because of the sharp decline of $\exp(-2r/l)$ with distance.

The ionic strength dependence of the rate of transfer from uninegative Tb(EDTA) to uninegative Co(EDTA) is shown in Figure 1. The striking finding is that the observed transfer rates fit the curve calculated for exchange transfer, given that $a = 0.8$ nm and $k_0 = 8.35 \times 10^6$ M^{-1} s^{-1}. It should be noted that there are no adjustable parameters in this fit; k_0 is the observed rate of transfer from uncharged Tb(HED3A) to Co(EDTA). In contrast, the ionic strength dependences calculated for dipole–dipole transfer with $a = 0.3$ or 0.8 nm do not fit the experimental points, nor do they accurately predict the effect of ionic strength.

Energy transfer between uninegative Tb(EDTA) and dinegative Cu(EDTA) provides further evidence that exchange interactions are dominant (Figure 2). The observed transfer rates for this donor–acceptor pair agree closely with the curve calculated for the expression

$$k_{ex} = 3.17 \times 10^6 \text{ M}^{-1} \text{ s}^{-1} \exp[-w(0.7)/kT] \quad (5)$$

This donor–acceptor pair has an R_0 value = 1.3 nm, which corresponds to a k_{dd} of 3.3×10^4 M^{-1} s^{-1} in the absence of ionic interactions. Thus, the observed rate of transfer from Tb(EDTA) to Cu(EDTA) is almost 2 orders of magnitude larger than that calculated for dipole–dipole transfer.

From the observed transfer rates and the expressions for k_{dd} and k_{ex}, we calculate that the contribution of exchange interactions to transfer between these chelates becomes dominant when the donor–acceptor distance is less than about 1.1 nm. Preliminary studies suggest that exchange interactions are also important in rapid-diffusion energy transfer between donors and acceptors with large R_0 values when their distance of closest approach is less than about 1 nm. Energy transfer by the exchange mechanism is more responsive to the difference between exposed and buried energy acceptors than is transfer by the dipole–dipole mechanism, because it has a steeper distance dependence. Our experiments also demonstrate that electrostatic effects can readily be taken into account by using the expressions given in eq 3 and 4. It should be feasible to ascertain the sign and perhaps also the magnitude of the electric charge around a chromophore in a macromolecule or membrane by measuring the effect of ionic strength on transfer rate and also by using a series of donors with different net charges.

Observation of a Fully Protected Oligonucleotide Dimer at m/z 12 637 by ^{252}Cf-Plasma Desorption Mass Spectrometry

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The new technique of ^{252}Cf-plasma desorption mass spectrometry (^{252}Cf-PDMS)[1,2] has had some success in producing large gas-phase organic molecular ions extending beyond m/z 4000.[3,4] We wish to report evidence of a positively charged ion at m/z 12 637 ± 10 in the mass spectrum of a chemically blocked synthetic deoxydodecanucleotide which we believe to be a dimer ion of this molecule. This is the largest organic molecular ion that has been identified, thus far, by ^{252}Cf-PDMS and, to the best of our knowledge, one of the largest molecular ions produced by any mass spectrometric method. There is considerable interest in the unblocked forms of molecules of this type for use as probes into the structure and function of DNA and RNA. For example, specific sequences have been chemically synthesized to study the control regions of a gene. Synthetic oligonucleotides have also been designed to function as primers for various endonucleases and for use as connectors and adaptors in cloning segments of DNA.[5] We became interested in the possibility of utilizing the method of ^{252}Cf-PDMS to confirm the chemical structure of the chemically blocked oligonucleotide intermediates because of the lack of other suitable procedures. Our initial investigation demonstrated that ^{252}Cf-PDMS could be used to produce and detect intact positive molecular ions and nested sets of negatively charged fragment ions which were used to identify the molecular weight and confirm the base sequence.[3] In addition we found that the blocked deoxyribo- and ribooligonucleotides provide an excellent source of molecules of regularly increasing size which are amenable to ionization and desorption by ^{252}Cf-fission fragments and which can therefore be used to obtain information on the production and detection of massive ions.

The dodecanucleotide reported in this communication was synthesized in the laboratory of S. A. Narang by using the modified triester method.[6] The abbreviated structural formula is shown in Figure 1. Each internucleotidic linkage is protected with the *p*-chlorophenyl group. Free 5′- and 3′-hydroxyl groups are protected with the dimethoxytrityl and benzoyl esters, respectively. Amino groups on cytidine and adenine residues are benzoylated (represented by the bz superscript above the base); the amino group on all guanine residues is present as the isobutyryl amide (symbolized by G^{iso}). The molecule has an average molecular weight of 6275.04 u (based upon C = 12.01115). A detailed description of the ^{252}Cf-PD mass spectrometer has been previously reported.[1,2] A thin-solid film of the protected oligonucleotide was prepared by electrospraying[7] a methanol–chloroform–acetone solution ($\sim 2 \times 10^{-4}$ M) onto a 1.1-cm^2 aluminized polypropylene foil, 6.25 μm in thickness. The time-of-flight mass spectrometer was operated using a flight path length of 45 cm. The mass resolution at this distance is ~600 $M/\Delta M$. The CEMA (channel electron multiplier array, Galileo Electro-Optics Corp.) detectors were operated at near saturation to maximize the detection efficiency for massive ions. Ions were accelerated to 10 keV. A 20-μCi californium-252 source was used giving an average fission fragment flux through the sample of 2000/(cm^2 s).

The mass calibration procedure used for this study was developed utilizing the precise timing capabilities of the ^{252}Cf-PDMS method.[2] The time-of-flight (TOF) of two ions in the spectrum (H^+ and Na^+) were precisely determined to within 0.001 ns (1 part in 2×10^6) by using a fast digital clock (TDC 100, Ortec). From these two calibration points we evaluate the slope and y intercept of a linear equation. This is then used to evaluate $M^{1/2}$ from the TOF.[8] The TOF for a peak in the spectrum corresponds to the centroid of the distribution evaluated using the mean value theorem. For ions above m/z 1000 the unresolved isotopic satellites result in a folded distribution and the centroid represents the isotopically averaged m/z. This procedure has been tested for known molecules up to 5000 units and an average precision of the order of 1 part in 2000 has been obtained. Because the TOF measurement is digital and not analog, no extrapolation uncertainity is introduced by extending this procedure to higher masses.

The positive ion spectrum of the dodecanucleotide is shown in

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(8) Due to the large initial kinetic energy of the H^+ ion, the effective mass of this ion was used in the calculation.

0002-7863/81/1503-1609$01.25/0

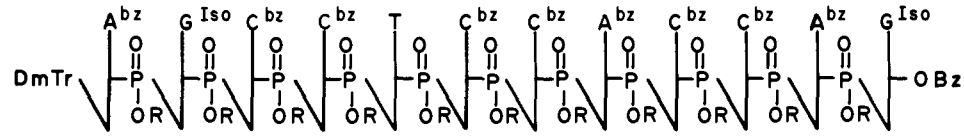

Figure 1. Abbreviated structural formula of the fully protected dodecanucleotide used in this study. Vertical lines represent the deoxyribose units. Horizontal lines represent 3′-ester linkages and slanted lines represent the 5′-ester linkages. Standard symbols are used to indicate the bases and protecting groups, R = *p*-chlorophenyl.

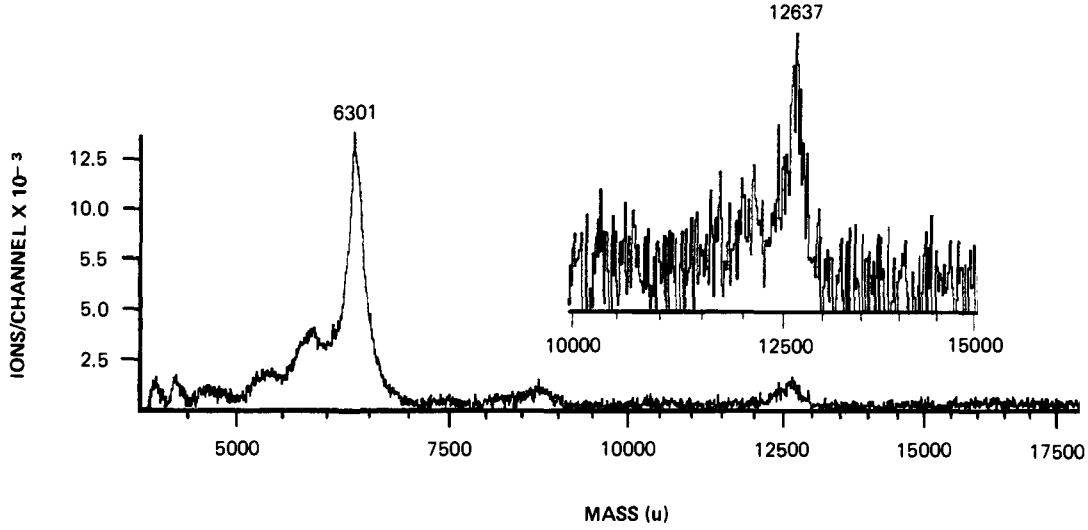

Figure 2. ^{252}Cf-PDMS positive ion spectrum of a fully protected dodecanucleotide above 4000 u. The dimer ion region is shown in more detail in the inset.

Figure 2. A peak of good intensity was observed at m/z 6301 ± 3 which is a molecular ion adduct of the form $(M + Na)^+$. This species of ions has been observed previously with the lower order oligonucleotides and the identity verified by substituting a different alkali metal ion (Cs^+) in the matrix.[3] On the basis of the known structure of this molecule, the elemental composition of this ion is $C_{279}H_{249}O_{82}N_{45}P_{11}Cl_{11}Na$ which gives an isotopically averaged mass of 6297.9 u. Within the limits of our error, the experimental result is in agreement with this value. This is the largest monomer ion that has been reported in the mass spectral literature. Negative ions were also observed at m/z 6277 ± 3 which are believed to be $M^{-}\cdot$ and/or $(M - H)^-$.

The lower order fully protected nucleotides in this series have been observed to produce oligomer ions extending to the tetramer producing ions primarily of the type $(2M + Na)^+$ and $(2M + 2Na - H)^+$, although additional species containing more sodium atoms were also present. Anticipating that this molecule might also form a dimer ion which would have a molecular weight in excess of 12 000 u, we extended the normal irradiation time a factor of 10 to obtain better statistics in the mass range above the monomer. This was made possible by a property of ^{252}Cf-PDMS, that the sample utilization is so small that repetitive scans can be made for many hours or days, if necessary, without sample degradation, in order to resolve weak ion peaks from the background by signal averaging. Figure 2 shows the spectrum obtained after an irradiation period of 13.9 h involving 100 million mass scans (or fission fragment–sample interactions). The monomer ion peak is clearly apparent at m/z 6301 but, in addition, two weak peaks at m/z 8709 ± 8 and m/z 1263 ± 10 were also observed. A statistical analysis of the region using a computerized digital filter technique[9] showed that both peaks were statistically significant. No peaks were detected above m/z 13 000 in this spectrum. In addition, no peaks above m/z 6300 were detected in the negative ion spectrum. The m/z 12 637 peak shown in the inset of Figure 2 is in the region expected for the dimer ion. The maximum in the peak intensity is five standard deviations above the background fluctuation. The measured value of m/z differs from that expected for $(2M + Na)^+$ by m/z 64 ± 10. Since no corresponding ions were observed in the negative ion spectrum, it is unlikely that these ions are from monomeric impurity species. Further, if a molecule of this mass, present in only trace levels, produced a detectable peak, it would imply that the probability for its formation is extremely high. This does not seem likely. For the m/z 12 637 species, it is therefore highly probable that multiple sodium attachment has occurred. The attachment of four Na atoms could account for the observed mass. This phenomenon has been observed in the ^{252}Cf-PDMS of large molecules, particularly when the Na content of the sample is high and several acidic hydrogens exist on the molecule.[1] Replacement of the acidic hydrogens by sodium can occur, for example, on the isobutyrlguanine residues in the molecule used in this study.[3] The ion at 8709 m/z may be a fragment of the dimer. This type of fragmentation has observed in the ^{252}Cf-PDMS of chlorophyll *a* which forms particularly stable oligomers.[4]

While we can not be definitive as to the composition of the m/z 12 637 species, it is likely that it is a dimer-adduct ion. What is remarkable is that an ion having a mass of this magnitude can be desorbed from a solid film by a nuclear fission fragment and can be detected.

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