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5-Fluorodeoxyuridine as an alternative to the synthesis of mixed hybridization probes for the detection of specific gene sequences

(mismatched base pairs/oligonucleotide synthesis/specific DNA hybridization/screening gene libraries)

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ABSTRACT **Synthetic complementary oligonucleotides are useful hybridization probes for the detection of mRNAs and genes encoding proteins for which only a partial amino acid sequence is known. Usually this involves the synthesis of mixtures of oligonucleotides complementary to all possible bases in degenerate positions of codons. As an alternative we have prepared and characterized a series of unique oligonucleotides containing a pyrimidine analog, 5-fluorodeoxyuridine (F). Thermodynamic parameters and the melting temperatures of hybrid duplexes containing A·F and G·F base pairs showed that they are considerably more stable than duplexes containing A·T and G·T base pairs. The stability of a duplex decreased linearly with the number of mismatches introduced at positions at least a codon apart. A 5-fluorodeoxyuridine-substituted oligonucleotide cDNA detects rat liver pyruvate carboxylase mRNA on a RNA gel blot with a dissociation temperature only 10°C below the measured melting temperature in solution. We suggest that the complexity of oligonucleotide cDNAs used for screening gene libraries can be reduced by the design of single hybridization probes containing the substituted bases—5-fluorodeoxyuridine to pair with adenosine or guanosine, guanosine to pair with cytidine or thymidine, and deoxyinosine to pair with adenosine or cytidine at positions of codon degeneracy—and still retain near-maximum stability of hybrid duplexes.**

Recombinant DNA technology allows the isolation of genes encoding specific proteins. When the partial amino acid sequence of the protein is known, an approach to isolation of the genes is the synthesis of mixtures of 8–64 oligonucleotides of 14–17 bases that are complementary in sequence to all possible codons predicted for the corresponding amino acid sequence (1–3).‡ A difficulty in these syntheses, however, is that coupling efficiencies of the nucleotides at each step are not uniform resulting in unequal representations of the oligonucleotides in the mixture; certain of the sequences may be practically absent (4). In general the successful detection of the correct sequence has been possible only when it is present in the screened library at a relatively high abundance (>0.1–1% of sequences). A complex mixture of oligonucleotide probes may lead to spurious hybridization to incorrect sequences, particularly in attempts to identify a correct single-copy sequence in mammalian gene libraries with a complexity approaching 10^9 base pair.

To circumvent these problems, base analogs have been designed that can potentially base pair with any of the four natural bases (adenosine, thymidine, guanosine, and cytidine) in DNA. For example, deoxyinosine (5), 2-amino-2′-deoxyadenosine (4), and phenyl derivatives (5) have been used to prepare unique probes at positions of base degeneracy, but base pairing is significantly less stable than the normal Watson–Crick base pairing (6). Similarly, deoxyguanosine has been used in positions opposite to cytidine or thymidine ambiguities because the G·T wobble base pair is believed to be one of the most stable mismatches (7). The design of longer, unique oligonucleotides making use of species-preferred codon frequencies to increase the probabilities of correct base pairing are not useful alternatives when amino acid sequence information is limited and strong codon preferences are not available.

We examined the feasibility of using 5-fluorodeoxyuridine (F) for pairing to adenosine or guanosine ambiguities. We selected 5-fluorodeoxyuridine because of the observations that the mutagen 5-bromodeoxyuridine mispairs with guanosine at a much higher frequency than that of the G·T mismatch (8–11). Mispairing may arise by tautomerism of 5-bromodeoxyuridine from the *keto* to the *enol* form by way of the electron-withdrawing effect of the electronegative bromine atom (12). Because fluorine is the most electronegative atom of the halogen series, it may have a strong effect on the tautomeric equilibrium. In addition, the radius of fluorine more closely matches that of hydrogen. Base-stacking stabilities of double-helical polynucleotides appear to be enhanced when halogenated pyrimidines are substituted for the naturally occurring bases (13).

Here we show that A·F and G·F base pairs are significantly more stable than A·T and G·T base pairs, respectively, based on thermodynamic studies of double-helix formation of several 19-mer oligonucleotides and on RNA gel blot analysis. In addition, we have obtained thermodynamic parameters on mismatched natural base pairs. These data allow us to evaluate the relative stabilities of the pairing and mispairing of the natural bases.

MATERIALS AND METHODS

Oligonucleotides were synthesized by an automated synthesizer (model 380A, Applied Biosystems, Foster City, CA) (14). Concentrations of oligonucleotide solutions were determined using a molar extinction coefficient of 9.0×10^3 at 260 nm (15).

Synthesis of 5-Fluorodeoxyuridine Derivatives. Because the unprotected O-4 group of the uracil ring is reactive toward nucleophilic displacement resulting in chain branching during synthesis, we used the *p*-nitrophenylethyl group to protect the O-4 atom of 5-fluorodeoxyuridine (16, 17). 5-Fluorodeoxyuridine was obtained from Sigma and other reagents were from Aldrich. Purification of the chemically modified

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Abbreviations: F, 5-fluorodeoxyuridine; t_m, melting temperature in °C; t_d, dissociation temperature in °C of hybrid duplexes in solution.
‡The number of oligonucleotides depends upon the particular codon sequence and the corresponding base degeneracy.

base at steps 3 and 5 of the synthesis was by HPLC (Waters Delta-Prep 3000) using silica columns (separation capability of 2 g). The steps in the synthesis of 5-fluorodeoxyuridine were:

Step 1. 5-Dimethoxytrityl-5-fluorodeoxyuridine: 5-Fluorodeoxyuridine (42 mmol, 9.74 g) was suspended in 100 ml of dry pyridine in a 200-ml round-bottom flask attached to a drying tube. 4-Dimethylaminopyridine (244 mg), triethylamine (7.6 ml), and dimethoxytrityl chloride (16.4 g) were added. After 3 hr, 100 ml of water was added, and the product was extracted five times with ethyl ether (500 ml total). The organic phase was washed with five 100-ml portions of water and was concentrated *in vacuo*. The product, with a glossy foam appearance, was dissolved in methylene chloride and concentrated several times. The yield was 23 g.

Step 2. 3′-*O*-Acetyl-5-*O*-dimethoxytrityl-5-fluorodeoxyuridine: The 23 g of product in 44 ml of dry pyridine (from step 1) was dissolved in 83 ml of acetic anhydride. The solution was stirred at 25°C for 3 hr and dried. The product was dissolved in 100 ml of methylene chloride, and the organic phase was washed with three 100-ml portions of water. The solid material was obtained as described above at a yield of 21.9 g (37 mmol).

Step 3. 3′-*O*-Acetyl-5-*O*-dimethoxytrityl-4-(*p*-nitrophenylethyl)-5-fluorodeoxyuridine: The solid material obtained in step 2 (21.9 g) was treated as described by Himmelsbach *et al.* (17). The reaction continued for 3 days at 40°C. Purification of the product was by HPLC at a yield of 6.8 g.

Step 4. 5-*O*-Dimethoxytrityl-4-*O*-(*p*-nitrophenylethyl)-5-fluorodeoxyuridine: The 6.8 g of product obtained from step 3 was stirred in 500 ml of 1 M ammonium hydroxide in methanol at room temperature overnight. The mixture was concentrated to dryness, redissolved in 200 ml of methylene chloride, and evaporated to dryness *in vacuo* at a yield of 5.9 g.

Step 5. 5-*O*-Dimethoxytrityl-4-*O*-(*p*-nitrophenylethyl)-5-fluorodeoxyuridine-3′-*O*(*N*,*N*-diisopropylamino)phosphoramidite: The 5.9 g of product obtained from step 4 was treated as described by Pon *et al.* (16) to give a product, which was purified by HPLC. A mixture consisting of ethyl acetate/dichloromethane/triethylamine, 45:45:10 (vol/vol), was used as the eluent. The product was monitored by chromatography on thin-layer plates observed under UV light, and the least polar component was assigned by NMR as the phosphoramidite compound at a yield of 5.4 g.

Step 6. Oligonucleotides were prepared by solid-phase synthesis.

Step 7. Deprotection of O-4 groups of fluorodeoxyoligonucleotides: The oligonucleotides were dried and redissolved in 1 M 1,8-diazabicyclo[5.4.0]undec-7-ene (Aldrich) in pyridine (1 ml, total volume) in a sealed tube. The tube was incubated at 50°C for 3 days. The final crude product was further purified by filtration on Sephedex G-25 in water.

Melting Experiments. Solutions of oligonucleotides were prepared at a concentration of 0.3 A_{260} unit/ml for each oligonucleotide in 0.1 mM Na_2EDTA/0.2 M NaCl/10 mM sodium phosphate, pH 7.0. The thermal denaturation curves were measured in a Beckman DU-6 spectrophotometer at 260 nm. The temperature, controlled by a circulating water bath, was increased linearly as a function of time at a rate of 0.5°C/min from 15°C to 95°C (Neslab MTP-5 programmer). The accuracy of the unit was ±0.5°C. Absorbance values were sampled at a rate of 10 points per min and stored in a Beckman data transporter (Beckman 597171). The DNA melting data were reduced by a DU-7 Data-Capture Software program (Beckman 597041). Each curve generated consisted of 1600 data points. The derivative at each point on the curve was determined by fitting a least-square regression line to the point and its neighbors in a dynamically specified "window" on both sides. The slope of this line was the derivative at that point. The typical window size was 50 points.

Differential melting curves were generated using the theory of DNA melting based on an all-or-none two-state model (18, 19). The transition enthalpy is calculated from the equation

$$\Delta H = -18.28/(1/T_{1/2} - 1/T_{3/4}), \quad [1]$$

where $T_{1/2}$ is the temperature (in degrees Kelvin) at the maximum of the differential melting curves and $T_{3/4}$ is the temperature at which the differential melting curves have fallen to half of $T_{1/2}$; the maximum occurs at $a = 0.414$, where a is the fraction of DNA molecules present in double-stranded form.

In these experiments, equimolar amounts of both strands are present. Thus the equilibrium for duplex formation is expressed as

$$K = 2a/[(1 - a)^2 C_t], \quad [2]$$

where C_t is the total strand concentration. At $T = T_{1/2}$, where $a = 0.414$, it can be seen that

$$K_{1/2} = 2.411/C_t. \quad [3]$$

The standard free energy at $T = T_{1/2}$ is calculated from the equation

$$\Delta G = -RT_{1/2} \ln K_{1/2}. \quad [4]$$

The entropy of transition is derived using the relation

$$\Delta S = (\Delta H - \Delta G)/T_{1/2}. \quad [5]$$

RNA Gel Blots. Poly(A)$^+$ RNA from livers of male rats was separated by electrophoresis in 0.8% formaldehyde/agarose gels, transferred to nitrocellulose paper, and hybridized to ^{32}P-5′-end-labeled oligonucleotides (20). Hybridizations were done for 24 hr at various temperatures. Blots were washed twice at 23°C and then at various temperatures with successive solutions of 2× SSC/0.1% $NaDodSO_4$ and twice with 1× SSC/0.1% $NaDodSO_4$ (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0). Autoradiography of the blots was for 1–5 days at −70°C using an intensifying screen.

Rat Pyruvate Carboxylase cDNA. A unique oligonucleotide (probe 1) and oligonucleotides with three cytidine or thymidine mixed bases (probe 3) or with three 5-fluorodeoxyuridine substitutions (probe 2) (Fig. 1, set 4) were constructed based on the sequence of two overlapping recombinant cDNAs for rat pyruvate carboxylase (obtained from a library provided by Michael Muechler).

RESULTS

Four sets of oligonucleotides were synthesized (Fig. 1). The first three sets were used in the thermal denaturation studies in solution. The fourth set was used for the RNA gel blot analysis. The oligonucleotides used in the melting experiment consisted of 19 nucleotides; the reference oligonucleotides have perfect 2-fold symmetrical, but not self-complementary, sequences. The symmetry of the molecules prevents them from forming hairpin or concatamer structures that may otherwise complicate the interpretation of the DNA melting data. The high G+C content (≈80%) of the oligonucleotides allowed us to incorporate as many as four mismatches into the hybrids and still obtain accurate measurements of the relevant thermodynamic parameters. In oligonucleotide sets 1 and 2, G·T and G·F mismatches were introduced into the hybrids at various positions. Set 2 is identical to set 1 except that G·F is substituted for G·T mismatches. A typical example

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SET  1
1)   3'-C.G.T.G.G.C.G.C.A.G.A.C.G.C.G.G.T.G.C-5'
2)   5'-G.C.A.C.C.G.C.G.T.C.T.G.C.G.C.C.A.C.G-3'
3)   5'-G.C.A.C.C.G.T.G.T.C.T.G.C.G.C.C.A.C.G-3'
4)   5'-G.C.A.C.C.G.C.G.T.T.T.G.C.G.C.C.A.C.G-3'
5)   5'-G.C.A.C.C.G.T.G.T.C.T.G.T.G.C.C.A.C.G-3'
6)   5'-G.C.A.C.C.G.T.G.T.T.T.G.C.G.C.C.A.C.G-3'
7)   5'-G.C.A.C.C.G.T.G.T.T.T.G.T.G.C.C.A.C.G-3'
8)   5'-G.C.A.T.C.G.T.G.T.T.T.G.T.G.C.C.A.C.G-3'

SET  2
1)   3'-C.G.T.G.G.C.G.C.A.G.A.C.G.C.G.G.T.G.C-5'
3F)  5'-G.C.A.C.C.G.F.G.T.C.T.G.C.G.C.C.A.C.G-3'
4F)  5'-G.C.A.C.C.G.C.G.T.F.T.G.C.G.C.C.A.C.G-3'
5F)  5'-G.C.A.C.C.G.F.G.T.C.T.G.F.G.C.C.A.C.G-3'
6F)  5'-G.C.A.C.C.G.F.G.T.F.T.G.C.G.C.C.A.C.G-3'
7F)  5'-G.C.A.C.C.G.F.G.T.F.T.G.F.G.C.C.T.G.C-3'

SET  3
X)   3'-C.G.T.G.G.C.T.C.A.X.A.C.T.C.G.G.T.G.C-5'
Y)   5'-G.C.A.C.C.G.A.G.T.Y.T.G.A.G.C.C.A.C.G-3'

RULER   1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9

SET  4
mRNA sequence:  5'-CCU AUG UGG AGG CUA ACC-3'

Probe-1:        3'-GGA TAC ACC TCC GAT TGG-5'

Probe-2:        3'-GGA TAF ACF TCF GAT TGG-5'

Probe-3:        3'-GGA TA(C/T) AC(C/T) TC(C/T) GAT TGG-5'
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FIG. 1. Sequence of synthetic oligonucleotides used in the denaturation experiments and in RNA gel blot analysis. Bold letters indicate sites at which 5-fluorodeoxyuridine or other bases were substituted in the synthesis of the oligonucleotides.

of the melting curves obtained is depicted in Fig. 2. The hybrid duplex has a sharp melting transition indicative of a well-behaved helix-to-coil dissociation (Table 1). Duplexes carrying G·F mismatches are significantly more stable than the corresponding duplexes carrying G·T mismatches (Table 1). Differences in stacking enthalpy between G·T and G·F mismatches in the duplexes 1.4 and 1.4F (Table 1) are ΔH(G/C) − ΔH(G/T) = 125.7 kJ/mol, and ΔH(G/C) − ΔH(G/F) = 40.8 kJ/mol. These data indicate that a G·T or a G·F mismatch contributes an ≈20% and ≈6.5% decrease in stacking enthalpy, respectively, due to interactions by neighboring bases.

The melting data (melting temperature in °C, t_m) indicate

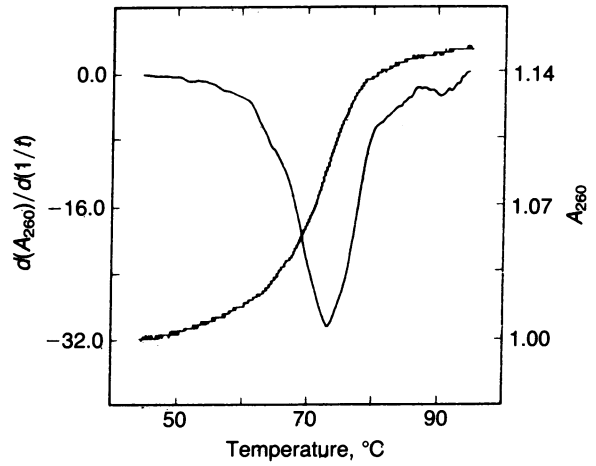

FIG. 2. Denaturation curves of duplex 1.3 (from Fig. 1, set 1) in integral and differential forms. Values of absorption are normalized to 1 A_{260} unit at 25°C. Smooth line, differential; stepped line, integral; t, temperature in °C.

Table 1. t_m values for all combinations of the 10 strands of set 3 in Fig. 1

Duplex	t_m, °C	ΔG*, kJ/mol	ΔH, kJ/mol	ΔS
1.2	80.5	26.59	628.8	1.68
1.3	73.0	26.03	545.4	1.50
1.4	72.3	25.98	503.1	1.38
1.5	65.2	25.44	430.1	1.20
1.6	63.2	25.29	395.8	1.10
1.7	53.0	24.52	259.3	0.70
1.8	44.6	24.12	166.0	0.45
1.3F	76.8	26.32	604.8	1.65
1.4F	75.6	26.23	588.0	1.61
1.5F	71.8	25.94	540.2	1.52
1.6F	71.5	25.92	526.0	1.45
1.7F	64.2	25.37	421.4	1.17

*ΔG value was calculated at $T_{1/2}$ for each duplex. The thermodynamic parameters were given for the double-strand formation of the duplex.

that mismatches at positions six bases apart are no more stable than mismatches that are one codon apart; duplex 1.5 is slightly more stable than duplex 1.6 by 2°C in t_m; and duplex 1.5F is almost equally as stable as duplex 1.6F by 0.3°C in t_m. Neighboring effects of base sequences are also evident because duplexes carrying mismatches at different locations assume different degrees of stability. The G+C-rich oligonucleotides consisting of the two sets of G·T and G·F mismatches exhibit a range of thermal stability that is linear with regard to the number of mismatches introduced into the sequences. The t_m decreases linearly as more mismatches are incorporated within a distance of at least three bases from each other. These results agree with those of earlier observations (1). Also the stacking enthalpy is linear with respect to t_m (Fig. 3). This linear relationship allows a prediction of the stability of hybrid duplexes containing base mismatches.

Set 3 includes 10 oligonucleotides used for measuring the thermodynamic parameters for the 25 hybrid duplexes with each of the bases adenine, cytosine, thymine, guanine, and 5-fluorouracil at the positions labeled X and Y. The sequences in this set are identical to those of sets 1 and 2 except for substitutions of A·T for G·C at positions 7 and 13 to reduce the G+C content to a more practical level of 70%. The base pairs in the nearest neighboring positions of the mismatch are similar in positioning to those studied earlier (21). t_ms of the helix-to-coil transition of all 25 duplexes are listed in Table 2.

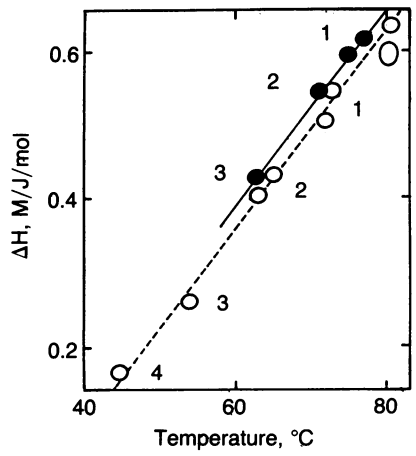

FIG. 3. A plot of the stacking enthalpy (H) versus the t_m with respect to the number of G·T (dashed line) and G·F (solid line) mismatches in the molecules. Numbers refer to number of mismatched base pairs.

Table 2. Thermodynamic parameters (t_m) for duplexes of set 1 and set 2 in Fig. 1

Base Y	Base X				
	A	C	G	T	F
A	67.0	66.2	69.0	72.2	73.2
C	65.0	64.0	75.0	65.2	66.2
G	67.8	74.6	69.0	69.4	72.5
T	72.8	66.6	68.6	65.0	66.2
F	74.2	68.1	72.0	66.0	66.7

Values are t_m in °C.

The immediate environments of the X·Y pair in the duplexes are asymmetric; the X base is between two purine bases and the Y base is between two pyrimidines. Some of the base pairs show large differences in the stabilities of the two opposite orientations. For example, T·G stabilizes the duplex (69.4°C) more than does G·T (68.6°C). This general observation of the dependence of t_m on neighboring sequences agrees well with the results reported by others (21–23). The general order of stability G·C > A·T > G·T, G·G > G·A > A·A > C·T > C·A, T·T > C·C is consistent with earlier findings in studies of A·T duplexes (21). Because both systems share the same nearest neighboring bases, the same order of stability of mismatches occurs in the two systems, indicating that the immediate neighboring bases exert the strongest influences at mismatched positions. Also the duplexes carrying 5-fluorodeoxyuridine are always more stable than the corresponding duplexes with thymidine substituted for 5-fluorodeoxyuridine (Table 2). Specifically, A·F and F·A base pairs are more stable than A·T and T·A base pairs by 1.0°C and 1.4°C, respectively. Likewise, G·F and F·G mismatches are 3.1°C and 3.4°C more stable than the corresponding G·T and T·G mismatches.

RNA Gel Blot Analysis with Oligonucleotides Containing 5-Fluorodeoxyuridine Residues. The calculated t_d (dissociation temperature of hybrids in solution) for probe 1 to pyruvate carboxylase mRNA was 57.8°C (17). DNA melting data yielded a t_m of 60°C for probe 1 and 52°C for probe 2. Probe 1 gave the clearest signal at both 52°C and 42°C, as expected based on its t_m (Fig. 4). Probe 2 gave a comparable signal at 42°C but a lesser signal at 52°C. The probe 3 signal was less than that of probe 1 and probe 2 at all temperatures tested (Fig. 4). These data suggest that despite significant G·F mismatching in probe 2, hybridization to complementary RNA immobilized on nitrocellulose filters occurs at temperatures 10°C below its t_m in solution and gives a similar stability for RNA·DNA duplex formation as that seen for the perfectly matched probe 1.

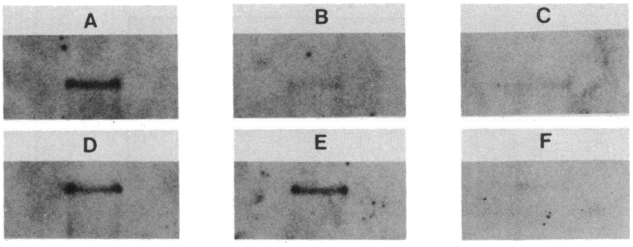

FIG. 4. RNA gel blot analysis of rat pyruvate carboxylase mRNA using probes with sequences shown in set 4 (Fig. 1). Upper and lower rows of filters were hybridized and washed at 52°C and 42°C, respectively, with probe 1 (*A* and *D*), probe 2 (*B* and *E*), and probe 3 (*C* and *F*). Probe 1 (*A* and *D*) is a perfect 18-base complement of the pyruvate carboxylase mRNA. Probe 2 is a unique 18-base oligonucleotide with 5-fluorodeoxyuridine substituted at positions 6, 9, and 12. Probe 3 is a mixture of eight oligonucleotides, synthesized individually and reconstituted as a mixture consisting of equal representations (one-eighth) of each oligonucleotide. Addition of eight times more of the labeled probe 3 during hybridization increased background considerably but did not increase the intensity of the RNA band.

DISCUSSION

For the most efficacious applications, base analogs complementary to multiple possible bases in degenerate positions must be nondestabilizing and nonselective when paired with the target bases. Rather than seeking a "universal base" that might base pair equally well with any of the four natural bases, we are attempting to develop individual "spacer" bases for purines and pyrimidines. Our studies show that 5-fluorodeoxyuridine exhibits superior stability and nonselectivity in pairing to adenosine or guanosine ambiguities in codons compared to the natural pyrimidines cytidine or thymidine. The superior nonselectivity of 5-fluorodeoxyuridine over thymidine arises from our observations of the relative stabilities of A·F compared to A·T and of G·F compared to G·T.

Our data provide evidence that 5-fluorouracil residues alter base-stacking interactions and enhance the conformational stabilities of double-helical polynucleotides in aqueous solution and thus give rise to more stable mispairing between 5-fluorouracil residues and purines. In double-helical DNA, halogenated bases produce specific mutations that arise from their occasional mispairing with guanine during genetic replication (24, 25).

Deoxyinosine is the least destabilizing and least discriminating purine when paired to adenosine or cytidine ambiguities (21, 26). The use of both deoxyinosine and guanosine, and 5-fluorodeoxyuridine as purine and pyrimidine spacer nucleotides, respectively, presents a possible strategy for the design of unique probes for screening cDNA libraries. Based on this strategy, 11 of the 20 amino acid codons are amenable to the synthesis of single-sequence cDNAs. Use 5-fluorodeoxyuridine for the 2-fold adenosine or guanosine degeneracy in the third positions of the codons for glutamic acid, glutamine, and lysine. Use guanosine for the 2-fold cytidine or thymidine degeneracy in the third positions of the codons for tyrosine, phenylalanine, aspartic acid, asparagine, histidine, and cysteine. Dual syntheses can be carried out utilizing either 5-fluorodeoxyuridine or guanosine at the third positions for the 4-fold (adenosine/guanosine/cytidine/thymidine) codons for isoleucine, alanine, valine, threonine, glycine, and proline. When a leucine is encountered, guanosine can be used as a complement to the position of first base degeneracy (cytidine or thymidine) and 5-fluorodeoxyuridine or guanosine for the 4-fold degenerate third base. For arginine, deoxyinosine is the preferred complementary base for the degenerate first position adenosine or cytidine and 5-fluorodeoxyuridine or guanosine at the third position. Serines should be avoided because of the poor stabilities of any of the base analogs in pairing with either adenosine or thymidine (first position) and guanosine or cytidine (second position) of the serine codon.

Our studies of the t_d of synthetic probes bound to low-abundance target RNA on nitrocellulose filters clearly show that a 5-fluorodeoxyuridine-containing probe gives a higher signal-to-noise ratio than do mixed-oligonucleotide probes containing the natural bases but that the t_d is 10°C less than the t_m predicted from solution phase melting. The difference between the t_d and the t_m observed is consistent with previous observations; t_ds of hybrids immobilized on filters are often lower than that predicted from solution hybridization (21, 27).

Our results on the RNA gel blot analysis of the pyruvate carboxylase mRNA system clearly indicate that a 5-fluorodeoxyuridine-substituted single probe is superior to the mixed-probe approach. We have also demonstrated a similar

result using a different complementary probe (a 19mer with the 5-fluorodeoxyuridines to mRNA (unpublished results).

NMR studies have been carried out on two self-complementary DNA dodecamers containing 5-fluorouracil residues, and it was suggested that the A·F base pair was less stable than the A·T base pair in the analogous position of the control helix (28). However, no measurement of t_m of those two molecules was described, though it was stated that there is a lack of destabilization of the helix as a whole (28). Our melting data described here concerning the involvement of 5-fluorouracil residues are not inconsistent with the dynamic behavior of the A·F base pair observed by the NMR studies.

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