that of ^{31}P in phosphate ion.[48] The complexity of the system renders an exact interpretation of the data inconclusive, but both SR and CSA are indicated by the data to be important relaxation mechanisms for the selenite ion. The possibility of a SC mechanism from rapid exchange of the acidic protons is unlikely for reasons presented earlier; it was also verified that the T_1s are virtually the same in both H_2O and D_2O solutions. The temperature dependence of the T_1 value for selenate ion (Table I) resembles that for selenite ion but must be regarded with the same discretion.

A very recent report by Koch et al.[8] has appeared concerning

^{77}Se FT NMR studies of H_2SeO_3, Na_2SeO_3, $NaHSeO_3$, and Na_2SeO_4 in H_2O. Their NOE determinations are in complete agreement with this study in that, for H_2SeO_3 and Na_2SeO_4, no NOE was observed. It is interesting to note, however, that for a 4.0 *m* solution of Na_2SeO_3, an enhancement of 0.4 was observed. The T_1 measurements reported by Koch et al.[8] were not obtained under comparable conditions, and a direct comparison of the values is not possible. For Na_2SeO_4 (0.5 *m*, pH 1-4) a T_1 = 10.2 s was obtained at ambient temperature compared to our values of 6.5 s (61 °C) and 16.8 s (15 °C) using 0.5 M Na_2SeO_4 in D_2O at pH 6.6. For H_2SeO_3 (4.0 *m*, pH 1-4), Koch et al. report[8] T_1 values of 1.1 s (H_2O) and 1.4 s (D_2O) at ambient temperature. The concentration of H_2SeO_3 in our study was held at either 1.0 or 0.5 M, and for a 1.0 M H_2SeO_3 solution (pH 1.5 in D_2O) we obtained T_1 values of 2.8 s (63 °C) and 8.5 s (10 °C). Our T_1 values are substantially longer in all H_2SeO_3 samples studied. It is possible that the samples of Koch et al. contained dissolved oxygen, which would shorten the T_1 values, and it is also known that at concentrations of ~4 M or greater, pyroselenate ions, $Se_2O_5^{2-}$, are formed. Thus, further studies of relaxation times of seleno oxyacids are clearly needed.

The relaxation times of dimethyl selenoxide and *N,N*-dimethylselenourea are included in Table I as further examples of multiply bonded selenium. It appears that SR is the dominant relaxation mechanism for ^{77}Se in these cases. The generality of this result awaits further experimentation, however, not only because they are both low molecular weight examples of this class but also because hydrogen bonding, hydration, and enolization are characteristic properties of these functionalities and must be taken into consideration.

Summary and Conclusions

For the molecules studied in this work the spin-rotation and chemical-shift anisotropy mechanisms were found to be the most important means of spin-lattice relaxation for the selenium-77 nucleus. The spin-rotation mechanism is very effective in reducing the T_1s of the small molecules, but this mechanism requires very rapid molecular rotation and freedom of movement and quickly becomes attenuated when the selenium atom is part of a large molecule. Solvation and/or aggregation also tends to reduce the efficiency of SR, and this accounts for much of the difference in T_1 between organic and aqueous solutions of these selenium compounds. With the exception of perhaps the selenols, this mechanism will likely be unavailable for selenium-containing biopolymers. It was, however, illustrated that in these systems the CSA mechanism, which was evidenced in some of the larger molecules studied herein, will play an important role in determining the T_1.

The dipole-dipole mechanism, which is so beneficial to ^{13}C NMR studies, was found to be totally absent in the compounds studied. Thus, in spite of a potentially large NOE, no enhancement was observed upon proton decoupling. This is a consequence of not only the dominance of the more efficient SR and CSA mechanisms but also the marked inefficiency of the ^1H-^{77}Se DD mechanism. The long Se-H internuclear distances, even for directly bound protons, account for much of this ineffectiveness. This DD process has a strong dependence on the molecular correlation time, however, and it was shown that in biological macromolecules T_1 (DD) can be on the order of tenths of a second. These considerations indicate that the study of such large molecules by ^{77}Se FT NMR will not be encumbered by exceptionally long recycle times. It may be noted that under the conditions where DD relaxation becomes most effective (i.e., long correlation times) the maximum observable NOE is reduced to 1.1.

Prototropic ionization is a characteristic of several selenium compounds of potential biological interest. It was illustrated that this property has a definite effect on the observed T_1, although secondary in the sense that it does not contribute directly through a scalar coupling mechanism but controls the nature of the species under investigation. It was beyond the scope of the present study to provide a thorough interpretation of the data in terms of all the relevant physicochemical aspects, but the data serve adequately to demonstrate the difficulties that may be encountered in analyzing the ^{77}Se T_1s of ionizable compounds in aqueous solution. In a more optimistic vein, this type of data could lead to an understanding of the pK_a of the ionizable group and information on the pH effects at a catalytic enzyme site containing selenium.

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Laser Temperature Jump Study of Solvent Effects on Proflavin Stacking

T. G. Dewey, Dorothy A. Raymond, and Douglas H. Turner*†

Contribution from the Department of Chemistry, University of Rochester, Rochester, New York 14627. *Received February 26, 1979*

Abstract: The Raman laser temperature jump technique has been used to determine rate constants for proflavin dimerization in aqueous solutions of methanol, ethanol, 1-propanol, glycerol, and urea. Forward rates for solutions in aqueous ethanol are quantitatively analyzed using a specific solvation model. The reverse rates are characterized by the molecularity of ethanol attack on the dye dimer. Thionine requires approximately three ethanols to disrupt a dimer, whereas proflavin requires only one. Poor correlations are found between the reverse rates and bulk solvent properties. The results suggest that solvent effects on dye stacking are determined by specific dye–solvent interactions. Comparison with previous results for thionine stacking indicates the detailed electronic structure of the dye determines these interactions, and that solvent–solvent contributions are relatively unimportant.

Stacking interactions play a fundamental role in nucleic acid chemistry and in the solution chemistry of dyes. Despite an ever increasing amount of thermodynamic data on stacking systems, these interactions are still poorly understood. While quantum chemists have emphasized electronic interactions,[1-3] experimentalists have demonstrated the importance of the aqueous environment.[4,5] Initially, the requirement of water for stacking was thought to implicate traditional hydrophobic interactions.[6,7] However, this hypothesis is contradicted by thermodynamic investigations that have shown that the driving force has a large favorable enthalpy and a significant unfavorable entropy.[8,9] Sinanoglu suggested a model in which the reduction of surface area in the solvent cavity upon stacking resulted in a favorable enthalpy contribution in liquids of very high surface tension.[10] Unfortunately, there has been little experimental work on solvent effects on stacking. Thus, the relative contributions of these various interactions remain unknown.

A more detailed picture of stacking can be obtained by studying the kinetics of the reaction as a function of solvent. In this paper, we present the kinetic results on the association of a cationic dye, proflavin, in various mixed aqueous solvents (see Figure 1). These results are compared with our previous study on thionine.[11] The comparison of these two dyes is of interest, since they both have essentially the same size and shape, while their equilibrium constants for stacking differ by almost an order of magnitude.

Experimental Section

Materials. Proflavin, 3,6-diaminoacridine, was obtained from Aldrich, and recrystallized from water as described previously.[12] Gravimetric analysis showed 1 mol of water per mol of proflavin. Concentrations were made by weight using a molecular weight of 227.26. Water was doubly distilled and absolute ethanol was used. Methanol was spectral grade from Mallinckrodt, 1-propanol "distilled in glass" from Burdick and Jackson, and urea Ultra Pure grade from Schwarz/Mann.

Kinetics. The association of proflavin has a relaxation time of less than 1 μs, thus requiring the use of the Raman laser temperature jump method described previously.[13,14] The probe beam was filtered with a Corning CS5-57 filter and monitored at 440 nm, close to the absorption maximum for the monomer. For studies involving small equilibrium constants, the probe beam was intensified by pulsing the Xe arc lamp to increase the light intensity a factor of ten.[15] As a check for photochemical effects, a 3.96×10^{-3} M solution in D_2O was tested. The temperature jump in D_2O is over 100 times smaller than in H_2O, and no signal was observed. Relaxation data were photographed on 35-mm film, projected onto a Tektronix 4662 plotter, and digitized and analyzed with a Tektronix 4051 terminal. The data were fit to a single exponential by a nonlinear least-squares procedure. Each relaxation time represents an average of at least 12 shots. The estimated error in the rate constants is ±15%. All solutions contained 0.01 M KH_2PO_4.

Results

The stacking of planar dye molecules has been demonstrated for acridine orange and thionine by the characteristic upfield shift of the NMR peaks of the ring protons.[11,16] Although no NMR measurements have been made on proflavin, it is expected to behave similarly. Proflavin does exhibit deviations in absorbance from Beer's law at high concentrations, which suggests that stacking does occur.[17-23]

Solvent effects were studied with aqueous mixtures, since dye stacking has not been observed in nonaqueous solvents.[24] Attempts were made to observe relaxations for both proflavin

† Alfred P. Sloan Fellow.

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