blization with a mismatch near the 5'-terminus may be balanced by intramolecular intercalation, but a similar effect would not be available at the 3'-end. The mismatch results, therefore, are consistent with intramolecular intercalation by the dppz complex tethered to the 5'-terminus.

The results taken together establish that an oligonucleotide functionalized with a dppz complex of ruthenium can be used to target single-stranded DNA in a sequence-specific fashion. This complex could be extremely valuable in the development of novel hybridization probes both for heterogeneous and homogeneous assays.

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Multiple Forms of Thioacetyl Coenzyme A Binding to Citrate Synthase. Resonance Raman Evidence

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Citrate synthase catalyzes the stereospecific condensation of the acetyl group from acetyl coenzyme A (acetyl-CoA) with oxaloacetate to generate citrate. The reaction occurs in a ternary complex wherein both acetyl-CoA and oxaloacetate are bound and is thought to proceed via generation of the enol form of acetyl-CoA, which then acts as a nucleophile to attack the C_2 carbonyl carbon of oxaloacetate.[1,2] A reactive ternary complex can be generated where acetyl-CoA is replaced by thioacetyl-CoA, a substrate analogue of acetyl-CoA in which the C=O group of the acetyl moiety is replaced by C=S. The enzyme catalyzes the exchange of the acetyl methyl protons of thioacetyl-CoA with solvent presumably through the required enol intermediate. While the exchange reaction is 250-fold faster for thioacetyl-CoA than for acetyl-CoA, k_{cat} for the condensation of thioacetyl-CoA with oxaloacetate is only ca. 0.0002% (pH 7.4) that of acetyl-CoA.[3] Thus a slowly-reacting ternary complex can be generated with an acetyl-CoA analogue which, because of its dithio ester moiety, is amenable to resonance Raman spectroscopic characterization.

In order to gain further insight into the details of acetyl-CoA binding, we have obtained resonance Raman (RR) spectra of thioacetyl-CoA bound in a ternary complex with oxaloacetate to citrate synthase. The dithio chromophore has an absorption band near 306 nm,[4] and excitation into this electronic transition gives rise to RR features associated with normal modes of the $H_3CC(=S)SCH_2CH_2NH$ moiety, analysis of which provides information on the rotational isomers involving this group. The data show that the bound form of thioacetyl-CoA is present as at least two rotational isomers.

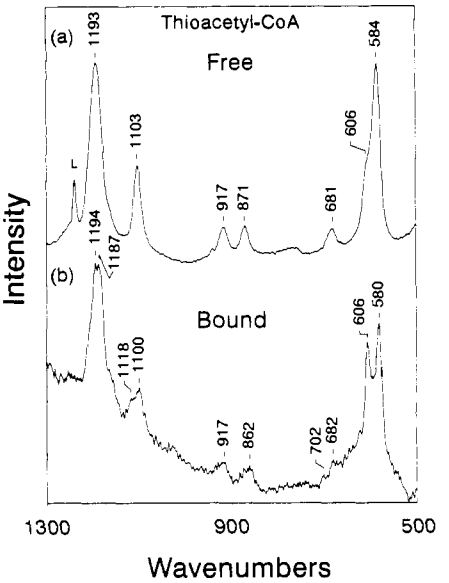

Figure 1. Resonance Raman (RR) spectra of free and bound thioacetyl-CoA were obtained at room temperature using 180° back-scattering geometry, 70-mW 324-nm Kr^+ laser excitation, 7-cm^{-1} spectral resolution, and published detection procedures.[11] Spectra were accumulated in memory blocks each 4 × 6 s, and identical data sets were co-added. (a) Resonance Raman spectrum of thioacetyl-CoA 0.9 mM in 0.1 M Tris pH 7.5; 48-s acquisition time. (b) Resonance Raman spectrum of thioacetyl-CoA bound to citrate synthase in 0.1 M Tris pH 7.5; 72-s acquisition time. For RR data collection the sample contained 0.65 mM citrate synthase, 2 mM oxaloacetate, and 0.2 mM thioacetyl-CoA. Using K_M = 60 μM for thioacetyl-CoA,[3] it is estimated that ca. 90% of the dithio ester is bound. A RR spectrum of enzyme/oxaloacetate in the absence of chromophore has been subtracted. Citrate synthase (EC 4.1.3.7) (lot numbers 77F-9560, 69F 9585) from Sigma Chemical Co. was purified by ion-exchange HPLC. Two milliliters of the enzyme suspension in ammonium sulfate was centrifuged in an Eppendorf microfuge. The pellet was dissolved in 1 mL of 15 mM Tris pH 8.0 and chromatographed on two (2 × 0.5 mL) Sephadex G-25 (fine) 5-mL "centrifuge" columns. Subsequently the protein (1 mL, 25 mg) was chromatographed on a Tosohaas DEAE 5PW column (21.5 mm × 15.0 cm) attached to a Gilson HPLC. The buffers used were 15 mM Tris-HCl pH 8.0 (A) and 15 mM Tris-HCl, 0.5 M KCl pH 8.0 (B). From t = 0–15 min, a gradient of 0–17% buffer B was run at 4 mL/min, the flow rate was reduced (over 1 min) to 2 mL/min, and the protein eluted isocratically with 17% B (retention time 50 min). The main fractions from the protein peak at 50 min were pooled and concentrated (Amicon Centricon-10). During concentration the protein was exchanged into 0.1 M Tris-HCl pH 7.5. Citrate synthase concentration was determined using ϵ_{280} = 70 000 M^{-1} cm^{-1} per active site. Immediately before the resonance Raman experiments, a 200 mM oxaloacetic acid solution was prepared in 0.1 M Tris-HCl at pH 7.5. Thioacetyl-CoA was dissolved in 0.1 M Tris-HCl pH 7.5 and its concentration determined using ϵ_{306} = 11 000 M^{-1} cm^{-1}.

In Figure 1 the 324-nm-excited RR spectrum of thioacetyl-CoA contains bands due solely to the (thioacetyl)thio group. The spectrum of the unbound ligand (Figure 1a) closely resembles the RR spectrum of ethyl dithioacetate reported by Teixeira-Dias et al.,[4] and band assignments can be made by analogy to their analysis. Most bands are due to complex motions, and simple group frequency assignments do not apply. One exception to this may be the band at 681 cm^{-1}, which has a high degree of S-CH_2 stretching character. Teixeira-Dias et al.[4] identified the intense feature at 1193 cm^{-1} as having a significant (but not predominant) $\nu_{C=S}$ contribution and the 1103-cm^{-1} feature as due to a mode with ν_{C-C} (CH_3—C(=S) bond) and ν_{C-S} (C(=S)—S bond) contributions, and the 917- and 871-cm^{-1} bands were assigned to CH_3—C(=S) rocking modes. The intense band at 584 cm^{-1} is highly mixed in character, with contributions from ν_{C-S}, $\nu_{C=S}$, and ν_{C-C} (CH_3—C(=S) bond). These assignments are supported by a 29-cm^{-1} shift to lower frequency in the 1193-cm^{-1} band and smaller shifts in the other bands in the [1-^{13}C]thioacetyl-CoA RR spectrum (unpublished work, this laboratory).

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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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