Table I. Best Fits to Hg-MerR EXAFS[a]

sample	condition	fit[b]	R, Å	N_S	$\Delta\sigma^2 \times 10^3$, Å^2	R, Å	N_N	$\Delta\sigma^2 \times 10^3$, Å^2	F[c]
1	ppt	S	2.42	*d*					
2	ppt	S	2.43	3	−2.3				4.5
		S + N	2.42	3	0.5	2.36	1	−7.5	2.7
3	lyoph	S	2.43	3	0.9				4.4
		S + N	2.43	2	−1.0	2.27	2	8.6	4.0
4	ppt	S	2.43	3	−0.8				4.5
		S + N	2.44	2	−3.1	2.21	2	5.5	2.4
average	-	S	2.43	2	−2.7				3.8
		S	2.43	3	−0.6				2.5
		S	2.43	4	+1.2				4.2
		S + N	2.44	2	−2.6	2.25	2	10.2	2.2

[a] Fits used a range of fixed integer coordination numbers (N) with bond length (R) and Debye-Walter factor ($\Delta\sigma^2$) as freely variable parameters. [b] Fits using sulfur only (S) and using sulfur + nitrogen (S + N) are reported. For samples 2-4, tabulated fits are for values of N giving the best fit for a given fit type (S or S + N). [c] Goodness of fit $F = [(\chi_{calcd}k^3 - \chi_{exptl}k^3)^2/(\mathrm{NPTS} - 1)]^{1/2}[(\chi k^3)_{max} - (\chi k^3)_{min}](100\%)$, where χ_{exptl} and χ_{calcd} refer to measured and simulated EXAFS and max and min refer to the maximum and minimum in the weighted, experimental data. [d] Data were extremely noisy and could be fit with one or two shells of sulfur. Subsequent data gave the same average Hg-S distance but did not confirm the two-shell HgS_2S_2' model.

number. However, the strong dependence of Hg-S bond length on coordination number clearly excludes simple HgS_2 or HgS_4 structures. Hg-S bond distances in mononuclear $Hg(SR)_2$ complexes are found from 2.32 to 2.36 Å (R_{av} = 2.34 Å) while mononuclear $Hg(SR)_4$ complexes exhibit Hg-S distances from 2.50 to 2.61 Å (R_{av} = 2.54 Å).[19] Although there are few crystallographically characterized mononuclear $Hg(SR)_3$ complexes, the examples that are known have Hg-S distances from 2.40 to 2.51 Å (R_{av} = 2.44 Å), consistent with the distance found in MerR.[9,19,20] A Fourier transform of the averaged Hg-MerR data from samples 2-4 is shown in Figure 1B. No detectable contribution from scatterers at >2.5 Å is observed for Hg-MerR; thus there is no EXAFS evidence for secondary bonding interactions.[21]

We see no evidence for two unresolved shells of scatterers in MerR. Although it is difficult to rigorously exclude contributions from a weak scatterer (Hg-N or Hg-O) in the presence of strong Hg-S EXAFS, two-shell fits (Hg-S + Hg-N) give only modest improvement over one-shell Hg-S fits. Improvement is only seen for the noisiest data, and the refined Hg-N distances vary from sample to sample. This argues against Hg-N ligation, although additional structural data on mercuric complexes having mixed sulfur/nitrogen ligation are necessary. No improvement is observed for two-shell (Hg-S + Hg-S′) fits. Chloride or exogenous buffer thiol ligation are unlikely on the basis of spectrophotometric titrations and gel filtration studies using radiolabeled thiols;[9] thus the EXAFS results suggest coordination by three endogenous sulfur ligands.

Chemical modification experiments corroborate this model. DTNB titrations[22] repeated in triplicate on the apoprotein reveal that 6.3 (SD = 0.3) of 8 cysteines per dimer are accessible, consistent with results of Schewchuk et al.[7] In contrast to that report, titration of the Hg-MerR samples prepared as described for EXAFS reveal that 3.2 (SD = 0.4) cysteines per dimer are available for reaction with DTNB, yielding a net protection of three cysteines per dimer in the Hg-protein.

One of the striking attributes of MerR is its avidity for mercuric ion; the binding is stoichiometric for nanomolar protein and Hg(II) concentrations, even in the presence of 10^5-fold excess thiol. The tridentate model for Hg-MerR coordination suggests a structural and thermodynamic rationale for the ability of this receptor to discriminate between Zn(II), Cd(II), and Hg(II) while maintaining a nanomolar sensitivity to the latter.[5] Work aimed at further characterizing MerR metal binding is in progress.

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(21) Weak interactions (e.g., R > 3 Å) are often not detectable by EXAFS; however, the 2.86-Å Hg-thiolate interaction in $[NEt_4][Hg_3(SCH_2CH_2S)_4]$ (Henkel, G.; Betz, P.; Krebs, B. *J. Chem. Soc., Chem. Commun.* **1985**, 1498-1499) is readily detectable (data not shown), suggesting that any outer-shell thiolates in MerR are more than ca. 2.8 Å from the Hg. Such weak secondary bonding interactions would not lengthen the two-coordinate Hg-S bond distance (in a hypothetical HgS_2S_n' structure) sufficiently to account for the observed first-shell Hg-S distance. EXAFS cannot address the question of secondary bonding interactions at longer Hg-S distances. For further discussion of the secondary bonding interactions in mercuric thiolate complexes, see ref 19.

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Recognition of Mixed-Sequence Duplex DNA by Alternate-Strand Triple-Helix Formation

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Oligodeoxyribonucleotide-directed triple-helix formation offers a chemical approach for the sequence-specific binding of double-helical DNA that is 10^6 times more specific than restriction enzymes.[1,2] Because triple-helix formation by pyrimidine oligonucleotides is limited to purine tracts, it is desirable to find a general solution whereby oligonucleotides could be used to bind all four base pairs of intact duplex DNA (37 °C, pH 7.0). Approaches toward such a goal include the following: the search for other natural triplet specificities, such as G·TA triplets;[1f] the design of nonnatural bases for completion of the triplet code; the incorporation of abasic residues for nonreading of certain base

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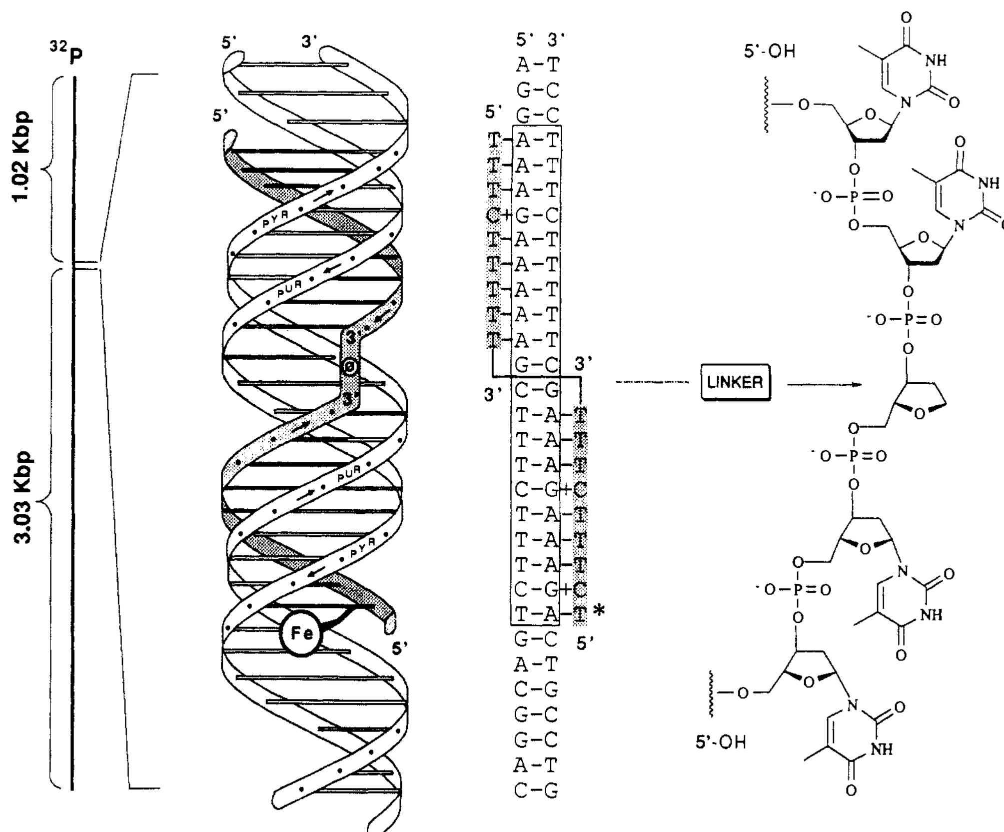

Figure 1. Linearized ^{32}P-labeled plasmid (4.05 kbp) showing double-strand-cleavage products 1.02 and 3.03 kbp in size (left). Simplified illustration of an alternate-strand triple-helix complex in which oligonucleotide **1** binds to a 5'-(purine)$_9$NN(pyrimidine)$_9$-3' sequence (center). The bidirectional pyrimidine oligonucleotide binds parallel to both purine strands of the DNA duplex site in opposite orientation. Each subunit of the oligonucleotide comprises nine pyrimidine nucleotides linked with a 3'–3' phosphodiester and 1,2-dideoxy-D-ribose.

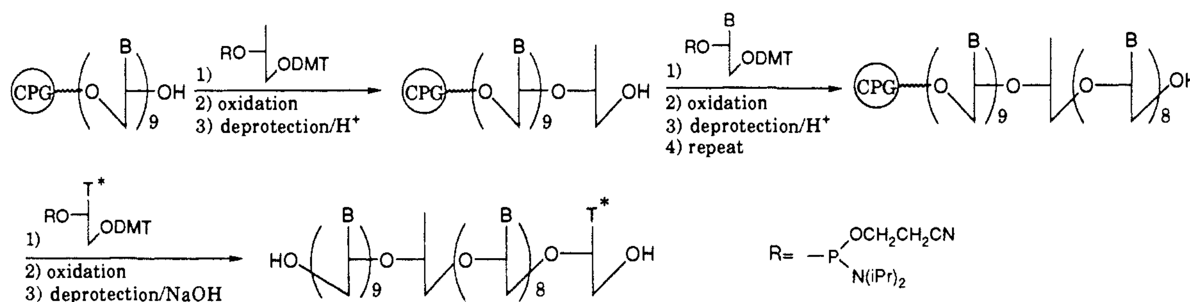

Figure 2. Scheme for the automated synthesis of oligodeoxyribonucleotide–EDTA·Fe **1**.

pairs; and the design of oligonucleotides capable of binding alternate strands of duplex DNA by triple-helix formation. We report that a pyrimidine oligodeoxyribonucleotide–EDTA·Fe containing a 3'–3' phosphodiester and a 1,2-dideoxy-D-ribose linker binds and cleaves a mixed-sequence double-helical DNA target site by alternate-strand triple-helix formation.

Within the pyrimidine triple-helix motif, oligonucleotides bind in the major groove parallel to the purine strand of the Watson–Crick double helix.[1a] Hoogsteen hydrogen bonding directs triplet specificity wherein thymine (T) binds adenine–thymine base pairs (T·AT triplet) and protonated cytosine (C^+) binds guanine–cytosine base pairs (C^+·GC triplet).[1-4] Extension of this structural motif to mixed sequences of the type (purine)$_m$(pyrimidine)$_n$ requires simultaneous binding of the pyrimidine oligonucleotide to adjacent purine tracts on alternate strands of the Watson–Crick duplex in parallel but opposite orientations (Figure 1). Pyrimidine oligonucleotides coupled 3'–3' or 5'–5' possess the requisite orientation for alternate-strand purine binding of

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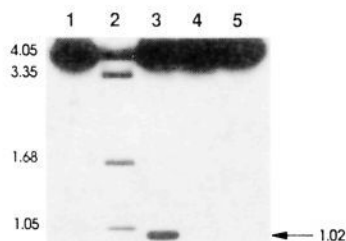

Figure 3. Site-specific double-strand cleavage of plasmid pHMPQ2 (4.05 kbp). In a typical experiment, a buffered solution containing tris(acetate) (25 mM, pH 7.0), oligonucleotide–EDTA (2 μM), $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (2 μM), NaCl (100 mM), spermine (1 mM), ^{32}P-labeled pHMPQ2, and calf-thymus DNA (100 μM bp) was equilibrated at 37 °C. After 1 h, sodium isoascorbate (1 mM) was added to the reaction mixture, initiating strand cleavage. The reaction was allowed to proceed for 8 h (37 °C), followed by ethanol precipitation. Gel electrophoresis (1% agarose) separated the double-strand-cleavage products, which were visualized by autoradiography. Product yields were determined by scintillation counting of isolated bands. Lane 1: pHMPQ2 linearized with *Sty*I and uniquely labeled with [α-^{32}P]TTP at the 3' end. Lane 2: DNA size markers obtained by digestion of linear pHMPQ2 with *Eco*RI (1055 bp), *Pvu*I (1680), *Pvu*II (3350), and undigested DNA (4049). Lane 3: plasmid with oligonucleotide **1** (2 μM). Lane 4: plasmid with oligonucleotide **2** (2 μM). Lane 5: plasmid with oligonucleotides **2** and **3** (2 μM each). Arrow indicates the labeled 1.02-kbp double-strand-cleavage product.

5'-(purine)$_m$(pyrimidine)$_n$-3' and 5'-(pyrimidine)$_m$(purine)$_n$-3' sequences. Essential to the design of these bidirectional oligonucleotides is the nature of the covalent linker. Based on model-building studies, an abasic deoxyribose analogue[5] (ϕ) should maintain overall structural continuity in the 3'–3' motif for alternate-strand binding. The stereochemistry of the (2*R*,3*S*)-3-hydroxy-2-(hydroxymethyl)tetrahydrofuran moiety properly orients the two subunits for the necessary crossover within the major groove of the Watson–Crick double helix (Figure 1). The linking distance appears to be optimal for bridging two base pairs, in a nonspecific manner, at the junction of a 5'-(purine)$_m$(pyrimidine)$_n$-3' target sequence.

The 3'-3' oligonucleotide (5'-T_3CT_5-3'–3'-$\phi T_3CT_3CT^*$-5') **1** incorporating nine pyrimidines flanking the 1,2-dideoxy-D-ribose was synthesized by automated methods starting with the 5'-oxygen of thymidine attached to the solid support (Figure 2).[6] After synthesis of the first nine bases in the 5'–3' direction, the 10th residue was introduced in opposite orientation by a 3'–3' coupling using 5-*O*-(4,4'-dimethoxytrityl)-1,2-dideoxy-D-ribose 3-(β-cyanoethyl *N*,*N*-diisopropylphosphoramidite). The next eight bases were added in the conventional 3'–5' orientation, and the protected thymidine–EDTA (T*) was incorporated as the last nucleotide.[7] As controls, oligonucleotides (**2** and **3**) capable of binding to only one half-site within the target sequence were synthesized with and without T*, respectively.

Oligonucleotide–EDTA·Fe **1** at 2 μM concentration (37 °C, pH 7.0) produced specific double-strand cleavage at the target site, 5'-$A_3GA_5GCT_3CT_3CT$-3', 1.02 kbp from the end of plasmid DNA, 4.05 kbp in size[8] (14% yield) (Figure 3). No additional cleavage at partially homologous sequences was detected. No detectable cleavage was observed (<1.5%) with 9-mer **2** (lane 4) or the combination of oligonucleotides **2** and **3** (lane 5). These results indicate that oligonucleotide **1** occupies both half-sites in a one-to-one complex. In data not shown, if the 1,2-dideoxy-D-ribose is replaced by an acyclic chain, 1,3-propanediol, binding is 3 times less efficient, indicating that the rigidity and stereochemistry of the dideoxyribose ring are likely important.

This work demonstrates that binding to alternate strands of double-helical DNA by oligonucleotide-directed triple-helix formation extends recognition to mixed DNA sequences of the type (purine)$_m$NN(pyrimidine)$_n$.[10] This result is one example of a larger class of potential *multimeric crossover oligonucleotides*. One could imagine the synthesis of 5'–5' linked oligonucleotides for binding (pyrimidine)$_m$(purine)$_n$ tracts of duplex DNA. Combinations of 5'–5' and 3'–3' linked oligonucleotides should then make multiple crossovers in the major groove possible for binding DNA sequences of the type 5'-(purine)$_m$(pyrimidine)$_n$-(purine)$_p$-3' and 5'-(pyrimidine)$_m$(purine)$_n$(pyrimidine)$_p$-3'. Incorporation of triplet specificities for TA[1f] and CG base pairs within the alternate-strand triple-helix motif should allow targetng to an even broader range of mixed DNA sequences.

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(8) The plasmid containing the target sequence was prepared by ligation of a short duplex containing the 20-base-pair site into the 4.02-kilobase-pair (kbp) *Bam*HI–*Hin*dIII fragment of pBR322.[9] This sequence occurs once in 4049 base pairs and lies 1.02 kbp upstream from the *Sty*I restriction site.

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(10) In a formal sense, alternate strand triple helix formation of the type (purine)$_n$NN(pyrimidine)$_m$ (n = 1–7 and $n + m$ = 14) affords recognition of 967 044 sequences.

[2-(Benzyloxy)-1-(*N*-2,6-xylylimino)ethyl]samarium as a Synthetic Equivalent to α-Hydroxyacetyl Anion

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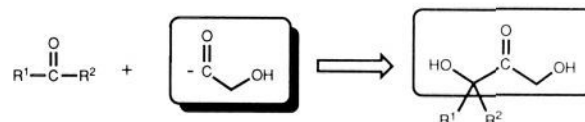
A synthetic equivalent to acyl anion is of great interest as an intermediate for nucleophilic introduction of the acyl group into organic molecules. In particular, α-hydroxyacetyl anion is attractive because the dihydroxyacetone unit, which would result from an addition reaction of α-hydroxyacetyl anion to a carbonyl compound, is a feature of a broad range of natural products such as keto sugars, corticosteroids, and anthracycline antibiotics.



Although a variety of acyl anion equivalents have been reported,[1] a synthetic equivalent to α-hydroxyacetyl anion is not well developed so far, and therefore, multistep procedures have been used for the introduction of a dihydroxyacetone side chain in the syntheses of corticosteroids[2] and adriamycin.[3] We now report

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