Determination of Secondary Structure in Rabbit Globin Messenger RNA by Thermal Denaturation†

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ABSTRACT: The secondary structure of highly purified globin messenger RNA has been investigated by alkaline hydrolysis, nuclease digestion, and thermal denaturation. The thermal denaturation properties of globin messenger have been compared to poly(U), poly(A), and a synthetic random sequence RNA copolymer. From these studies it is concluded that globin mRNA contains considerable secondary structure and that the amount of helical structure is greater than that which occurs with a random sequence polyribonucleotide. Globin mRNA contains, by comparison to the secondary structures of native DNA, tRNAs, or 18S rRNA, helices which involve 55–62% of the bases or 58–68% if a correction is made for the 3′-terminal poly(A) segment. The helices of globin mRNA appear to be unique as differences in the NaCl stabilization of this RNA have been noted when compared to other naturally occurring and synthetic RNAs. Comparison of the hyperchromicity maxima, obtained at 260 and 280 nm for globin mRNA and 18S rRNA, indicates that the helices of the two RNAs contain similar numbers of G·C base pairs. Differential analysis of NaCl stabilization curves indicate three discrete thermally denaturable helix types in globin mRNA.

The secondary structure of eukaryotic mRNAs is of interest as it has been suggested that bacteriophage mRNAs such as f2, R17, MS2, and QB exhibit a high degree of secondary structure (Min Jou et al., 1972; Adams et al., 1972; Henkens and Middlebrook, 1973; Phillips and Bobst, 1972) and that this structure may play an essential role in the function of these viral mRNAs (Lodish, 1968a,b, 1970; Felsenfeld, 1967; Fukami and Imahori, 1971; Mills et al., 1973; Voorma et al., 1971). In contrast to these bacteriophage mRNAs, eukaryotic mRNAs have not yet been studied in such detail.

Examinations of the amino acid sequences (by computer techniques) of cytochrome *c* and the α variants of human hemoglobin, Constant Spring and Wayne, have revealed sequences in the constructed mRNA for these proteins which could base pair to form helices (White et al., 1972; Laux et al., 1973). Also the amino acid sequences (e.g., in cytochrome *c* from various sources) have been found to be constant in regions where the putative mRNA sequence can be made to fit into helices.

Although the above considerations suggest that secondary structure might exist in eukaryotic mRNAs, direct experimental proof must be obtained. The availability of a highly purified mRNA, such as the one for rabbit globin, provides the opportunity for a detailed analysis of the secondary structure of an animal mRNA. This paper describes the thermal denaturation of the globin mRNA and compares it to the thermal denaturation of both synthetic and naturally occurring nucleic acids.

Materials and Methods

Nucleic Acids Used for Comparison. Poly(U),[1] 1:1:1:1 random sequence ribonucleic acid copolymer, and poly(A) were purchased from Miles Laboratories, Inc., Kankakee, Ill. tRNA and 18S and 28S rRNAs were prepared from rabbit reticulocyte ribosomes. Ribosomes were treated with pyrophosphate (Holder and Lingrel, 1970) releasing tRNA and ribosomal subunits. The tRNA was further purified by phenol extraction while the rRNAs were prepared by dissociation of rRNA and protein by 1% sodium dodecyl sulfate followed by separation on 5–20% sucrose gradients (Holder, 1973). Calf thymus DNA was a gift from Dr. R. Krueger, University of Cincinnati. Experimentally determined extinction coefficients were used as previously described in detail (Holder, 1973).

Preparation of Globin mRNA, Test for Purity, and Chain Breaks. Globin mRNA was prepared by methods described previously (Aviv and Leder, 1972; Gorski et al., 1974). The purification included both oligo(dT)-cellulose affinity chromatography and sucrose density gradient centrifugation. The purified RNA gave two bands, corresponding to the α- and β-globin mRNAs, when electrophoresed in polyacrylamide gels at 25° in a 99% formamide solvent (Morrison et al., 1974) and Figure 1. As no small molecular weight bands are seen in these denaturing gels the absence of hidden breaks in the mRNA is assumed.

Thermal Denaturation of Nucleic Acids. Thermal denaturation was performed by direct heating in an aluminum cuvet holder with embedded electrically controlled heating coils. The precision of heating was ±0.2°. In a typical experiment the quartz cells were charged with 85–90 μg of

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[1] Abbreviations used are: poly(U), poly(uridylic acid); poly(A), poly(adenylic acid); h_{260}, ratio of the absorbance at 260 nm at any given elevated temperature to absorbance at 260 nm at 20.0°; T_m, median melting temperature is that temperature exhibited at one-half the maximal value of h_{260}; interquartile range, the range in temperature exhibited between 1/4 and 3/4 of the maximal value of h_{260}.

FIGURE 1: Denaturing polyacrylamide gel analysis of purified globin mRNA. Polyacrylamide gels (4.8%) were made and run in 99% formamide as previously described (Morrison et al., 1974). Gels were subjected to electrophoresis for 30 min at 1 mA/gel, then for 2 hr at 5 mA/gel. The gels were stained with Toludine Blue O for 1 hr and destained with H_2O.

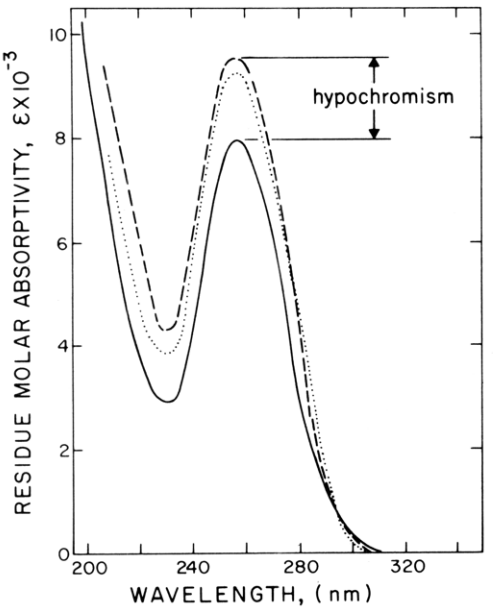

FIGURE 2: Ultraviolet adsorption spectra of globin mRNA. Spectra of globin mRNA in 0.1 m*M* NaCl–5 m*M* sodium phosphate (pH 7.40) at 20° (—); calculated adsorption spectra (Beaven et al., 1955) for an equimolar solution containing ribonucleotides in the same proportions as in intact globin mRNA (- - -); spectra of globin mRNA at 90° after being heated from 20° at a rate of 0.5°/min (• • •).

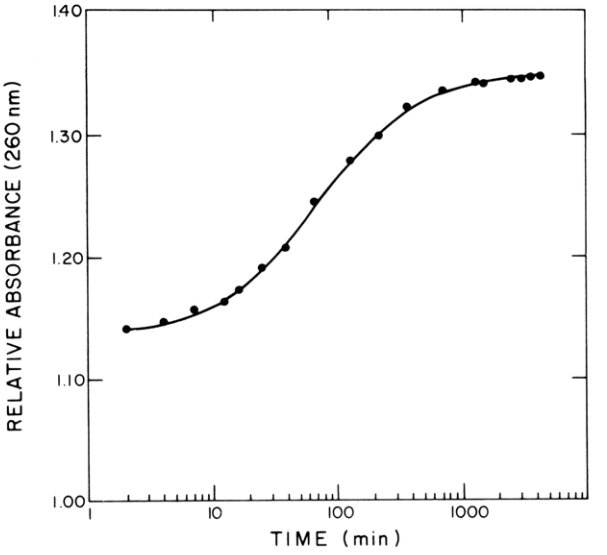

FIGURE 3: Time course of alkaline hydrolysis of globin mRNA. Globin mRNA in 5 m*M* Tris-HCl (pH 7.40) was made 0.4 *N* KOH at 37°. The A_{260} is measured at various times after neutralization with monosodium phosphate buffer and compared to the A_{260} of an untreated globin mRNA control (in the same resultant buffer as each hydrolysis time point) to give relative absorbance increase due to hydroxide hydrolysis.

nucleic acid in 2 ml of standard buffer: 0.1 *M* NaCl–5 m*M* sodium phosphate buffer (pH 7.40). To prevent solvent losses caused by prolonged heating drops of silicone oil were placed on top of the solution in the cuvet. The rate of heating or cooling was 0.4–0.5°/min and A_{260} measurements were made at 0.3° intervals. All thermal data were corrected for the "dilution effect" caused by the thermal expansion of water. The differential heating curves were calculated by determining the slope of the h_{260} (relative increase in A_{260}) vs. temperature plots at 0.3° intervals between 20 and 95°.

Results

Hypochromism in Globin mRNA. It is known that most ribonucleic acids absorb less in the ultraviolet in the polymeric form than an equivalent amount of free ribonucleotides in solution. This phenomenon is called hypochromism and is due to overlap of the π electrons of heterocyclic bases (Michelson, 1963; Pullman, 1968).

It is seen in Figure 2 that rabbit globin mRNA exhibits a hypochromic effect with respect to an equivalent amount of free nucleotides. The hypochromism suggests an organized structure within this mRNA. If this structure is the result of short range interbase forces of hydrogen bonding and stacking typical of other nucleic acids previously studied (Doty et al., 1959), then the hypochromic effect should be capable of reversal. When purified globin mRNA was slowly heated increased ultraviolet absorbance (hyperchromism) was observed at 260 nm compared to the native state at 20° indicating the bases were going from a more organized state to a less organized state. The A_{260} of the heated globin mRNA does not reach the full adsorption exhibited by the free ribonucleotides possibly due to helical regions of particularly high (G + C) content (Doty et al., 1959; Rich and Kasha, 1960) or residual vertical stacking interactions (Brahms et al., 1967; Warsaw and Tinoco, 1965a,b; Cantor and Tinoco, 1965) which are an obligatory function of the ribonucleotides being covalently linked in the globin mRNA chain (nearest neighbor interactions).

In order to directly determine the total amount of secondary structure in the globin mRNA an exhaustive alkaline hydrolysis in 0.4 *N* KOH was performed at 37°. Kinetics of hydrolysis (Figure 3) shows initially a rapid rise (39% of the total A_{260} increase) in the first 2 min. This is most probably due to rapid denaturation of the labile secondary structures by the hydroxide ion which would be expected to compete for the protons shared between the bases. The lag in A_{260} increase (5.9%) in the 2–10-min period agrees with results reported for 18S and 28S rRNA where it was found that RNA initially undergoes a tenfold reduction in size by hydroxide ion with only minimal disruption of RNA secondary structures (Cox et al., 1968). These results are consistent with the concept that amorphous regions are degraded before structured regions. A rapid increase in A_{260} occurs in the 10–200-min hydrolytic time period (43.4%) which indicates the hydrogen-bonded loops of the globin mRNA are rather frayed to such an extent that they are melted at 37°. The maximum and limiting value of h_{260} was 1.355, indicating that a total hyperchromism of 35.5% is associated with the total disruption of globin mRNA into its component ribonucleotides.

Another method used to investigate the existence of secondary structure in globin mRNA is exhaustive ribonuclease degradation. Limiting values of relative A_{260} increase for exhaustive pancreatic and T_1 ribonuclease digestions

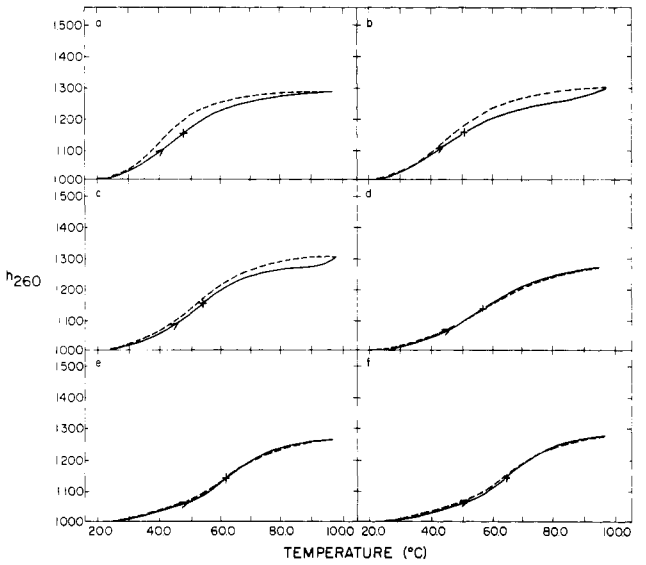

FIGURE 4: Thermal denaturation of globin mRNA. Helix-coil transitions were made in the following solvents: (a) 0.5 m*M*, (b) 15 m*M*, (c) 50 m*M*, (d) 100 m*M*, (e) 250 m*M*, and (f) 500 m*M* NaCl in 5×10^{-4} *M* sodium phosphate (pH 7.40) buffer.

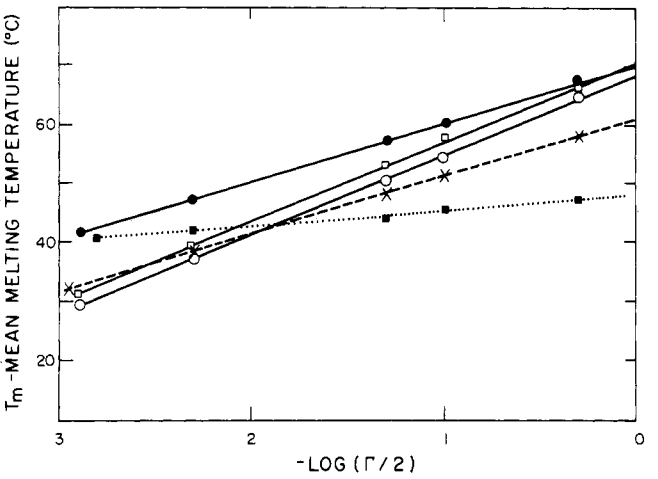

FIGURE 5: Salt stabilization of globin mRNA (O-O) compared to the naturally occurring cytoplasmic tRNAs (●-●) and 18S rRNA (□-□), and the synthetic polynucleotides poly(A) (■ · · ■) (a fully stacked but non-hydrogen bonded polymer at pH 7.4) and RNA copolymer (x- -x) (a RNA generated randomly in a nontemplate synthesis which contains each of the ribonucleotides in approximately equal proportions). An appropriate amount of NaCl was added to each of the nucleic acid solutions in 0.5 m*M* sodium phosphate (pH 7.4) prior to thermal denaturation as in Figure 4. The mean melting temperatures were calculated and plotted against the logarithm of the buffer ionic strength (Doty et al., 1959).

were 1.256 and 1.242, respectively. This small difference suggests that relatively few of the secondary structures in globin mRNA are stable without the participation of guanosine residues in either base stacking or base pairing.

Thermal Denaturation and Renaturation of Globin mRNA in Solvents of Increasing NaCl Concentration. When globin mRNA is placed in aqueous solvents which are 0.5, 15, 50, 100, 250, and 500 m*M* in NaCl concentration, a progressive increase in the median melting temperature (T_m) is observed (Figure 4). The mean melting temperatures are 48.3, 50.4, 53.7, 57.9, 61.6, and 63.3° for each of the respective NaCl concentrations. The cooling curves indicate some hysteresis at the lower salt concentrations ($Na^+ \leq 0.05$ *M*). In experiments where the globin mRNA was cooled and placed at 0-4° for 2 hr virtually identical curves were obtained upon reheating as was obtained for the original denaturation curves. The thermal denaturation appears to be a readily reversible process indicating short, proximally located regions of homology (Lingrel et al., 1971; Holder, 1973).

The addition of salt to solutions containing RNA usually promotes an ultraviolet hypochromic effect as well as an increase in T_m. This effect is manifest by the salt at neutral pH by cationic shielding of the negative charges on the RNA phosphates resulting in decreased monopole-monopole repulsion interactions.

Figure 4 shows that with increasing salt concentration there is an increase in the median melting temperature of globin mRNA. However, when rRNA and synthetic RNA are compared to globin mRNA it is evident that the increased stability does not show the same proportionality for each RNA (Figure 5). The shielding of the negative charges potentiates the formation of more secondary structure in some RNAs than in others. This result is consistent with the concept that each RNA contains different types of helical structures as stacked structures like poly(A) (Felsenfeld, 1967) are relatively unaffected by changes in NaCl concentration.

Transfer and ribosomal RNA were chosen to be compared to globin mRNA as representatives of naturally occurring RNAs known to contain helical and stacked regions connected by single-stranded amorphous regions (Cox et al., 1968; Cox, 1970). tRNA shows an increased T_m with added NaCl indicated by a positive slope but not as large as the slopes for 18S rRNA or globin mRNA. This could be due to a compactness of tRNA secondary structure which reduces the salt stabilization response in contrast to the more open structured 18S rRNA and globin mRNA secondary structures.

The 18S rRNA and globin mRNA have similar proportional increases in T_m as a function of NaCl concentration, though at all salt concentrations tested the globin mRNA showed less thermal stability than the 18S rRNA. The similarity in slopes suggests that similar types of thermally denaturable secondary structures are present in both the 18S rRNA and globin mRNA but that those of the globin mRNA are of lower thermal stability. This lowered stability in globin mRNA could be due to either shorter helical loops, greater mismatching within loops, or loops which contain a lower (G + C) content than the loops in 18S rRNA. It has been determined that the base composition of globin mRNA is lower in (G + C) content than for 18S rRNA (Figure 7).

The random sequence RNA copolymer indicates less of a dependency of T_m on NaCl than either 18S rRNA or globin mRNA (Figure 5). This observation supports the observed lack of cooperativity (Figure 6b and discussed below) in the thermal denaturation of the random RNA copolymer.

Thermal Denaturation of Globin mRNA. (a) Comparison to tRNA, Calf Thymus DNA, and Poly(U). Globin mRNA has an appreciable amount of secondary structure when compared to tRNA which is known to have a very specific structure related to biological function (Figure 6a). The T_m for globin mRNA, which appears to be an approximate function of helical (G + C) content (Figure 7), was 57.9° compared to 60.0 and 89.5° for tRNA and DNA, respectively. The maximum h_{260} which is proportional to the total number of internucleotide bonds denatured was 42%

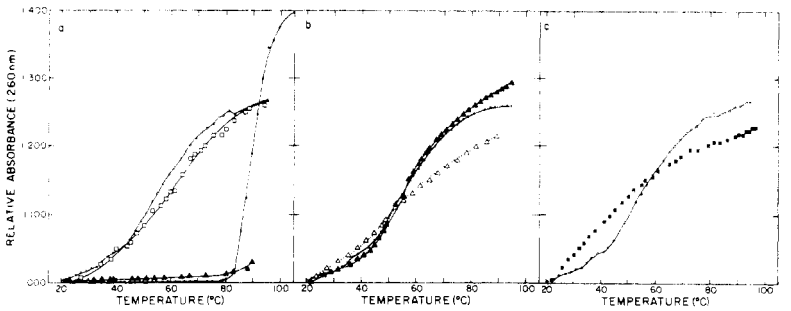

FIGURE 6: Thermal denaturation of globin mRNA (O-O) in 0.1 *N* NaCl-5 m*M* sodium phosphate (pH 7.4) compared to (a) tRNA (□-□), poly(U) (▲-▲), and calf thymus DNA (●-●); (b) 18S rRNA (rabbit) (▲-▲) and a 1:1:1:1 random sequence compolymer (△ · · △); (c) poly(A) (■ ■). For tabulated thermal denaturation parameters see Table I.

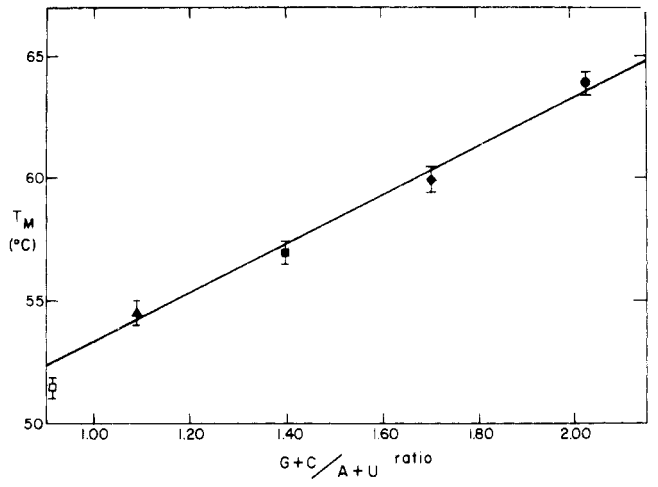

FIGURE 7: Thermal denaturation in 0.05 *M* NaCl-0.5 m*M* sodium phosphate (pH 7.4) as a function of base composition: (▲) rabbit globin mRNA; (■) rabbit 18S rRNA; (♦) rabbit tRNAs; (●) rabbit 28S rRNA; and (□) the random RNA copolymer. T_m values are the mean denaturation temperatures for the entire 20–95° range.

Table I: Summary of Thermal Denaturation of Various Nucleic Acids Compared to Globin mRNA.

Nucleic Acid[a]	T_m (°C)	Maximum Change in h_{260} (%)	Interquartile Range (°C)
Poly(U)	~5–8		
Poly(A)	46	23.6	30.5
1:1:1:1 random RNA copolymer	51.5	22.2	30.0
rA·rU	56.5	50.0	5.0
Globin mRNA	57.9	26.8	22.5
18S rRNA	59.0	29.5	22.0
tRNA	60.0	26.5	25.5
28S rRNA	64.0	30.8	28.5
Calf thymus DNA	89.5	42.5	7.5
rG·rC[b]	120.0	50.0	

[a] The nucleic acids used in the thermal denaturation studies are arranged in order of increasing T_m or stability. Each nucleic acid was thermally denatured in 0.1 *M* NaCl–5 m*M* sodium phosphate (pH 7.40). [b] T_m estimated as well as the change in absorbance (Doty, 1959).

of DNA while the h_{260} for tRNA and globin mRNA were 26.5 and 26.8%, respectively. A rather simplistic estimation of 63% helical secondary structure for globin mRNA may be made by direct comparison of the h_{260} for globin mRNA to native calf thymus DNA assuming that the native DNA is 100% double helical. The interquartile range is smaller for DNA than for both tRNA and globin mRNA (Table I). Both parameters, h_{260} and interquartile range, indicate by comparison to DNA and tRNA that globin mRNA is a partially structured polyribonucleotide whose helical regions are interconnected by less restricted amorphous regions. Poly(U) has little or no secondary structure at 20° or higher (Lipsett, 1960), and therefore represents a single-stranded RNA at the temperature employed to demonstrate the globin mRNA thermal transition. The data in Figure 6a indicate that unlike poly(U), globin mRNA has secondary structures which can be heat denatured in the 20–96° thermal range.

(b) Comparison to 18S rRNA and 1:1:1:1 Random Sequence RNA Copolymer. Cox has estimated 61–69% of the bases in 18S rRNA are located in double-helical segments (Cox, 1970) while Gould and Simpkins have estimated 68% (Gould and Simpkins, 1969). Because the melting profiles of 18S rRNA and globin mRNA are similar, but *always* found to be significally different as typically shown in Figure 6b, it is concluded that a correspondingly proportional amount of secondary structure is present in the globin mRNA. A comparison of h_{260} values indicates globin mRNA contains 55–62% secondary structure using the 18S rRNA results of Cox or 61% using the weighted average results for 18S rRNA of Gould and Simpkins.

The globin mRNA exhibits a higher T_m and a more cooperative thermal transition when compared to a random sequence RNA containing the four ribonucleotides A, G, C, and U in approximately equal proportions. Comparison of h_{260} values suggests that globin mRNA may contain as much as 20% more helical structure than the RNA copolymer. The interquartile range, a reflection of the intramolecular helical interactions (Cox, 1970), is greater for the random sequence RNA copolymer than for rabbit 18S rRNA, tRNAs, or globin mRNA while the h_{260} maximum is less for the synthetic RNA than for the h_{260} maxima of native RNAs. These observations suggest that globin mRNA not only contains more secondary structure than a random sequence RNA but also contains a higher organization of helical structures which fold in a cooperative manner. Apparently, the randomly occurring regions of homology in the random sequence copolymer have an equal probability of being distal as well as proximal to each other.

It has been reported that G·C base pairs make a different contribution to the melting absorption spectra than the A·U base pairs (Felsenfeld and Sandeen, 1962; Fresco et al., 1963). The $\Delta A_{280}/\Delta A_{260}$ ratio indicates the relative amount of G·C base pairs per helix (Cox and Kanagalingam, 1967). Upon thermal denaturation 28S rRNA gives a

ratio of 0.81 while 18S rRNA and globin mRNA ratios are 0.65 and 0.67, respectively. Such results indicate that the globin mRNA has as high (G + C) content within the helical regions as 18S rRNA while Figure 7 demonstrates the globin mRNA melts according to the overall (G + C) content as do other nucleic acids of comparable interest. These data are compatible with the concept that G·C pairs are most important to helical thermal stability. The T_m for the random copolymer is also indicated in Figure 7 for comparison though it is likely that there are fundamental differences between native and synthetically generated nucleic acids (Ricard and Salser, 1975).

(c) Comparison to Poly(A). Poly(A) is known to be fully stacked and contains no hydrogen-bonded bases at neutral pH (Brahms et al., 1966; Cox, 1970). In Figure 6c high molecular weight poly(A) (1×10^5) is compared to globin mRNA. Poly(A) shows a noncooperative melting typical of stacked bases behaving independently of each other in the polyribonucleotide (except for nearest neighbors) (Leng and Felsenfeld, 1966). In contrast globin mRNA shows a departure from the monotonic melting function exhibited by poly(A) again suggesting the presence of helical structures in the globin mRNA.

Differential Melting Curves of Globin mRNA. The melting curves in Figure 4 were found to be quite reproducible which provided justification to calculate (at sufficiently small intervals) the slope of the melting curves, $\Delta h_{260}/\Delta T$. The slope differential was plotted against temperature (Figure 8). It is obvious that at low salt concentrations a nondiscrete melting pattern resulted. However, upon elevating the NaCl concentration, two and possibly three peaks become prominent. These peaks are indicated in Figure 8f. The data would suggest that there are discrete helical species within the globin mRNA which can be independently stabilized by addition of NaCl and that thermally denature independently of each other similar to the process of independent melting species in tRNA (Römer et al., 1970). Peak 1 is representative of helices which are relatively enriched in A·U base pairs while peaks 2 and 3 are progressively enriched with G·C base pairs. This inference of helical base content in the three peaks is based on assumptions that the degree of mismatching within the three helix types is approximately the same as well as the percent bases which are stacked. These assumptions need not be strictly true, however. It is possible that each mRNA isolated will show unique differential melting profiles which could prove to be a useful method to discern what types of helices are present in each mRNA sequence.

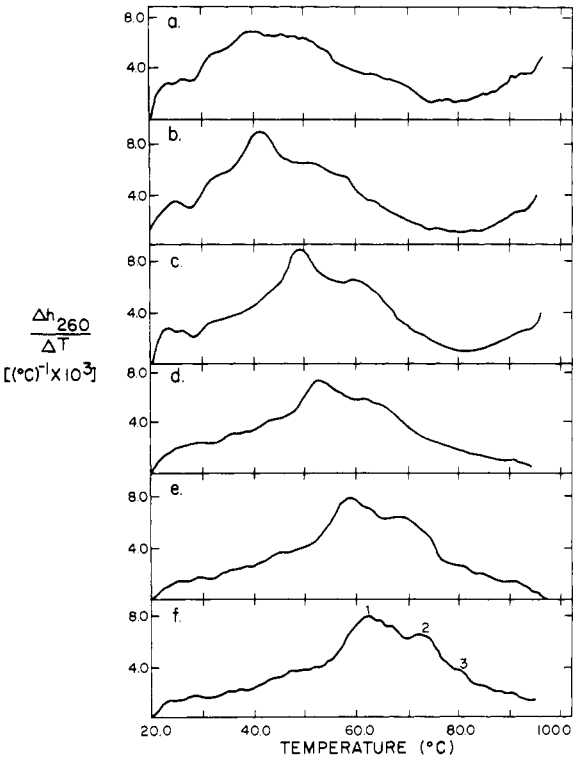

FIGURE 8: Differential melting curves of globin mRNA in 0.5, 1.5, 50, 100, 250, and 500 m*M* NaCl in 0.5 m*M* sodium phosphate (pH 7.40). Derivatives of denaturation curves in Figure 4 were made at 0.3° intervals and plotted against temperature. Each point used to calculate the increment of hyperchromicity in a 0.3° interval was the average result of four simultaneously determined thermal denaturations. The variation of each experimentally determined point never exceeded 1.5%.

Discussion

The globin mRNA used for the thermal denaturation studies reported here has been prepared in such a manner that it is free of protein, sodium dodecyl sulfate, and hidden breaks. Such a preparation can bind efficiently to oligo(dT)-cellulose, can be incorporated into protein synthesizing polysomes (J. W. Holder, unpublished results), and can promote the specific synthesis of rabbit globin α and β chains in a duck cell-free system (Gorski et al., 1974). Such studies indicate that the thermal denaturation studies presented here are representative of a highly purified mRNA containing a high percentage of functionally competent mRNA sequences for rabbit globin. Although globin mRNA can be resolved into two electrophoretic bands in 99% formamide corresponding to the α- and β-globin mRNAs (Morrison et al., 1974) some microheterogeneity probably exists in both mRNAs as different sizes of poly(A) occur at the 3′-termini (Gorski et al., 1974).

Theoretical predictions (Rich, 1966; Figueroa et al., 1972) have indicated that mRNA sequences would be expected to contain a low degree of autocomplementarity. Such regions might form intrastrand helices and could impede the movement of the ribosomes in translation. Seemingly in support of these predictions, there has been presented preliminary thermal denaturation data (Hunt and Laycock, 1969) which suggested that rabbit globin mRNA showed a lack of hydrogen bonding. In direct contrast to the preceding predictions and thermal denaturation data we report that globin mRNA from rabbit reticulocytes contains thermally denaturable helices which disorder in a cooperative and reversible process. In support of the present work it has been reported that rabbit globin mRNA (Lingrel et al., 1971) and mouse globin mRNA (Williamson et al., 1971) contain at least 50% helical secondary structure while Bobst et al. (1974) report values of 65–70% helical content for rabbit globin mRNA. It should be noted, however, that the previous studies suffer somewhat in that the globin mRNA preparations were not as highly purified as the one used here. Further evidence for secondary structure has also been obtained using highly purified rabbit globin mRNA by circular dichroism studies and ethidium bromide binding (J. W. Holder and J. B. Lingrel, unpublished).

Comparison of the thermal denaturations of poly(A) and poly(U) shows a difference indicative of a fully stacked non-hydrogen bonded RNA polymer compared to a polymer which possesses neither stacking nor hydrogen bonded interactions (in the 20–95° range) while comparison of glo-

bin mRNA to poly(A) shows a difference between an RNA which has contributions of both hydrogen bonding and base stacking and an RNA polymer which has only base stacking. Further comparisons of globin mRNA to the naturally occurring tRNAs, 18S rRNA, and native DNA allow relative comparisons to be made to nucleic acids whose secondary structures have been the object of numerous investigations. Finally it is apparent that since globin mRNA has more helical secondary structure than a random sequence RNA copolymer it may be inferred that globin mRNA contains more than a random amount of secondary structure (Holder, 1973).

As 26.8% maximum hyperchromicity is the result obtained for globin mRNA, comparison to 40.8% hyperchromicity estimated for fully structured duplexes containing 50% (G + C) content (Doty, 1962) should indicate how many bases in globin mRNA are involved in secondary structure, i.e., stacked and hydrogen bonded bases. This calculation indicates that approximately 65.7% of the bases are structured. The previous estimate of 55–62% helical content in globin mRNA by comparison to 18S rRNA should be considered a minimal estimate since it is known that globin mRNA contains an 8–10% poly(A) tract at its 3′-terminus and that poly(A) stacks but is known not to form base pairs at pH 7.4, the conditions which were used for the melting studies presented here. Correction for the percentage poly(A) content in globin mRNA suggests that the range of helical content is 58–68% for the portion of globin mRNA minus the 3′-poly(A) isostich segment.

The importance of viral RNA secondary structure to viral function has been previously demonstrated (Lodish, 1970; Fukami and Imahori, 1971). These authors through selective perturbation of the viral RNA secondary structure could differentially alter the quantitative functions of the polymerase and maturation mRNA sequences within bacteriophage f2 and R17 RNAs. Interestingly the mode of translation of the tricistronic R17 RNA can be changed between simultaneous and sequential according to the pretreatment of the viral RNA prior to assay for mRNA activity in a cell-free system (Fukami and Imahori, 1971). These studies suggest that secondary structure not only directs the ribosomes to the proper reading frame but controls the number of ribosomes gaining access to each respective initiation site on the viral RNA. Although no direct correlation has yet been established between globin mRNA function and secondary structure the hypothesis has been advanced that the globin mRNAs as well as the non-globin reticulocyte mRNAs are differentially initiated due to different secondary structures in these mRNA (Lodish and Desalu, 1973).

The secondary structural data given here demonstrate the average physicochemical properties of both α- and β-globin mRNAs. The distribution of the secondary structures within these mRNAs is unknown at this time and will have to await the primary sequencing of these mRNAs. If the secondary structure is within the translated region then this might offer the first indication that there exists information in eukaryotes extending beyond the triplet codon involving many bases. Secondary structure in eukaryote mRNAs is probably not needed for packaging as is necessary for the bacteriophage RNAs. If the secondary structure lies in the extracoding regions the possibility remains that these structures could control translation. One plausible control mechanism has been proposed whereby specific mRNA helical structures bind specific proteins (Scherrer, 1973). However, definite proof for these types of specific associations in eukaryotes remains to be demonstrated.

Although the presence of 58–68% helical structures in globin mRNAs may restrict the number of possible nucleotide sequences, it is believed that this restriction could be necessary to preserve potentially important regions of the globin mRNA and as a consequence preserve the amino acid sequences coded by these helical mRNA regions. Such preservation of amino acid sequences could maintain the essential regions basic to globin structure in the formation of functional hemoglobin.

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Purification of SV-40 Messenger RNA by Hybridization to SV-40 DNA Covalently Bound to Sepharose†

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ABSTRACT: SV-40 DNA sheared form was coupled in a stable covalent bond to cyanogen bromide activated Sepharose. Under the conditions used at least 80% of the DNA was bound to Sepharose. The $T_{1/2}$ of hybridization of 0.5 μg/ml of SV-40 cRNA to SV-40 DNA-Sepharose was 1 hr. This rate of hybridization is sufficiently rapid to purify SV-40 sequences from solutions containing as little as 0.05–0.1 μg/ml. Nonspecific hybridization of RNA is in the range of 0.1–0.2% of the total input RNA. The DNA-Sepharose is fairly stable and can be reused several times to purify RNA. The SV-40 DNA-Sepharose was used to select large quantities of virus specific RNA from SV-40 infected BS-C-1 cells. The virus specific RNA when added to cell-free extracts from wheat germ was shown to direct the synthesis of the major viral structural protein VP-1.

Some DNA viruses, such as Simian Virus 40 (SV-40) (Oda and Dulbecco, 1968), do not inhibit host cell RNA synthesis after infection. In order to purify virus specific RNA, procedures utilizing hybridization of RNA from infected cells to virus DNA have been reported (Bautz and Hall, 1962; Gillespie and Spiegelman, 1965; Bautz et al., 1966; Riggsby, 1969; Weinberg et al., 1972; Prives et al., 1974a; Coffin et al., 1974; Eron and Westphal, 1974). As RNA-DNA hybridization competes unfavorably with DNA–DNA reannealing, the denatured viral DNA has been immobilized on nitrocellulose filters preventing the renaturation of the DNA. When RNA from infected cells is incubated under suitable conditions with virus DNA thus immobilized, only viral RNA forms a stable duplex with the DNA. The virus specific RNA can be subsequently eluted from the filters under hybrid denaturing conditions. This procedure has been used to isolate viral RNA from SV-40 infected cells in order to study viral RNA metabolism (Weinberg et al., 1972, 1974) and its cell-free translation into virus specific polypeptides (Prives et al., 1974a). However, there are several problems associated with this procedure. RNA eluted from the filters is translated very inefficiently most probably due to the coelution of inhibiting contaminants including possibly a small amount of viral DNA (Prives et al., 1974b). It was found that virus-specific RNA must undergo a further purification through oligo(dT)-cellulose before it could be effectively translated and this invariably results in a considerable loss of RNA. Furthermore, filters can be used only once in a selection procedure, a serious drawback when the amount of DNA is limited in

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