8–10 kcal/mol.[27]

Although the precise assignment of these species must await further experiments, the initially formed intermediate at both [**1**] could be assigned to the ion-radical pair **2** formed in reaction 3.[28] The energy of **2** can be estimated from redox potentials to be ~45 kcal/mol.[29] At low [**1**], the second intermediate produced could be assigned to the radical pair (**3**) formed in reaction 4. At high [**1**], the α-amino radical could further react with **1**, reactions 6 and 7. The energy of the ion pair generated by secondary electron transfer from α-amino radical to **1** (reaction 6) is estimated to be about 20 kcal/mol.[29,30] By using thermochemical cycle calculations, the enthalpies of reaction to produce the radical pair **3** and products according to reaction 10 are estimated to be 28 and 14.5 kcal/mol, respectively.[31]

These results indicate that for the present two-component system a combination of electron, proton, and/or H-atom transfer steps permits the efficient accumulation of reactive but metastable products which persist long enough to be usable as reagents in other dark processes without the necessity for sacrificial reagents or complex catalysts. The energy stored in the overall reaction (reaction 10) is modest (at most 20% of the threshold excitation energy of **1**), and the degree of reversibility is limited by the reactivity of the amine oxidation products. Nonetheless, the results suggest attractive possibilities exist for fashioning fairly simple organic reagents in homogeneous solution into potentially powerful systems for energy transduction and associated application.

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(27) Although the maximum quantum efficiency for QH$^\bullet$ production is 2, the observed value is ~1.4. As observed in the reductions of quinones by amines and aromatic hydrocarbons,[31] this inefficiency can be attributed to the partitioning of the ion-radical pair between back-electron transfer, reaction 5, and proton transfer, reaction 4. Consequently, the PAC values are calculated assuming the quantum yields for back-electron transfer and formation of the radical pair are 0.3 and 0.7, respectively. These quantum yields are unaffected by [**1**].

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(29) This estimate uses the equation[33]

$$\Delta E(\mathbf{2}) = E_{1/2}(\mathrm{TEA/TEA^+}) - E_{1/2}(\mathbf{1}^-/\mathbf{1}) + 0.2\ \mathrm{eV}$$

and the redox potentials of **1** ($E = -0.78$ ev vs SCE) and triethylamine ($E = 0.96$ ev vs SCE)[19] in acetonitrile. The 0.2 eV term is the empirical correction value obtained by Weller for exciplexes.[33] It is assumed that the energetics of the ion pair in benzene is quite similar to that of the exciplex. In addition, studies on the quenching of quinones by amines provide support for this equation.[28]

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Cross Polarization Magic Angle Spinning Proton NMR Spectroscopy of Solids

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Recently, proton magic angle spinning (^{1}H MAS)–NMR spectroscopy of solids has been used to probe the surface sites of silica gel[1] and silicaalumina catalysts[2] as well as hydrogen environments in calcium phosphates[3] and hydrous minerals.[4] Relatively narrow ^{1}H MAS spectra can be obtained with rapid spinning alone[5] or with slower spinning speeds in conjunction with either multiple-pulse averaging[6] or isotopic dilution[7] with ^{2}H. Since ^{1}H chemical shift assignments are frequently controversial for inorganic solids and surface species and scalar couplings typically cannot be resolved, the most important problem in ^{1}H MAS–NMR spectroscopy is the development of new experiments for the assignment of resonances to specific proton environments. *We have found that cross polarization*[8] *from low-γ nuclei to protons provides a convenient means of identifying resonances corresponding to relatively immobile protons closely associated with the low-γ nuclei.*

This experiment may at first seem counterintuitive in that cross polarization is normally performed by transferring magnetization from abundant, high-γ nuclear spins (γ_I, usually ^{1}H) to the isotopically or chemically dilute low-γ nuclear spins (γ_S) which are to be observed. Since the maximum theoretical enhancement in a cross polarization experiment[8] is γ_I/γ_S, cross polarization from a low-γ nucleus to ^{1}H will result in an *attenuation* of the observable ^{1}H magnetization. Furthermore, the recycle time will be dependent on the (typically long) T_1 of low-γ, spin-1/2 nuclei, so a potentially large reduction in sensitivity could occur in the proposed experiment. Fortunately, ^{1}H MAS–NMR is so intrinsically sensitive that typically only a few dozen scans are taken, and large penalties in sensitivity will be tolerable in return for information that will facilitate spectral assignments.

Our first experiments have involved cross polarization from ^{31}P to ^{1}H (^{1}H{^{31}P} CP/MAS–NMR), reflecting our interest in phosphorus chemistry,[9] the favorable properties of ^{31}P for the first demonstration of the experiment, and the availability of assignments for ^{1}H MAS–NMR spectra of a number of solid calcium phosphates.[3] All spectra were obtained on a Chemagnetics M-100S spectrometer equipped with a home-built magic angle spinning probe double tuned for ^{1}H and ^{31}P. The observation and decoupling channels of the probe are interchangeable. Contact times of 2 ms were used for the ^{1}H{^{31}P} CP/MAS spectra reported below, and the ^{1}H and ^{31}P 90° pulse lengths were each 6.5 μs. Spinning speeds on this probe are limited to 4 kHz, so we sometimes do not obtain the ^{1}H resolution reported for spinning speeds of 8 kHz or higher. Therefore, we sometimes use isotopic dilution with ^{2}H to reduce the ^{1}H–^{1}H homonuclear dipolar couplings to manageable levels.[7] Our probe has a broad ^{1}H background signal centered at 0 ppm which is noticeable only in spectra of dilute samples. The ^{1}H{^{31}P} CP/MAS spectra have all of the characteristics of true cross polarization spectra including sensitivity to the Hartmann–Hahn match, ^{31}P T_1, ^{31}P flip angle, and cross polarization contact time. We have not observed any improvement in the resolution of ^{1}H MAS or CP/MAS spectra by

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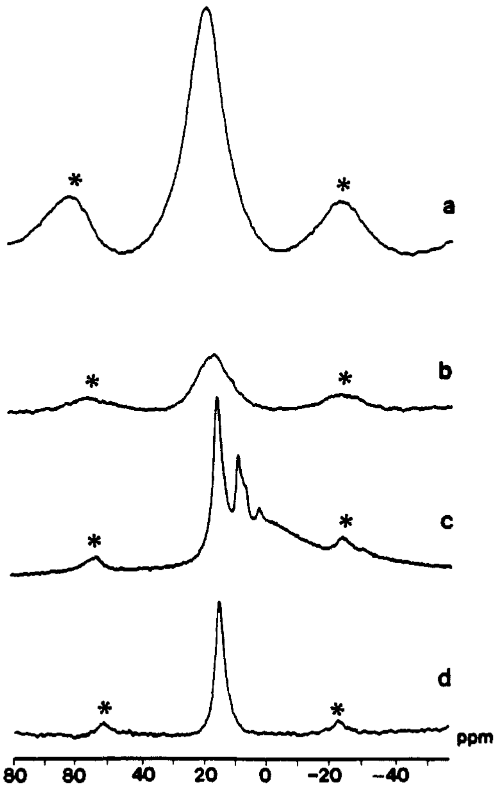

Figure 1. 99.5-MHz proton MAS-NMR spectra. (a) $CaHPO_4$, single-pulse excitation, eight scans, pulse delay 4 s. (b) $CaHPO_4$, obtained by cross polarization from ^{31}P to ^{1}H ($^{1}H\{^{31}P\}$ CP), eight scans, pulse delay 60 s. (c) Sample obtained by dissolving KH_2PO_4 in 95% D_2O/5% H_2O followed by evaporation, single-pulse excitation, 100 scans, pulse delay 1 s. (d) Same sample as in c, $^{1}H\{^{31}P\}$ cross polarization, 12 scans, pulse delay 60 s. * denotes spinning sideband.

applying ^{31}P decoupling during the observation of the ^{1}H free-induction decay.

Figure 1a is the ^{1}H MAS spectrum of natural-abundance $CaHPO_4$ obtained with conventional single-pulse excitation. This spectrum shows a single, broad isotropic peak with a chemical shift of 15.3 ppm and a series of spinning sidebands. This spectrum is essentially identical with that of a sample of monetite (natural $CaHPO_4$) reported by Yesinowski and Eckert[3] except that our spectrum is broader due to slower spinning and/or disorder in our sample. Figure 1b is the $^{1}H\{^{31}P\}$ CP/MAS spectrum of the same sample as in Figure 1a. Sufficiently long pulse delays were used to avoid partial saturation of the proton magnetization in Figure 1a or the phosphorus magnetization in Figure 1b. The signal intensity in Figure 1b is 0.25 that in Figure 1a which compares satisfactorily with the theoretical ratio $\gamma 31_P/\gamma 1_H = 0.40$, demonstrating that $^{1}H\{^{31}P\}$ cross polarization is reasonably efficient for this sample.

Figure 1c is the ^{1}H MAS spectrum of a sample that was prepared by dissolving KH_2PO_4 in 95% D_2O/5% H_2O and then evaporating to dryness. The spectrum consists of five isotropic resonances: a broad probe background signal centered at 0 ppm and four signals due to the sample. Drawing on the ^{1}H MAS-NMR shift assignments for calcium phosphates reported by Yesinowski and Eckert,[3] it is reasonable to propose the following assignments for Figure 1c. The intense peak at 14.0 ppm is assigned to the acid (POH) protons. The peak at 8.0 ppm and shoulder at 5.2 ppm are assigned to two or more types of mobile structural or surface water.[10] Finally, the small peak at 1.1 ppm is consistent with the presence of a small hydroxide impurity, probably as a distinct phase. The $^{1}H\{^{31}P\}$ CP/MAS spectrum of this sample (Figure 1d) shows a single intense isotropic resonance at 14.0 ppm, a result which is consistent with the above assignments. The other ^{1}H resonances in Figure 1c (including the probe background signal) are absent from Figure 1d, implying that they are due to protons that are either remote from ^{31}P nuclei or so mobile as to average ^{1}H–^{31}P dipolar interactions. Identical results were obtained for natural abundance samples of KH_2PO_4 except that the 14.0-ppm signal was broadened by homonuclear dipolar interactions that were incompletely averaged at 4 kHz (spectra not shown). The role of the new experiment in assigning ^{1}H MAS spectra is reminiscent of that of the interrupted-decoupling experiment[11] (also called dipolar dephasing) in ^{13}C CP/MAS-NMR spectroscopy in that both facilitate spectral assignment in return for a penalty in sensitivity.

It is easy to imagine extensions of this experiment involving, for example, $^{1}H\{^{13}C\}$ and $^{1}H\{^{27}Al\}$ CP/MAS experiments on catalyst surface species. In some cases it might be desirable to use the cross polarization process to drain transverse magnetization from protons to their associated low-γ nuclei which, in the case of quadrupolar spins, might have short $T_{1\rho}$ values. Finally, one could imagine using the standard tricks of spin gymnastics to tailor the low-γ magnetization prior to cross polarization or to exploit correlations between two or more nuclei.

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Identification of a Covalent α-D-Glucopyranosyl Enzyme Intermediate Formed on a β-Glucosidase

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The catalytic mechanism of enzymes which hydrolyse glycosides with net retention of anomeric configuration has been a subject of study for many years.[1-4] A double displacement mechanism involving an intermediate of some kind is fairly well-established, but some controversy has existed as to whether this intermediate is covalently bonded or exists as an ion pair and whether the sugar residue involved is cyclic or acyclic.[5] In this paper we describe a series of ^{19}F NMR experiments which identify the intermediate arising from hydrolysis of 2-deoxy-2-fluoro-β-D-glucopyranosyl fluoride by a β-glucosidase from *Alcaligenes faecalis* (pABG5 β-glucosidase).[6] In a separate ^{19}F NMR experiment we have proven the α-configuration of the anomeric linkage of this sugar to the enzyme.

A common feature of all the possible mechanisms is that both formation and hydrolysis of the glycosyl enzyme intermediate proceed via transition states with substantial oxocarbonium ion character. This is illustrated in Scheme I for the mechanism involving initial exocyclic bond cleavage and a covalent glucopyranosyl intermediate. We have capitalized on this in our use of 2-deoxy-2-fluoroglycosyl derivatives with good leaving groups, as mechanism-based inactivators,[7,8] since the fluorine at C-2 inductively destabilizes such positively charged transition states, slowing the rates of both glycosyl enzyme formation *and* hy-

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