There are four sources of information which shed some light on the effects of rotational isomerism about the $CH_3C(=S)SC-H_2CH_2NH$ linkages on the RR spectrum of thioacetyl-CoA. These are the variable temperature studies on the RR spectrum of $CH_3C(=S)SCH_2CH_3$,[5] the vibrational analysis of $CH_3SC-H_2CH_2CH_3$ by Nogami et al.,[6] the X-ray crystallographic-vibrational analysis of *N*-benzoylglycine ethyl dithio esters,[7] and a conformational analysis of (thioacyl)papain intermediates at low temperature.[8] These studies taken together suggest that the intense 584-cm^{-1} band in Figure 1a is due to a gauche form about the $CH_3C(=S)S—CH_2CH_2NH$ single bond in thioacetyl-CoA while the shoulder at 606 cm^{-1} attests to a minor population of a trans rotamer. Asymmetry of the 681-cm^{-1} ν_{S-C} band on the high-frequency side suggests that a weak band is present near 700 cm^{-1}, which would also emanate from the trans rotamer. More evidence for multiple rotamers is seen in the broad nature of the 1193-cm^{-1} peak, which may contain two or more unresolved components. Our analysis is probably too simplistic, given the potential for isomerism about the $CH_2—CH_2$ and $CH_2—NH$ bonds in the $CH_3C(=S)SCH_2CH_2NH$ moiety of thioacetyl-CoA. However, the evidence in Figure 1a for more than one rotamer is clear, and considering the conclusion from the work of Nogami et al.,[6] and from spectra-structure studies on dithio esters,[5,7,8] it is likely that trans and gauche isomers about $S-CH_2$ are involved.

The 324-nm-excited RR spectrum of thioacetyl-CoA bound to citrate synthase is shown in Figure 1b. Under the conditions employed, ca. 90% of the ligand is bound to the enzyme. The spectrum in Figure 1b offers overwhelming evidence for the presence of more than one bound form. Following the analysis undertaken above, the 580- and 682-cm^{-1} bands suggest that a major population of a gauche rotamer about the $S-CH_2$ linkage is present bound to the enzyme. However, the increase in intensity of the 606-cm^{-1} peak, compared to that for the free ligand (Figure 1a), indicates that an increased population of a trans-like rotamer is bound to citrate synthase, too. This conclusion is supported by the presence of the small but reproducible ν_{S-C} band near 702 cm^{-1}.

The observation of two bound conformers of thioacetyl-CoA is consistent with X-ray crystallographic analysis, which indicates that the acetyl portion of acetyl-CoA is bound heterogeneously in stable ternary complexes with D- or L-malate.[9] On the basis of the crystallographic data, Karpusas et al.[2] have proposed a reaction mechanism which involves rotation about the $CH_3C(=O)—SCH_2$ bond to allow the acetyl CH_3 group to attack (as the CH_2 enol) the carbonyl group of oxaloacetate. This necessarily requires that the acetyl methyl group can occupy at least two positions in the active site and may account, at least in part, for the observation of two bound conformers of thioacetyl-CoA from the RR studies and for the conformational heterogeneity of the acetyl group suggested by the crystallographic studies. The rotation discussed by Karpusas et al.[2] will result in the unfavorable movement of atoms out of the ester plane. However, repositioning of the acetyl methyl group can be equally accomplished via the energetically less expensive rotation about the $CH_3C(=O)S—CH_2CH_2NH$ bond proposed to explain the observation of two bound conformers of thioacetyl-CoA from the RR studies.

An alternative proposal is that there are two different conformers induced by different enzyme conformations. Two significantly different crystal structures of citrate synthase exist with CoA analogues bound, a C_2 symmetry form which crystallizes in the presence of carboxymethyl-CoA and oxaloacetate[9] and a $P4_32_12$ crystal form which crystallizes in the presence of *S*-acetonyl-CoA and oxaloacetate.[10] In any event the lability of the $CH_3C(=S)$ protons to solvent exchange indicates that the methyl group is close to a base which facilitates enol formation in one of the conformers. The extremely slow condensation requires that the resulting enol be poorly positioned with respect to the C_2 of oxaloacetate.

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NMR Evidence for DNA Bound Water in Solution

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X-ray crystallographic studies have long provided evidence for the presence of water molecules intimately associated with proteins and nucleic acids.[1,2] DNA crystal structures show the presence of a spine of hydration[3] and various other hydration features believed to stabilize the B-form. Historically, this has been a difficult phenomenon to verify in solution.[4-7] Recently it has been demonstrated that protein associated water molecules may be detected by NMR.[8-11] The success of this work has led us to investigate the hydration of nucleic acids in solution with the same methodology.[8,11] We provide evidence for the spine of hydration, in which water molecules have a relatively long lifetime, and also for other water molecules less strongly associated with nucleic acids in solution.

In solution NMR studies, detection of the NOE in multidimensional experiments is crucial to structure determinations. In the study of bound water by this technique, two major problems arise. The first is suppressing the intense water resonance without obscuring the NOE between water and DNA protons. The second is identifying interactions that arise directly from cross relaxation between DNA and water protons rather than from chemical exchange.[7] We have used methodology developed by Wüthrich and co-workers for investigating protein hydration,[9,11] in which a combination of NOESY and ROESY experiments is used to detect cross relaxation and distinguish it from chemical exchange.

We have recorded a series of NOESY and ROESY spectra[12] of the DNA dodecamer CGCGAATTCGCG (synthesized and purified with standard methods) at 5, 10, and 25 °C. At 10 °C

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(12) Spectra were recorded on a Bruker AMX-600 spectrometer using the solvent suppression exactly as described by Otting et al.[9]

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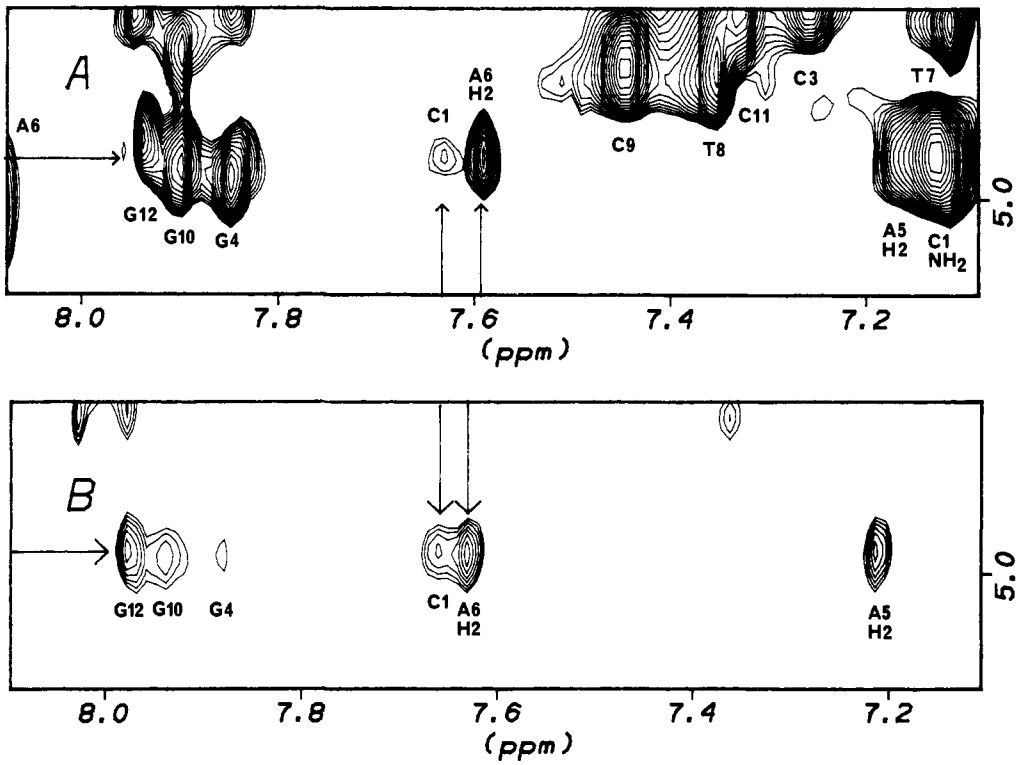

Figure 1. Identical regions of the (A) NOESY (200 ms mixing time) and (B) ROESY (50 ms mixing time) spectra recorded at 10 °C of a solution of 6 mM DNA at pH 6.0. (The sample was dialyzed 2 days against water, dried, and dissolved in 90% H_2O/10% D_2O.) The water chemical shift is indicated by the horizontal arrows. The vertical arrows indicate DNA–water crosspeaks (see text). Aromatic proton chemical shifts (H8 of purines, H6 of pyrimidines) are labeled by residue. Crosspeaks, other than to water, are either intraresidue or sequential peaks involving the sugar H3′ protons.

the spectra are particularly simple to interpret as no DNA protons resonate at the water chemical shift. The presence of an NOE at the water chemical shift therefore identifies a potential DNA–water interaction. Using ROE[13] it is possible to determine whether other nearby protons are exchanging with solvent, which could then lead to an exchange transferred NOE.[7] Because the sign of the NOE changes from negative to positive as the exchange rate with the bulk solvent increases, we can detect two distinct types of hydration water. Water that exchanges with the bulk solvent at a rate that is slow on the molecular tumbling correlation time scale (a few nanoseconds) gives rise to a negative NOE. We will call this a type I interaction. Exchange rates that are fast on the correlation time scale give rise to a positive NOE (a type II interaction). We observe several interactions of each type, two of type I are shown in Figure 1.

Water molecules in the type I exchange regime are detected near DNA protons: C1 H6, A6 H2, A5 H2, A6 H1′, and T7 H1′. All but the C1 H6 are minor groove protons.[14] The spine of hydration, first identified in this DNA dodecamer, lies in the minor groove in the AT region.[15] At least four water molecules are seen in crystal structures bridging the A-N3 and T-O2 positions across the floor of the minor groove by hydrogen bonding. The exact number and location of the water molecules cannot be determined from our data. However the observations are consistent with a spine of hydration in the minor groove which remains bound for a time longer than required for molecular tumbling. We find no evidence of slow exchange on the NMR time scale as the bound water has the same chemical shift as the bulk solvent. In proteins, an upper limit on the exchange lifetime of several type I (negative NOE) water molecules in the interior of the protein has been found to be around 20 ms (4 °C).[16] The more solvent exposed DNA bound water is almost certainly even shorter lived. Water in the spine region of the minor groove remains in the type I exchange regime through 25 °C as the NOE to the A6 H2 proton is still negative at this temperature (data not shown). There is evidence that type I bound water is also present at the ends of the helix. Both the C1 H1′ and the G12 H1′ show positive ROEs to the water; only the C1 position shows a clear negative NOE. The G12 H1′ is in a crowded region of the spectrum and may have a weaker NOE. These results are in agreement with the reported ribbons of water hydrating the ends of various B-form helices in crystal structures.[17] It is interesting to note that the end of the helix which is expected to be more open and solvent exposed, still shows evidence for type I ordered water at 10 °C.

Both the spine of hydration and ribbon structures are implicated in stabilizing the B-form of DNA. Spectra collected at higher ionic strength both by us (data not shown), and others,[7] show several formerly type I water interactions have entered the type II regime. Thus there appears to be a salt dependence on the kinetics of hydration water exchange. Many DNA protons in the major groove show type II interactions with the water regardless of ionic strength indicating they are hydrated by very loosely associated water molecules. These include the G12 H2′, T7 CH_3, and T8 CH_3 protons. This is also in general agreement with current ideas on the solvation of DNA.[18]

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