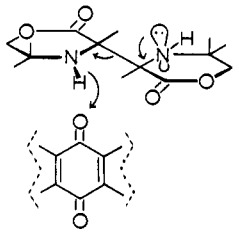
homolysis of **2** in methanol solvent under the reaction conditions. The rate constant for bond homolysis of **2** in methanol solvent at 9.9 ± 0.1 °C is (5.17 ± 0.02) × 10^{-4} s^{-1} and in methanol-*d* solvent (4.19 ± 0.04) × 10^{-4} s^{-1}. The homolysis rate constants were determined by using *N*-methylisatin as a trapping agent for the oxomorpholinyl radical **3**. The experimental technique was analogous to that previously described[9] in chloroform solvent except the destruction of *N*-methylisatin was monitored by visible spectroscopy at 420 nm. The hydrogenolysis of daunomycin hydrochloride by **2** in methanol solvent at 9.7 ± 0.2 °C is first order in daunomycin hydrochloride (**5**) and first order in *dl* dimer **2** when daunomycin hydrochloride is 1–2 × 10^{-4} M and occurs with a rate constant of 43 ± 7 M^{-1} s^{-1}. In methanol-*d* solvent at 9.8 ± 0.1 °C the rate constant is 8.1 ± 1.3 M^{-1} s^{-1}. The two rate constants yield a deuterium kinetic isotope effect of 5.3 ± 1.7. Destruction of daunomycin hydrochloride was monitored spectrophotometrically at 475 or 480 nm. The error in the measurements of the bimolecular rate constants resides predominantly in the determination of the infinity point. Each rate constant is the average of four measurements which followed bimolecular kinetics with correlation coefficients of better than 0.9995 for more than 70% of the reaction.

In the daunomycin hydrochloride concentration region of 1–2 × 10^{-4} M especially in methanol-*d* solvent, competitive first- and second-order reaction mechanisms can logically be inferred from the magnitude of the rate constants. The second-order rate constants were recalculated by using the integrated rate expression for competitive first- and second-order processes, the measured first-order rate constant for bond homolysis, and an iterative procedure. The data fit the competitive first- and second-order mechanisms as well as the second-order mechanism. The recalculated second-order rate constants in methanol and methanol-*d* solvents are 38 ± 6 and 4.4 ± 1.2 M^{-1} s^{-1}, respectively, giving a deuterium kinetic isotope effect of 8.6 ± 2.7.

Although the isotope effect for the bimolecular mechanism includes contributions from exchange of the various remote acidic protons of daunomycin hydrochloride with the solvent and a solvent isotope effect, the magnitude is still suggestive of cleavage of an N–H bond of the dimer in the transition state of the rate-controlling process. Note that the isotope effect for homolysis of



2, which also includes a solvent isotope effect, is only 1.23 ± 0.03. The N–H bond cleavage probably involves either a hydrogen atom or a hydride transfer, both facilitated by cleavage of the central 3,3′ C–C bond. If hydrogen atom transfer occurs, a subsequent electron transfer from radical **3** would be required to generate anion **12**.

The reduction of the 7-deoxyaglycon triacetate to the hydroquinone most likely occurs via a series of electron transfer followed by proton-transfer steps from radical **3** (Scheme I), since this reduction is visibly slower than bond homolysis of **1** or **2**. The process of electron transfer from radical **3** is analogous to that proposed earlier for the reduction of the carbon–nitrogen double bond of 5,6-dihydro-5,5-dimethyl-3-phenyl-1,4-oxazin-2-one,[2] the reduction of benzil to benzoin,[2] and the reductive dimerization of isatin to isatide[9] by the radical **3**.

Recently, Bachur and co-workers have proposed that the quinone-containing anticancer drugs including adriamycin (**4**) and daunomycin (**5**) serve as electron-transfer catalysts for the reduction of oxygen to superoxide by NADPH.[3,4] Since **4** and **5** bind effectively to DNA, probably by intercatalation, the toxicity and/or activity in chemotherapy has been proposed to result from the production of superoxide and subsequently peroxide and hydroxyl radicals in the vicinity of the DNA. In an anerobic environment **4** and **5** are reductively cleaved by NADPH to the 7-deoxyaglycons **6** and **7**, respectively, analogous with the reductive cleavage described here. The dramatic reduction in the toxicity of adriamycin by **1** and **2** may then result from the reductive cleavage reaction. Although the 7-deoxyaglycons themselves should be capable of serving as catalysts for the reduction of oxygen to superoxide, reactivity should be severely inhibited by solubility.

The reactivity of both the radical dimers (**1** and **2**) and the oxomorpholinyl radical **3** appear to resemble the reactivity of NADPH. The reactivity of the dimers might parallel the reactivity of NADPH in hydride donation, and the reactivity of the oxomorpholinyl radical **3** appears to parallel the free-radical reactivity of NADPH in electron transfer.[2,10,11]

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Exchange Interaction Contribution to Energy Transfer between Ions in the Rapid-Diffusion Limit[1]

Claude F. Meares* and Simon M. Yeh

Department of Chemistry, University of California Davis, California 95616

Lubert Stryer*

Department of Structural Biology, Sherman Fairchild Center Stanford University School of Medicine Stanford, California 94305

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Energy transfer is enhanced by translational diffusion occurring during the excited state lifetime of the fluorescent donor.[2,3] The increase in transfer efficiency becomes maximal when $D\tau/s^2 \gg 1$, where D is the sum of the diffusion coefficients of the donor and acceptor, τ is the lifetime of the donor, and s is the mean donor–acceptor distance. This rapid-diffusion limit has been experimentally realized[3] by the use of terbium chelates as long-lived energy donors ($\tau \sim 1$ ms). An attractive feature of rapid-diffusion transfer is its very strong dependence on the distance of closest approach of the donor and acceptor. Consequently, this technique can be used to ascertain the depths of chromophores in biological macromolecules and membrane systems.[4] The

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quantitative interpretation of such data requires a knowledge of the contributions of dipole–dipole coupling[5] (Förster transfer) and exchange interactions[6] to the transfer process. We have investigated energy transfer between metal chelates with well-defined structures to elucidate the roles of these transfer mechanisms. We show here that exchange interactions account for nearly all of the transfer between terbium chelate donors and chromophoric metal chelate acceptors in the rapid-diffusion limit.

The fluorescent energy donors in this study were the Tb(III) chelates of ethylenediaminetetraacetic acid (EDTA) and *N*-(β-hydroxyethyl)ethylenediaminetriacetic acid (HED3A). The acceptors were the Co(III) and the Cu(II) chelates of EDTA. These chelates are nearly spherical,[7] with the electric charge and the transition moment almost centered. Furthermore, the structure of the electrically neutral Tb(HED3A) chelate is very similar to that of the uninegative Tb(EDTA) chelate. Hence, we anticipated that a comparison of these chelates would reveal the effect of electric charge on the distribution of donors and acceptors and on the resulting transfer efficiency. Donors were excited with pulses of 488-nm light from an argon-ion laser, and their emission kinetics were measured as described previously.[3] Concentrations of donors and acceptors were about 10^{-3} M. $NaClO_4$ was used as the inert electrolyte in aqueous solution at 298 K.

The observed transfer rate from Tb(HED3A) to Co(EDTA) is 8.35×10^6 M^{-1} s^{-1}. The contribution k_{dd} (M^{-1} s^{-1}) from dipole–dipole transfer in the rapid-diffusion limit[3] is given by

$$k_{dd} = 6.023 \times 10^{-1} \int_a^\infty \frac{1}{\tau_0} (r/R_0)^{-6}(4\pi r^2)\, dr \qquad (1)$$

$$k_{dd} = 2.523 R_0^6 a^{-3}/\tau_0 \qquad (2)$$

where R_0 (nm) is the 50% transfer distance for a single donor–acceptor pair, a (nm) is the distance of closest approach of the donor and acceptor, and τ_0 (s) is the lifetime of the donor in the absence of transfer. For $R_0 = 2.1$ nm (obtained from the spectral overlap of the donor and acceptor and a donor fluorescence quantum yield of 0.2), $\tau_0 = 1.0$ ms and $a = 0.8$ nm (from space-filling models and data given in ref 7); $k_{dd} = 4.23 \times 10^5$ M^{-1} s^{-1}. This calculated dipole–dipole contribution is only 5% of the observed transfer rate. A closest approach distance of 0.3 nm would make k_{dd} equal to the observed rate, but such a value of a is clearly impossible for these chelates. Thus it seems likely that most of the observed transfer comes from exchange interactions arising from overlap of the electron clouds of the donors and acceptors.[8]

The transfer rate due to exchange interactions cannot be readily calculated a priori. However, the distance dependence of exchange transfer is very different from the r^{-6} dependence of Förster transfer. The rate of exchange transfer is proportional to $\exp(-2r/l)$, where l, the average effective Bohr radius of the atoms involved,[6] is about 10^{-1} nm. This difference provides an experimental basis for differentiating between the exchange and dipole–dipole mechanisms. The experimental strategy is to alter the spatial distribution of charged donors and acceptors by varying the ionic strength of the solution[10,11] and measure the resulting transfer rate. Electrostatic interactions change the radial distribution function by the factor $\exp[-w(r)/kT]$, where $w(r)$ is the potential of mean force between the donor and acceptor ions, k is Boltzmann's constant, and T is the absolute temperature.[11] For 1–1 and 2–2 electrolytes up to a concentration of 1 M, the potential of mean force is closely approximated by

$$w(r) = \left(\frac{Z_A Z_D e^2}{\epsilon(1 + \kappa a)}\right)\left(\frac{\exp[-\kappa(r - a)]}{r}\right) \qquad (3)$$

where Z_A is the acceptor charge, Z_D is the donor charge, ϵ is the dielectric constant, I is the ionic strength (M), and κ is the Debye-Huckel parameter, which is equal to $3.3I^{1/2}$ nm^{-1} in water at 298 K. Insertion of $\exp[-w(r)/kT]$ into the integrand of eq 1 then gives the ionic strength dependence of k_{dd}. The effect of ionic strength on the rate k_{ex} of exchange transfer can be calculated by substituting $\gamma \exp[-w(r)/kT] \exp(-2r/l)$ for $(r/R_0)^{-6}/\tau_0$ in eq 1. The constant γ for a particular donor–acceptor pair is in fact defined by the observed transfer rate k_0 involving an uncharged donor, for which $\exp[-w(r)/kT] = 1$. The resulting expression can then be closely approximated by

$$k_{ex} = k_0 \exp[-w(a)/kT] = k_0 \exp\left[\frac{-Z_A Z_D e^2}{\epsilon a(1 + \kappa a)kT}\right] \qquad (4)$$

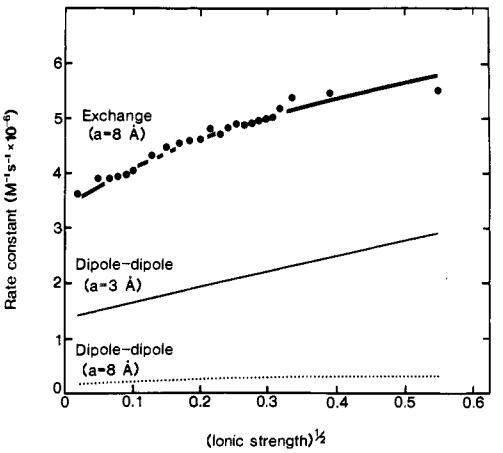

Figure 1. Ionic strength dependence of rate constants for energy transfer in the rapid-diffusion limit from uninegative Tb(EDTA) to uninegative Co(EDTA). The observed rate constants (solid circles) fit the curve (upper solid line) calculated for exchange interactions with $a = 0.8$ nm and $k_0 = 8.35 \times 10^6$ M^{-1} s^{-1}. The middle curve is calculated for dipole–dipole transfer with $a = 0.3$ nm and the lower one for $a = 0.8$ nm.

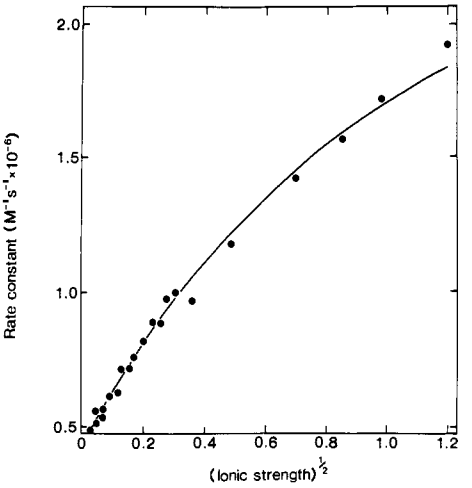

Figure 2. Ionic strength dependence of rate constants for energy transfer in the rapid-diffusion limit from uninegative Tb(EDTA) to dinegative Cu(EDTA). The observed rate constants (solid circles) fit the curve calculated for exchange interactions with $a = 0.7$ nm and $k_0 = 3.17 \times 10^6$ M^{-1} s^{-1}.

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(8) The contribution from exciton interactions, which have an r^{-3} distance dependence, is probably negligible because the interaction energy between these weak transitions ($<10^{-5}$ cm^{-1} at 0.8 nm) is much less than the vibronic band width. Also, it is interesting that the magnitudes of the observed rate constants (k_0) are consistent with those observed for organic triplet energy transfer.[9]

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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

Catherine J. McNeal* and Ronald D. Macfarlane*

Department of Chemistry, Texas A&M University
College Station, Texas 77843

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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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