mechanism involving heme release. The previously characterized heme reorientation about the α,γ-meso axis, which necessarily ruptures the Fe-His bond, occurs at a rate $\sim 10^5$ slower[13] than the presently characterized hopping.

Only the coprohemin II (**2**) metMbCN complex allowed measurement of all of the necessary parameters to quantitatively define the activation parameters and yields the values $E_a \sim \Delta H^{\ddagger} \sim 17$ kcal/mol, $A \sim 6 \times 10^{12}$, and negligible $\Delta S^{\ddagger}$. These parameters are consistent with the process occurring within the folded holoprotein without major disruption of the heme pocket structure. Previously we had shown that two isomeric forms of noncentrosymmetric dipropionate, hexamethylporphine-iron(III), with alternate orientations in the heme pocket of sperm whale metMbCN similarly interconverted via saturation transfer.[20] The similar relaxation rates for hemin methyls in all of these complexes dictate that all of these hemins rotationally hop about the iron-His bond at 1-10 s^{-1} at ambient temperture, which is some 10^4 slower than the rates qualitatively implied by the dynamic line broadening and chemical shift averaging observed[14,18] for the myoglobin complexes of the synthetic hemins without peripheral propionates. The difference in rates would translate into a lower rotational barrier by ~ 5.5 kcal/mol in the absence of propionates. Although the difference in rates in part reflects the steric influences due to the difference in the dize of the peripheral substituents, at least part of the decreased rotational rate in the present complexes is due to the importance of the propionate salt bridges in stabilizing a fixed orientation of the hemin in its pocket. Thus, it is clear that, while propionate-protein salt bridges modulate the hopping process by stabilizing a given orientation, breaking these salt bridges must represent only a fraction of the barrier to such rotations.

Comparison of Horse and Sperm Whale Mb. Comparison of the ^{1}H NMR spectra of the hemin **5** complexes of sperm whale and horse metMbCN in Figure 6A,C shows that the molecular/electronic structures are essentially indistinguishable, as found earlier for the two native proteins as well as for the proteins reconstituted with a number of different hemins.[5,11] The degree of saturation transfer, however, for essentially indistinguishable relaxation rates, is larger in the horse than sperm whale protein by a factor ~ 3, which translated directly to a similarly more rapid hopping rate and an implied lower activation energy of 0.6 kcal/mol for the horse protein. Hence, the heme pocket of horse metMbCN exhibits a reduced dynamic stability with respect to heme reorientation about the Fe-His F8 bond, relative to that in the sperm whale protein. The difference in the heme pocket between the two proteins is the nature of the donor residue in the salt bridge to the f-position propionate, which involves an Arg in sperm whale and a Lys in horse Mb.[23,31] The faster rate and implied lower activation energy to rotational hopping therefore indicate that the propionate-Lys CD3 salt bridge in horse Mb is weaker than the propionate-Arg CD3 salt bridge in sperm whale Mb. This different stability of the f-propionate salt bridge in horse and sperm whale Mb had been previously detected by distinct thermodynamic processes: the preferential formation of the invariant g-propionate-His FG3 salt bridges in monopropionate heptamethylporphine-iron(III) reconstituted sperm whale and horse Mb[19] and the lower free energy for opening the ligation channel to the protein surface, as detected by labile proton exchange,[11] which involves rupture of the f-propionate salt bridge.[32] Thus, the three types of experiments are remarkably consistent in providing a picture of a dynamically less stable closed pocket for horse than sperm whale Mb, on the basis of three divergent dynamic and structural properties, and indicated that the rate of rotational hopping of centrosymmetric hemins provides a valuable probe for detecting differential heme pocket dynamics among Mb genetic variants and synthetic point mutants.

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Characterization of Methyl α-Hydroxymethylacrylate Ether Cyclopolymer Using Nutation NMR Spectroscopy

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Abstract: A cyclopolymer of the ether of methyl α-hydroxymethylacrylate (MHMA) was synthesized from a doubly ^{13}C-labeled starting material. Nutation NMR spectroscopy was then used to determine whether the polymer contained five- or six-membered rings in the main chain generated by the cyclization step. Directly bonded ^{13}C spin pairs, which are detectable by the nutation NMR experiment, can be formed only in the case of a cyclopolymer containing five-membered rings. Experimental spectra indicate only isolated ^{13}C labels, suggesting that the cyclopolymer obtained under the current reaction conditions consists primarily of six-membered rings generated by strict head-to-tail addition of the methacrylate moieties.

We have previously described the facile synthesis of methyl α-hydroxymethlacrylate (MHMA)[1] and the unexpected dimerization of this material to its ether.[2] The cyclopolymerization of the ether to a soluble product indicated a high ratio of intramolecular over intermolecular addition of the initially formed radical intermediate.[3] Detailed investigation of the reaction mechanism of both MHMA and ether formation used ^{2}H- and ^{13}C-labeled formaldehyde (Figure 1).[4] During this study, the doubly ^{13}C-labeled cyclopolymer of the ether was obtained. While formation of either the five- or six-membered ring repeat units is possible during cyclopolymerization of the ether, a model di-

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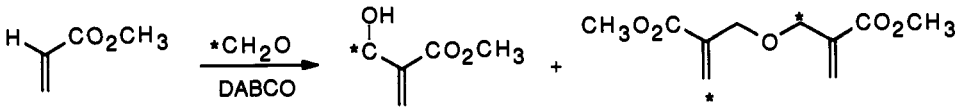

Figure 1. Synthesis of ^{13}C-labeled MHMA and one of three possible doubly ^{13}C-labeled ether dimers.

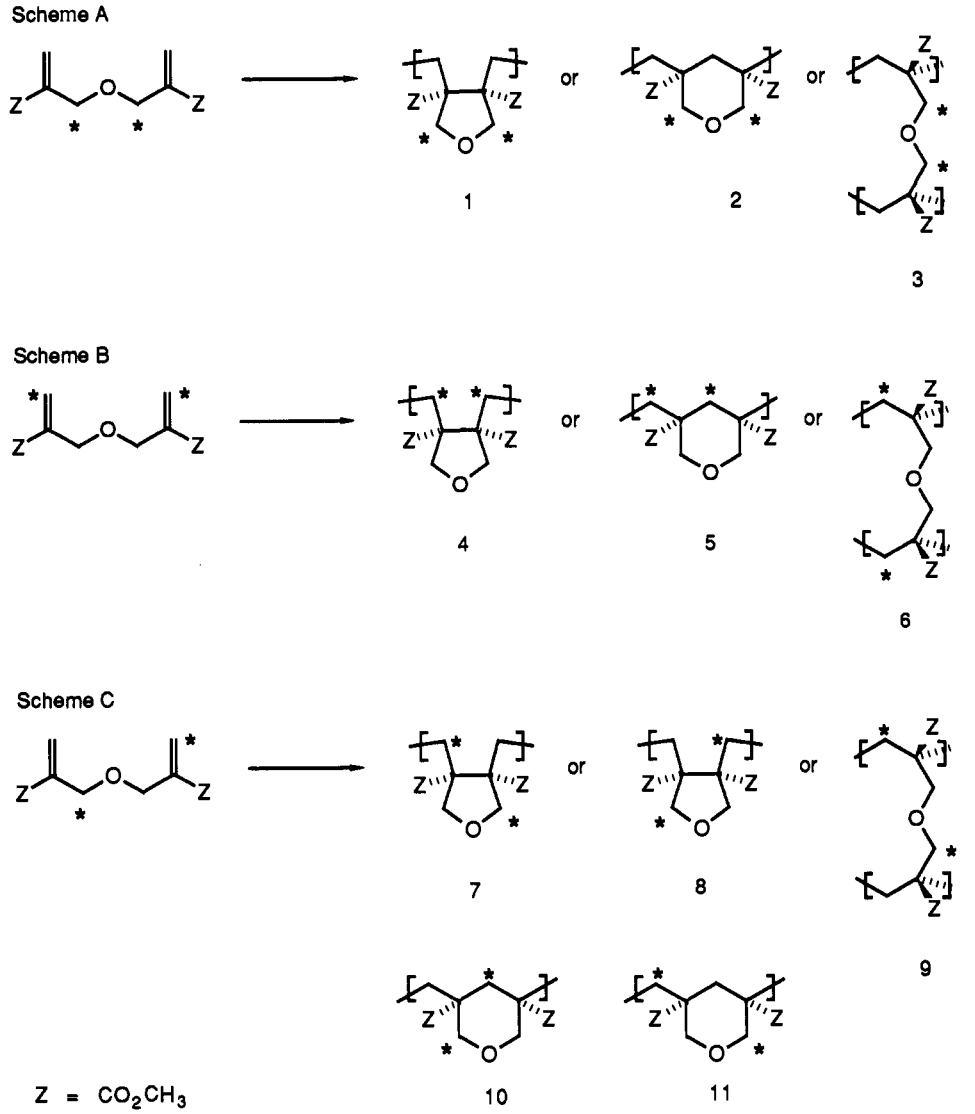

Figure 2. Possible polymerization structures from the three ^{13}C doubly labeled ether dimers.

functional monomer used by Stansbury supported six-membered ring formation.[5]

Factors which generally affect cyclopolymerization include monomer concentration, steric interaction of the ester functionality, and polymerization temperature.[6] Cyclopolymerization is the primary mechanism at low monomer concentration, while intermolecular 1,2-vinyl addition increases as the concentration is increased. The latter leads to a cross-linked, insoluble polymer even at low relative amounts of noncyclized intermolecular reaction. Ring size preference (for difunctional monomers capable of forming more than one cyclic repeat unit) is usually a sensitive function of all reaction variables with temperature playing a key role in reducing selectivity by overcoming differences in activation energies. Diacrylates are reported to prefer six-membered ring formation; however, probably owing to a combination of transition-state orientation effects and radical stability differences combined with reversibility of the head-to-head addition product, the five-membered ring may be formed.[6]

^{13}C nutation NMR spectroscopy has been shown to be useful in determining the bond lengths of directly bonded ^{13}C labels in amorphous and polycrystalline solids.[7] Using this technique, the dipole–dipole coupling is obtained from the Fourier transform of a forced precession (nutation) of nuclear magnetization excited by sudden application of a resonant rf field.[8] The nutation sequence eliminates the chemical shift anisotropy effect so that the dipolar interaction information can be acquired. This technique has been used in the determination of the Ziegler–Natta polymerization mechanism of acetylene, where it was demonstrated that double bonds in the polymer derive directly from the monomer triple bond without any rearrangement during or after polymerization.[9] A requirement for the nutation experiment is that the sample contain directly bonded ^{13}C spin pairs (^{13}C–^{13}C).

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Selection is achieved by enrichment with labeled material, followed by dilution (if necessary) with unenriched material to avoid interference from long-range spin coupling. The obtained spectrum will be a Pake doublet with a splitting that is directly related to the C–C bond length.

Figure 2 shows polymerization schemes leading to the cyclopolymer and 1,2-addition products, where **1** thru **11** are possible repeat groups for (generally) head-to-tail placement. By examining the routes to the cyclopolymer from the labeled monomer, only the mechanisms which form five-membered rings in the polymer will provide a directly bonded ^{13}C spin pair through intramolecular head-to-head addition. Repeat groups **5**, **6**, **9**, and **11** could undergo head-to-head placements which would lead to adjacent ^{13}C labels, although the probability of such head-to-head placements (other than within five-membered ring units) is expected to be small because of steric interactions. The highest concentration of adjacent labels occurs if only five-membered rings are formed. The relative probability of repeat groups **1**, **4**, **7**, and **8** is 1:1:1:1 based on complete equilibration of labels in the diacrylate ethers as was demonstrated in the previous solution NMR study.[4] Statistical analysis of all possible combinations of five-membered ring linkages indicates that a maximum of 25% of the total would contain adjacent labeled pairs. The nutation experiment would give a Pake doublet if any one of these combinations is found in the polymer to any extent. Alternatively, if the polymer is composed only of six-membered rings and few head-to-head linkages, only a single central peak will be seen in the NMR spectrum.

Experimental Section

Methyl acrylate, sodium acetate, sodium acetate-$^{13}C_2$, adamantane, and 1,4-diazabicyclo[2.2.2]octane (DABCO) were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used as received; ^{13}C-labeled paraformaldehyde was obtained from MSD isotopes (Montreal, Canada).

The reaction involving MHMA formation was carried out in a 5-mm NMR tube. Details of the reaction conditions are given elsewhere.[4] The acrylate and paraformaldehyde were introduced into the NMR tube with 3 wt % DABCO and a DMSO-d_6 capillary insert as the NMR lock and reference. The NMR tube was placed in an oil bath maintained at 70 °C. At selected intervals, it was removed from the oil bath and allowed to cool to room temperature; an NMR spectrum was acquired. The data from these spectra confirmed the synthesis mechanism previously postulated[2] and described in detail elsewhere.[4]

At longer reaction times, when ether content approached 80% of the total reaction mixture, a second phase formed in the bottom of the NMR tube containing essentially pure ether. This was due to the immiscibility of the diacrylate ether with the water-miscible MHMA in the presence of water. Complete label equilibration has occurred in the ether dimers giving a 1:1:2 ratio of species shown in Schemes A–C (Figure 2). Further heating led to spontaneous polymerization of the dimer ether layer despite the presence of DABCO which is an excellent radical inhibitor. The DABCO was constrained to the MHMA aqueous phase which did not polymerize. The insoluble but swellable cyclopolymer was washed thoroughly with chloroform and dried in a vacuum oven.

NMR Measurements. ^{13}C CP/MAS NMR spectra were acquired on a Bruker MSL-200 spectrometer operating at a frequency of 50.32 MHz for carbon using a Bruker cross polarization, magic angle spinning (CP/MAS) probe. The nutation experiment was run on a Bruker MSL-300 spectrometer at a frequency of 75.46 MHz for carbon. A nonspinning cross-polarization probe was used to obtain the CP-nutation NMR spectra. The experiment performed was a modification of the original technique of Kendrick and Yannoni.[10,11] To compensate for chemical shift and rf inhomogeneity effects, the nutation pulse train was changed to a modified Carr–Purcell–Meiboom–Gill (CPMG) spin-echo train with a 7.2-μs 180° pulse width and 11.5-μs window in the pulse train.[12,13] The modified CPMG pulse train involved reversing the rf phase of alternating pulse pairs.[14] The original work by Kendrick and Yannoni was done at a much lower field, where the chemical shifts are proportionately smaller. The sodium acetate standard for the nutation experiment was made by diluting the labeled material to a concentration of 3.7 wt % with natural abundance salt. Such dilution is only necessary if peak broadening due to *inter-* rather than intramolecular coupling is significant.[10] Broadening caused by the experimental procedure itself can be evaluated with an unenriched sample (adamantane in this case) which gave an acceptable line width of 104 Hz.

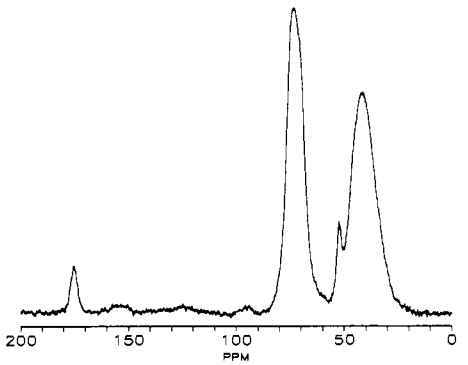

Figure 3. ^{13}C CP/MAS NMR spectrum of the ^{13}C doubly labeled cyclopolymer.

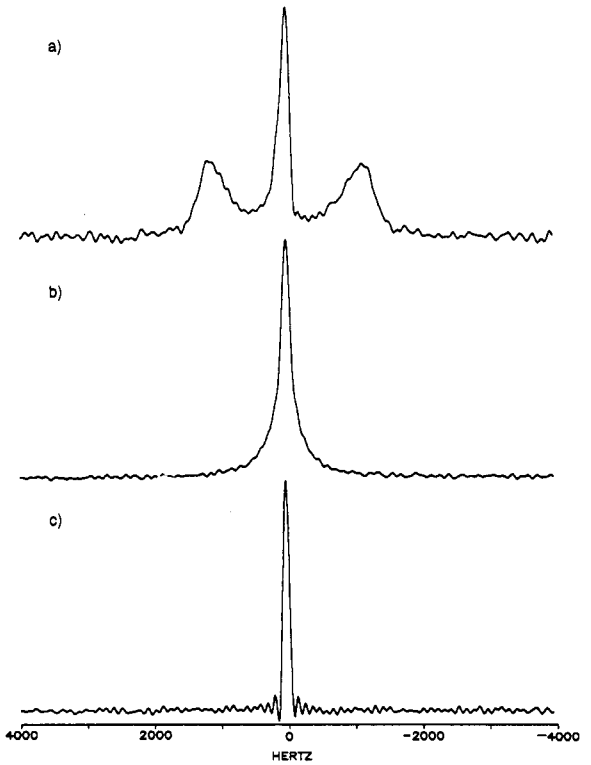

Figure 4. CPMG nutation NMR spectra of (a) sodium acetate standard with 3.7% ^{13}C doubly labeled material, (b) ^{13}C labeled MHMA ether cyclopolymer, and (c) unlabeled adamantane.

Results and Discussion

The cyclopolymer swelled but was not soluble in common organic solvents, suggesting that a small amount of cross-linking had occurred due to the high monomer concentration. Stansbury reported similar behavior for this material.[5] Efficient vinyl polymerization is evident from the absence of pendant vinyl peaks in the ^{13}C CP/MAS NMR spectrum (Figure 3). It is also clear that both backbone and ring carbons are ^{13}C labeled by the greater intensity of the corresponding peaks (41 and 74 ppm, respectively) compared with NMR spectra of unlabeled α-hydroxymethylacrylate ether cyclopolymers.[3]

Figure 4 shows the nutation NMR spectra of the sodium acetate standard, the MHMA cyclopolymer, and unlabeled adamantane. The standard gives the expected Pake pattern with a splitting of 2317 Hz due to ^{13}C directly bonded spin pairs. The central peak is due to isolated, noncoupled, natural abundance ^{13}C spins. Adamantane was used as a test of line-width resolution. The

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plastic crystal nature of adamantane should introduce motion to give a narrowed peak. The doubly labeled cyclopolymer gives a single peak with a line width of 176 Hz.

The nutation experiment has been shown to determine bond lengths to an accuracy of ~1% for directly bonded ^{13}C nuclei. For this particular project, the question was not to determine bond lengths, but to find whether any directly bonded labeled positions existed. From rough estimates determined by spectral addition, the limit of detection appears to be at a directly bonded spin-pair concentration of less than 5% of the total ^{13}C concentration. Thus, few or no adjacent ^{13}C labels exist confirming predominant formation of the six-membered ring repeat unit during cyclopolymerization under the conditions used here.

Summary

Nutation NMR was used to confirm the structure of a ^{13}C doubly labeled cyclopolymer. A labeling experiment used to give information regarding the reaction mechanism for α-hydroxymethylacrylate formation and dimerization also provided a serendipitous synthesis of the labeled cyclopolymer.[4] The cyclopolymer was shown to be composed of primarily six-membered rings through the absence of spin-pair coupling. These results confirm the potential of the nutation NMR experiment for mechanism and structure elucidation in characterization of suitably labeled materials.

Acknowledgment. This research was supported in part by the Petroleum Research Foundation, administered by the American Chemical Society, and the Division of Materials Research of the National Science Foundation through Grant DMR-8620138. We also gratefully acknowledge the Department of Defense instrumentation grant through the Office of Naval Research, which was used to purchase the Bruker MSL-200 NMR spectrometer.

Registry No. $H_2C{=}CO_2CH_3$, 96-33-3; $^{13}CH_2O$, 3228-27-1; $H_2C{=}C(CO_2CH_3)^{13}CH_2OH$, 131237-17-7; $H_2{}^{13}C{=}C(CO_2CH_3)CH_2O^{13}CH_2C(CO_2CH_3){=}CH_2$, 131237-18-8; $H_2{}^{13}C{=}C(CO_2CH_3)CH_2O^{13}CH_2C(CO_2CH_3){=}CH_2$ (homopolymer), 131237-30-4; $H_2C{=}C(CO_2CH_3)CH_2OCH_2C(CO_2CH_3){=}CH_2$ (homopolymer), 109669-57-0.

Solvent Effect on the Anomeric Equilibrium in D-Glucose: A Free Energy Simulation Analysis

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Abstract: The equilibrium between the α and β anomers of D-glucopyranose in aqueous solution has been investigated by free energy simulations that permit a separation of the intramolecular and intermolecular contributions to the free energy difference. The simulations correctly predict that the free energy difference between the two forms in aqueous solution is small; the calculated free energy difference, $\Delta G(\beta \rightarrow \alpha)$, is -0.31 ± 0.43 kcal/mol, in comparison with the experimental value of 0.33 kcal/mol. The calculated free energy difference is the result of near cancelation of two larger, statistically significant contributions, i.e., an intramolecular electrostatic term favoring the α anomer and an intermolecular solute–solvent interaction term favoring the β anomer. This result supports the conjecture that solvation stabilizes the β anomer in water. There is a large difference in the intramolecular contribution to the anomeric equilibrium calculated in solution from the free energy simulation and the gas-phase minimum; this suggests that conformational averaging, modulated by the solvent, is significant even for the internal terms. An examination of the rotamer distribution in the hydroxymethyl side chain shows that the trans, gauche conformer is strongly disfavored in aqueous solution, in accord with experiment.

Introduction

The origin of the anomeric equilibrium between the α (axial) and β (equatorial) cyclic forms of simple sugars, such as glucose, is one of the most widely studied questions in carbohydrate chemistry.[1] The equilibrium concentrations for the various tautomers in aqueous solution have been measured, and empirical "stability factor" rules have been developed to rationalize these equilibria.[2] For D-glucose in aqueous solution at 293 K, the anomer distribution is 36% α-D-glucopyranose:64% β-D-glucopyranose, as measured by optical rotation and NMR experiments; only negligible amounts of the linear and furanoid tautomers are present under these conditions. The relative contributions of the gas-phase intramolecular potential and solvation effects to the observed α and β equilibrium distribution remain unclear.[3]

It has long been assumed that the anomeric equilibrium is related to other aspects of the anomeric effect.[4,5] This term refers to the observation that, for *substituted* sugars (such as the methyl pyranosides), the α anomer is more stable than the β anomer and that there are characteristic structural features that are configuration-dependent. For example, both C–O bond lengths involving the anomeric carbon atom C1 (see Figure 1) are shortened relative to more typical C–O bond lengths, with the C1–O1 bond longer than the C1–O5 bond for the preferred α anomer;[6] corresponding results are observed for the bond angles. In the most commonly accepted explanation, the anomeric effect is assumed to result from a back-donation of electrons from the lone-pair orbitals of the oxygen atom to the antibonding σ* orbital of the adjacent C–O bond, with this overlap being more pronounced for α configurations. Although some doubts have been expressed about this explanation,[7] it is generally consistent with the results of quantum

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