If the ends of the growing chains contain bases that are less tightly bound than initiator chains the distribution will be quite sharp. It may be noted that all of these phenomena occur without a change in the mean chain length of the total distribution of material (that is, unreacted initiator is averaged in), but will affect the average product chain length. We suggest that the practical solution for oligomer synthesis, which means building as narrow a distribution as possible, can often be realized by using monomers that reduce the affinity of the growing chains for the enzyme. In the present experiments this is clearly demonstrated in only one case, by substituting dNacATP for dATP. Since dNacGTP, dNacCTP, or dNanCTP will also substitute for normal substrates, we assume this solution will be generally applicable. As chains are extended the presence of blocking groups will also prevent some aggregation problems. The experiments presented therefore provide a practical description of the rules for statistical synthesis of certain classes of oligodeoxynucleotide.

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Acid-Catalyzed Exchange of Hydrogen in Thiamine and Related Compounds*

D. W. Hutchinson

ABSTRACT: The protons of the C(2′)-methyl group of thiamine, oxythiamine, and imidazolethiamine undergo acid-catalyzed exchange in deuterium oxide. The position of exchange has been established by nuclear magnetic resonance studies on the sulfonic acids and 5-(2-hydroxyethyl)-4-methylthiazole produced by bisulfite cleavage of deuterated thiamine and oxythiamine. Confirmation of the position of exchange in thiamine and oxythiamine has been provided by mass spectrometry. A convenient method for the tritiation of thiamine has been developed.

The base-catalyzed exchange of the C(2′) proton of the thiazolium ring of thiamine (Breslow, 1958) has been the subject of a recent study when it was observed that the rate of exchange decreased rapidly with increasing acidity of the reaction medium (Mieyal *et al.*, 1967). In the current investigation, the exchange of the protons of the C(2′)-methyl group of the pyrimidine residues of thiamine chloride hydrochloride (I), oxythiamine chloride hydrochloride (II), and imidazolethiamine (III) has been observed in strongly acid solution.

Materials and Methods

Thiamine chloride hydrochloride (BDH) and oxythiamine chloride hydrochloride (Sigma) were commercially available. Imidazolethiamine (Masuda, 1961) was a gift from Dr. A. J. Knell. Nuclear magnetic resonance spectra were recorded at 60 MHz on a Perkin-Elmer R12 spectrometer, using sodium 2,2-dimethyl-2-silapentane-5-sulfonate as internal standard. The tritium content of samples was measured with a Packard 4000 scintillation counter using a dioxan-based scintillation medium which contained naphthalene (60 g/l.), 2,5-diphenyloxazole (4 g/l.), and 1,4-bis[2-(5-phenyloxazolyl)]benzene (1 g/l.).

Deuterium Exchange of Thiamine (I). The nuclear magnetic resonance spectrum of a freshly prepared solution of thiamine chloride hydrochloride (100 mg) in 6 N deuterium chloride in deuterium oxide (1 ml) was recorded when signals at τ 0.25 (1 H, s), 1.9 (1 H, s), 4.38 (2 H, s), 6.08 (2 H, t, J = 6 Hz), 6.78 (2 H, t, J = 6 Hz), 7.33 (3 H, s), and 7.42 (3 H, s) were observed. The spectrum was rerecorded at intervals over a period of 1 week, during which time the peak at τ 7.33 gradually disappeared ($t_{1/2}$ = 20 hr at room tempera-

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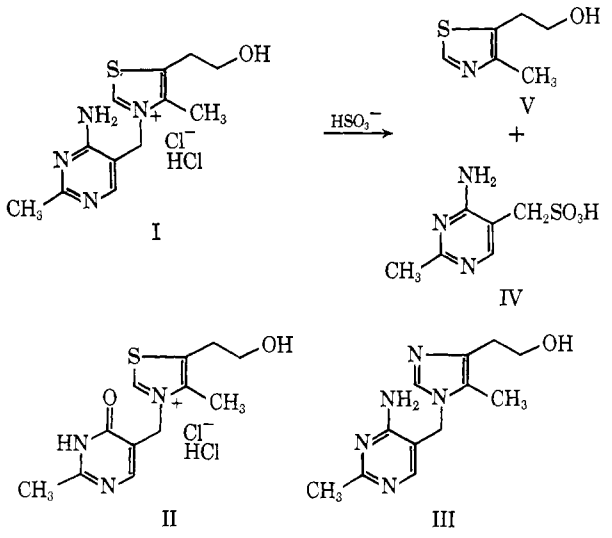

FIGURE 1: Thiamine and related compounds: I, thiamine; II, oxythiamine; III, imidazolethiamine; IV, 5-(2-hydroxyethyl)-4-methylthiazole; V, 4-amino-2-methyl-5-pyrimidinemethanesulfonic acid.

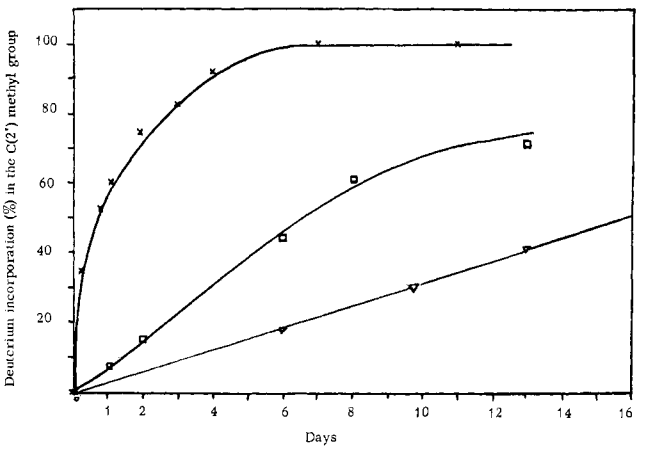

FIGURE 2: Deuterium incorporation into thiamine and oxythiamine: (—×—×—×—) thiamine, 6 N DCl; (—▽—▽—▽—) thiamine, 1 N DCl; (—□—□—□—) oxythiamine, 1 N DCl.

ture). When 1 N deuterium chloride was used as solvent the exchange reaction was much slower ($t_{1/2}$ = 19 days). There was no observable change in the peak at τ 7.33 in the spectrum of a solution of thiamine chloride hydrochloride in deuterium oxide after 2 weeks (see Figure 2).

A solution of thiamine chloride hydrochloride (400 mg) in 6 N deuterium chloride (4 ml) was evaporated to dryness after 7 days, the residue dissolved in water (10 ml), and lyophilised to dryness. The residue was again dissolved in water (10 ml) and lyophilized to dryness, and the solid dried *in vacuo*. The deuterated thiamine chloride hydrochloride (400 mg) was cleaved by sodium bisulfite at pH 5 (Williams *et al.*, 1935) to give 4-amino-2-methyl-5-pyrimidinemethanesulfonic acid (IV) (230 mg, 85%) and 5-(2-hydroxyethyl)-4-methylthiazole (V) (151 mg, 88%). The nuclear magnetic resonance spectrum of IV (NaOD–D_2O) obtained from undeuterated thiamine showed peaks at τ 2.05 (1 H, s), 5.95 (2 H, s), and 7.60 (3 H, s). The nuclear magnetic resonance spectrum of IV (NaOD–D_2O) from deuterated thiamine showed peaks τ 2.05 and 5.95, no peak at 7.60 was discernable. The nuclear magnetic resonance spectra (in DMSO-*d*) of V from both deuterated and undeuterated thiamine were identical showing peaks at τ 0.10 (1 H, s), 6.35 (2 H, t, J = 4 Hz), 7.00 (2 H, t, J = 4 Hz), and 7.50 (3 H, s).

Deuterium Exchange of Oxythiamine (II). Oxythiamine chloride hydrochloride (100 mg) was dissolved in 1 N deuterium chloride (1 ml) and the nuclear magnetic resonance spectrum recorded immediately when peaks were observed at τ 0.08 (1 H, s), 1.65 (1 H, s), 4.40 (2 H, s), 6.22 (2 H, t, J = 6 Hz), 6.85 (2 H, t, J = 6 Hz), 7.20 (3 H, s), and 7.44 (3 H, s). The peak at 7.20 slowly diminished with time ($t_{1/2}$ = 7 days at room temperature).

A sample of oxythiamine chloride hydrochloride fully deuterated in the C(2′) methyl group (325 mg) was cleaved with bisulfite as described above to give 4-hydroxy-2-methyl-5-pyrimidinemethanesulfonic acid (195 mg, 90%). The nuclear magnetic resonance spectrum (in NaOD–D_2O) of the sulfonic acid showed peaks at τ 1.88 (1 H, s) and 5.92 (2 H, s) but no peak at 7.65 which was present in the spectrum of undeuterated sulfonic acid.

Deuterium Exchange of Imidazolethiamine. Imidazolethiamine (100 mg) was dissolved in 6 N deuterium chloride (1 ml) and the nuclear magnetic resonance spectrum recorded immediately when peaks were observed at τ 1.05 (1 H, s), 1.37 (1 H, s), 4.55 (2 H, s), 6.05 (2 H, t, J = 5 Hz), 7.00 (2 H, t, J = 5 Hz), 7.27 (3 H, s), and 7.65 (3 H, s). The peak at 7.27 slowly diminished with time ($t_{1/2}$ = 60 hr at room temperature).

Mass Spectra of Thiamine (I) and Oxythiamine (II). The mass spectrum of thiamine chloride hydrochloride was recorded at 250° and showed peaks at *m/e* 85 (24%), 112 (100%), 113 (34%), 114 (8%), 122 (22%), 143 (24%), 144 (3%), and 157 (6%) (Hesse *et al.*, 1967). The mass spectrum of fully deuterated thiamine chloride hydrochloride, which had been washed twice with water as described above, showed peaks at *m/e* 85 (32%), 112 (100%), 113 (50%), 114 (15%), 124 (10%), 125 (22%), 126 (8%), 143 (39%), 144 (16%), and 160 (6%). No significant peaks were detectable at *m/e* 122 and 157. A comparison of the mass spectra of oxythiamine chloride hydrochloride and deuterated oxythiamine chloride hydrochloride showed little change in the regions *m/e* 85, 112, 113, 114, 143, 144, 145. However, a peak at *m/e* 122 in the spectrum of oxythiamine was absent in the spectrum of the deuterated compound being replaced by a peak at *m/e* 125.

Tritiation of Thiamine. Thiamine chloride hydrochloride (1.55 g) was dissolved in 6 N HCl (10 ml) and tritiated water (0.1 ml, 9.6 × 10^8 dpm) added. At intervals aliquots (0.5 ml) were removed, evaporated to dryness, dissolved in water (2 ml), set aside for 30 min, and evaporated to dryness. This procedure was repeated and the solid dried overnight *in vacuo*. The specific radioactivity of the [^{3}H]thiamine chloride hydrochloride was measured, a final specific activity of 20,000 dpm/mmole being obtained after 7 days.

Samples (1 ml) of a solution of [^{3}H]thiamine chloride hydrochloride (200 mg, 20,000 dpm/mmole) in water (20 ml) (pH 4) were removed at intervals, evaporated to dryness and the specific radioactivity of the residue measured. There was no change in the specific radioactivity of the thiamine after 8 days.

Discussion

The unambiguous assignment of the signals due to the two methyl groups in the nuclear magnetic resonance spectrum

of thiamine has not been made up to the present time. It has generally been assumed (Kotera, 1965; (Sable and Biaglow, 1965) that the signal at lower field was due to the C(2′)-methyl group while the signal at higher field was due to the C(4)-methyl group, although the reverse assignment has recently been made from a study of the nuclear magnetic resonance spectrum of C(2′)-ethylthiamine chloride hydrochloride (Biaglow, 1969). The present work demonstrates that the lower field signal at τ 7.33 is due to the C(2′)-methyl group as there is no signal due to a methyl group in the nuclear magnetic resonance spectra of the sulfonic acids IV derived from fully deuterated thiamine and oxythiamine. Moreover, the nuclear magnetic resonance spectra of the 5-(2-hydroxyethyl)-4-methylthiazole (V) derived from deuterated and undeuterated thiamine are identical, indicating that there is no deuterium incorporation in that part of the molecule. Confirmation that the position of exchange is the C(2′)-methyl group comes from mass spectrometry. It has been shown (Hesse *et al.*, 1967) that the peaks at *m/e* 85, 112, 113, 114, 143, and 144 in the mass spectrum of thiamine chloride hydrochloride are all derived from the thiazolium residue while the peaks at *m/e* 122 and 157 derive from the pyrimidine residue. In the mass spectrum of fully deuterated thiamine all peaks due to the thiazolium residue are the same as those in the mass spectrum of undeuterated material while the peaks at *m/e* 122 and 157 are replaced by peaks at 125 and 160, respectively. This indicates that three atoms of deuterium are incorporated into the pyrimidine residue.

In the nuclear magnetic resonance spectrum of oxythiamine chloride hydrochloride two singlets due to three protons appear at τ 7.20 and 7.42. The electron distribution of the thiazolium ring of oxythiamine should not be significantly different from that for thiamine and hence the position of the signals due to the C(4)-methyl group should be approximately the same for the two compounds. On the other hand, the charge distribution of the pyrimidine ring should be different in oxythiamine and the methyl group should be deshielded compared with thiamine. Thus the signal in the nuclear magnetic resonance spectrum due to the C(2)-methyl group of oxythiamine should appear at lower field than that observed for the C(2′)-methyl group of thiamine. Confirmation that the signal at τ 7.20 is due to this methyl group is provided by the nuclear magnetic resonance spectrum of the sulfonic acid obtained by bisulfite cleavage of deuterated oxythiamine when no signal due to the C(2)-methyl group can be observed. Mass spectrometric evidence also indicates that deuterium is incorporated into the pyrimidine residue as the peaks due to the thiazolium moiety of deuterated oxythiamine are not significantly different from those obtained in the mass spectrum of the undeuterated compound. However, the peak at *m/e* 122 in the spectrum of oxythiamine is absent in the spectrum of the deuterated material being replaced by a peak at *m/e* 125.

Imidazolethiamine also contains a methyl group which can undergo acid-catalyzed exchange in deuterium oxide. By analogy with thiamine and oxythiamine, it is proposed that this is the C(2′)-methyl group.

Methods currently available for the synthesis of ^{14}C-(Williams and Ronzio, 1952) and ^{35}S-labeled thiamine (Williams and Ronzio, 1952; Verrett and Cerecedo, 1957) involve lengthy synthetic procedures. The acid-catalyzed exchange reaction provides a convenient method for the preparation of isotopically labeled thiamine which could be used for both enzymatic and metabolic studies.

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