5)Pyr adduct. Class I secondary structure yielding the 8–(66–68) and the minor 8–(59–62) cross-links can be viewed as a mixture of $tRNA^{Phe}$ conformers in equilibrium on the time scale of the photoreaction as shown in Figure 8. It is more difficult to explain the origin of the 8–(40–43) cross-link on the basis of the extended structure of Figure 8. Folding of these structures to specifically bring the region $\Psi_{39}CCC$ in the vicinity of s^4U_8 appears unlikely in view of the strong electrostatic repulsions involved. We are then led to the possibility of an alternative minor secondary structure retaining the dihydrouracil and TΨC stems, the lower part of the 5′-CCA stem being now paired with the sequence $C_{42}C_{43}G_{44}$. This would bring $C_{40}C_{41}$ in the vicinity of s^4U_8.

One unexpected product of our kinetic studies is that, in form III $tRNA^{Phe}$, two classes of conformers coexist that are not in equilibrium on a relatively long time scale. This situation is not without precedent in the tRNA field since metastable inactive states of yeast $tRNA^{Leu}$ (Gartland & Sueoka, 1966) and *E. coli* $tRNA^{Trp}$ (Lindahl et al., 1966) can occur under conditions that favor the active forms. We interpret this finding as an indication that electrostatic repulsions may maintain as far apart as possible the bihelical regions and prevent major conformational rearrangements between, for example, the cloverleaf and the extended forms of Figure 8. It should be mentioned that the stability prediction would yield more weight to the cloverleaf than observed here. However, these predictions are derived from thermodynamic parameters obtained in medium of moderate ionic strength (Gouy et al., 1985) and do not take into account the electrostatic repulsions prevailing in a low ionic strength medium.

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Registry No. s^4U, 13957-31-8; Pdo(4–5)Cyd, 33602-88-9; Pdo(4–5)Urd, 103793-79-9; N_1N_1'-dimethyl-Pyo(4–5)Ura, 103793-77-7; N_1N_1'-dimethyl-Pyo(4–5)hUra, 103793-78-8; N_1N_1'-dimethyl-Pyo(4–5)hCyt, 68455-51-6; N_1N_1'-diriboside Pyo(4–5)hCyt, 103793-76-6.

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APPENDIX: COMPUTATION OF THE RADIUS OF GYRATION OF A CLOSED RING TO WHICH LINEAR SIDE CHAINS ARE ATTACHED

We have to compute the radius of gyration, R, of a closed ring of N beads to which linear side chains of I and J beads are attached by their end at the same point of the ring. In a first approach, the chains have the following properties. They follow a Gaussian statistics, whatever their length. They are infinitely thin and can cross one another like phantoms. Under these simplifying hypotheses, our problem can be solved by using the method developed by Zimm and Stockmayer (1949) to determine R in the case of linear molecules containing branches. For our very particular system, we find

$$\langle R^2 \rangle = \frac{b^2}{12}\left[N + 2\frac{(I+J)^2}{t} + 3N\frac{(I-J)^2}{t^2} \right] \quad (1)$$

Table V: Results for Squared Radius of Gyration

				R^2/b^2				
					unknotted rings		trefoil knots	
cross-link	N	I	J	formula 1	d/b = 0.1	d/b = 0.5	d/b = 0.1	d/b = 0.5
8–8	0	0	76	12.67	12.62 ± 0.3	16.73 ± 0.3		
8–13	5	8	63	12.13	12.28 ± 0.4	16.59 ± 0.2		
8–25	17	8	51	10.41	10.68 ± 0.3	14.24 ± 0.3		
8–35	27	8	41	8.79	9.00 ± 0.3	12.00 ± 0.3		
8–45	37	8	31	7.27	7.52 ± 0.3	9.53 ± 0.3	7.11	8.57
8–55	47	8	21	6.11	6.67 ± 0.3	8.09 ± 0.6		
8–60	52	8	16	5.74	6.21 ± 0.3	6.51 ± 0.3	4.35	5.50
8–65	57	8	11	5.56	6.30 ± 0.3	6.74 ± 0.3		
8–75	67	8	1	5.90	6.70 ± 0.4	6.05 ± 0.5	4.49	4.60

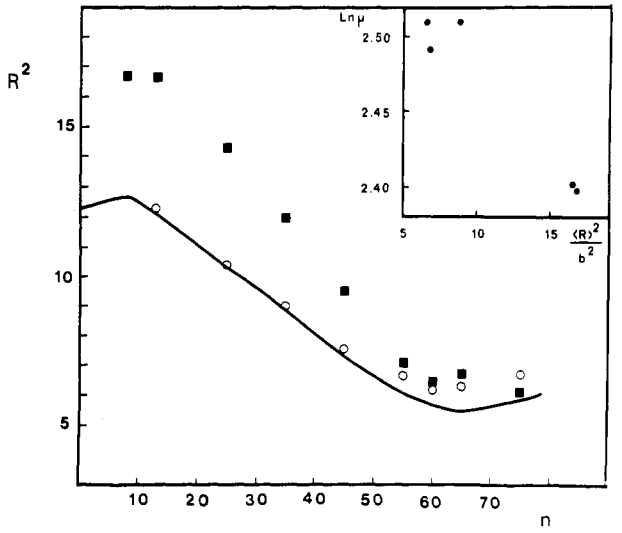

FIGURE 9: Variation of the mean squared radius of gyration R^2 reported to the square of the distance separating two adjacent beads in the simulation of a tRNA of 76 nucleotides, as a function of the number of the nucleotide, n, to which nucleotide 8 is cross-linked. Solid line refers to the phantom infinitely thin Gaussian chain (formula 1), (○) Monte Carlo simulation with d/b = 0.1, and (■) d/b = 0.5. The insert shows a plot of Ln μ vs. $(R_G/b)^2$, values calculated for d/b = 0.5.

where t is the total number of beads ($t = N + I + J$).

In order to evaluate the validity of eq 1, we performed a Monte Carlo simulation that will be reported elsewhere in greater detail. The ring is simulated by N freely jointed bonds of same length b. Two beads are randomly selected A_i and A_j. These two beads separate the ring into two arcs. The shorter arc is rotated by a random angle about an axis passing through A_i and A_j. The found conformation is accepted if the moving arc has not passed through the fixed arc and if any two beads are separated by more than a distance d. These two conditions imply that the knot type of the chain is preserved throughout the simulation and that the chain has a finite thickness. To each conformation of the ring, two linear chains of finite thickness of I and J freely jointed bonds of length b are attached to the same bead on the ring. The radius of gyration of the $N + I + J$ beads is then computed. The first thousand conformations are discarded. The average of the radius of gyration is computed on the next 2000 conformations. The results are reported in Figure 9 and Table V. The first column recalls the position of the cross-link. The next columns show the values of the squared radius of gyration obtained through formula 1 and the Monte Carlo simulations for two values of the bead diameter to bond length ratio d/b. When the beads are thin, the Monte Carlo results are very close to the Gaussian approximation, although there is a slight discrepancy when one of the linear chains has few beads. The next columns in the table show some results concerning trefoil knots. These results compare well with earlier studies (Le Bret, 1980).

Using the analysis of the distribution of spaces available to a spherical object placed within a three-dimensional network of fibers (Ogston, 1958), Cobbs (1981) showed that the mobility μ, reported to some standard μ_0, of a particle having a squared radius of gyration, R^2, is given by

$$\ln (\mu/\mu_0) = -R^2/R_0^2 \quad (2)$$

Here R_0 is interpreted as some characteristic size of a pore in the gel (Lumpkin et al., 1985), although more classically it is expressed as some function of the concentration of the gel (Cobbs, 1981). The fact that ln μ is linearly related to R^2 is well supported experimentally (Dingman et al., 1972) but cannot be checked here since the five experimental points are gathered in two groups. If the bond length, b, is taken as 16 Å, so that eq 1 gives a radius of gyration of 54 Å for a denatured tRNA in agreement with the data of Pilz et al. (1970), expression 2 leads to a characteristic size of a pore of 130 ± 20 Å, which is in the expected range.

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