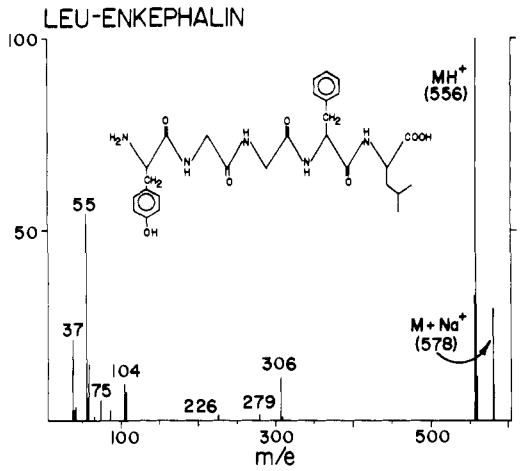

Figure 5. Positive ion mass spectrum of the pentapeptide leucine enkephalin by the new ionization technique.

Spectra obtained on two dinucleotides are shown in Figures 3 and 4. These spectra are very similar to those obtained by Schulten with field desorption.[9] As shown in the FD work, these spectra are sufficient to uniquely determine the structure of the dinucleotide. In particular, the protonated cyclophosphates (mass 306 for CpG and mass 330 for ApU) are observed for the nucleoside in position 1, but the corresponding cyclophosphate containing the other nucleoside (mass 346 for CpG and mass 307 for ApU) is not detected.

The new ionization technique has been applied to a number of other interesting classes of compounds. All of the common amino acids have been investigated as well as several di- and tripeptides and one pentapeptide. A mass spectrum of the pentapeptide, leucine enkephalin, is shown in Figure 5. This spectrum is typical of all the amino acids and peptides investigated in that the protonated molecular ion is the base peak in the spectrum; a small amount of alkali addition also occurs, but very little fragmentation is observed. The di- and trisaccharides which have been studied give only $M + Na^+$ and $M + K^+$ with very little fragmentation and no protonated molecular ions. Preliminary studies have been conducted on a number of other interesting systems, including antibiotics, vitamins, and fatty acids. In general, protonated and/or cationized molecular ions are observed as major peaks in the spectra. More detailed results will be presented later.

At present, the overall ionization and detection efficiency for the new ionization technique is between one and two orders of magnitude lower than for conventional chemical ionization in our apparatus; however, the background ionization of the solvent is lower by 4 or 5 orders of magnitude in the new technique. This latter fact coupled with the reduced amount of fragmentation that is generally observed led to the surprising result that the detection limit for the protonated molecular ion of most of the involatile substances investigated is substantially lower with the electron beam turned off! Typically, sample input rates in the range of 1–10 ng/s are sufficient to obtain reproducible, "clean" spectra when scanning at rates on the order of 100 amu/s over the full spectral range. Higher sensitivities are obtained by using single-ion monitoring or scanning over a limited mass range. Some results obtained for adenosine by scanning over a 5-amu range centered on the MH^+ ion at mass 268 are shown in Figure 6. In these experiments, 200 pg injected gave an integrated response about five times the noise level.

Clearly, a large amount of additional work will be required before the full value of the new ionization technique can be determined. Some of the details of the ionization process are not yet fully understood, and it is unlikely that our present techniques and apparatus are optimum. Even at the present primitive stage of development, the new technique appears to provide many of the advantages of field desorption or plasma desorption without some of the disadvantages. In particular, the new technique is compatible with on-line LC; it is fast, sensitive, relatively simple, and inexpensive.

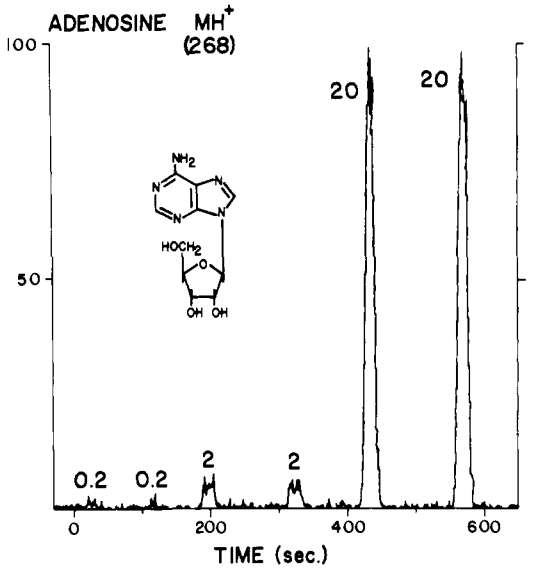

Figure 6. Response of the mass spectrometer by using the new ionization technique to a series of injections of adenosine. The plot gives the total ion current (arbitrary units) measured by scanning (1 s) over a 5-amu range centered on the MH^+ ion at mass 268. Each evolution peak is labeled with the quantity of adenosine injected in ng. The peaks are 20-s wide fwhm, and the noise level between peaks summed over the width of a peak corresponds to the extrapolated response for 40 pg.

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Time-Resolved Proton Magnetic Resonance Studies of Polynucleotides

Sir:

Examination of the proton magnetic resonance spectrum of yeast transfer RNA^{Phe} by the spin–echo sequence gives a simplified subspectrum with enhanced resolution. The normal proton magnetic resonance spectrum of biopolymers generally shows few resolved resonances because of the line widths of individual resonances and because of the very large number of proton resonances in the spectrum. As a result, much of the information intrinsically present in the proton magnetic resonance spectrum is unavailable. In essence, the problem is one of separability, how to separate and separately access information from single resonances. The 360-MHz proton magnetic resonance spectrum of yeast $tRNA^{Phe}$ shown in Figure 1 illustrates this problem.[1] Focusing, for example, on the 7–9-ppm region where resonances from the adenine A8 and A2, guanine G8, cytosine C6, and uracil U6 protons are found,[2] it can be seen that there are only a couple of resolved or partially resolved resonances at the high- and low-field extremes of the region plus some sign of structure throughout the main body of the feature, a poorly resolved spectrum. However, just as the

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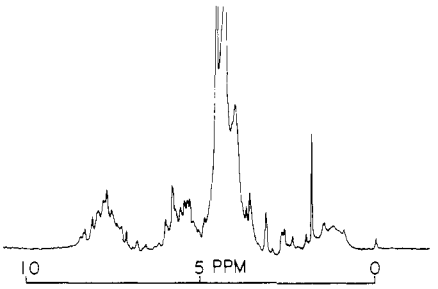

Figure 1. The proton magnetic resonance spectrum of yeast $tRNA^{Phe}$ at 40 °C in D_2O solution. The sample of 2 mg of yeast $tRNA^{Phe}$ (Boehringer Mannheim lot 1298133) per 50 μL was prepared by dialysis against 100 mM NaCl, 10 mM cacodylate, pH 6.0, 10 mM $MgCl_2$, and 1 mM EDTA.

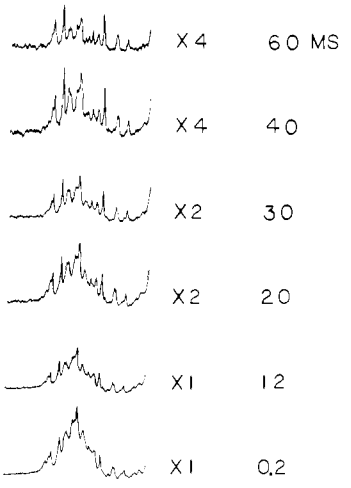

Figure 2. Examples of spectra obtained with a $(90\text{–}\tau\text{–}180\text{–}\tau\text{–FID–10-s delay})_n$ sequence, showing the decay of the aromatic resonances, 7–9-ppm region. The notation X1, X2, and X4 indicates the relative scale expansion used to plot the corresponding spectra. The decay times denoted 60 MS, 40, 30, 20, 12, and 0.2 are the values of 2τ in ms.

unusual chemical shift of a resonance can lead to some resolution, other unusual characteristics of particular resonances may be similarly useful.[3]

The 7–9-ppm regions of spectra obtained with a $90\text{–}\tau\text{–}180\text{–}\tau$ sequence are shown in Figure 2, where it can be seen that with increasing delay times (2τ) a spectrum with greatly enhanced resolution is obtained. The features of the relaxed spectrum correspond closely to the hints of structure observable in the normal spectrum, indicating that the highly relaxed spectrum is composed of a subset of the resonances normally observed in the 7–9-ppm region, i.e., a subspectrum. Because of its simplicity and enhanced resolution, the subspectrum is a partial solution to the problem of separability, and as such it provides improved access to information about the properties and processes of these molecules.

In addition to giving an interesting subspectrum, the data also provide some characterization of the properties of the resonances in the subspectrum. The resonances making up the underlying, broad background decay with a composite lifetime of 15 ms whereas most of the resolved features have decay times which average around 65 ms, more than four times longer. One of the resolved resonances has a lifetime of 150 ms, clearly longer than any of the others. The highly relaxed spectrum at the top of Figure 2 is similar to the convolution difference spectrum,[4,5] which can be computed from the normal spectrum (data not shown), but not identical. The differences appear to be largely due to the way rapidly decaying components of the spectrum are discriminated against. For example, for a simple spectrum known to contain just two kinds of resonances, those with lifetimes of 65 and 15 ms, the convolution difference parameters could be optimized to give roughly a 7:1 discrimination[4] against the short-lived component while the discrimination would be 20:1 for the conditions of the top spectrum in Figure 2. Besides obtaining lifetimes as a characterization of the properties of the subspectrum, it is also of interpretive value to estimate the number of resonances contributing to the subspectrum.

Using the integrated intensity of the subspectrum and an average lifetime to correct for the extent of decay from time zero indicates that 16 ± 3 protons contribute to the subspectrum whereas visual inspection of the subspectrum indicates 12 features. The correspondence of the number of visually identifiable features and the integrated intensity supports the very basic premise that while a couple of features have two resonances contributing most of the subspectrum is composed of nearly resolved single resonances.

In studies of mononucleotide[6] relaxation, adenine A2 proton resonances have been found to have unusually long relaxation times because of spatial isolation from other protons and therefore from efficient dipole–dipole relaxation processes. The adenine A2 resonances of tRNA would also be expected to have spin–spin relaxation times notably longer than most other aromatic resonances on the basis of interproton distances calculated from tRNA crystal structure data and the assumption of a single correlation time.[7] For $tRNA^{Val}$, it has been suggested that A2 protons account for about half of the sharper lines in the normal and convolution difference spectra.[7] Consequently, A2 proton resonances might also be expected to predominate in these subspectra, with the ratio of decay times indicating interproton distances up to 30% longer. That most of the resonances are at least of the same type is supported somewhat by the near uniformity in observed decay times. In addition, the number of adenines[8] in yeast $tRNA^{Phe}$, 17, is close to the number of resonances in the subspectrum. Thus, the notion that the subspectrum is mainly due to adenine A2 proton resonances is reasonable in itself and is consistent with the available facts, but of course such data cannot prove that this is the correct assignment. Not ruled out, for example, is the possibility that some resonances from protons in bases with facile local internal motion[9,10] also contribute. The one resonance with the exceptionally long decay time of 150 ms could be such an example. However, the possibility that unresolved doublets from U6 or C6 protons contribute can be ruled out. With the pulse sequence used here, these resonances would show a pronounced modulation in intensity due to the U5–U6 or C5–C6 three-bond scalar coupling,[3] but none were observed, despite the fact that the intensities of the subspectrum resonances can be followed long enough to have observed a full (positive intensity–null–negative intensity) cycle of the modulation.

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