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# OPTICAL STUDIES ON THE STRUCTURE OF HEMOGLOBIN MESSENGER RIBONUCLEIC ACID

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SUMMARY

The secondary structure of hemoglobin mRNA (HbmRNA has been investigated by optical rotatory dispersion (ORD), circular dichroism (CD) and ultraviolet absorbance spectrophotometry. The dependence on temperature or reaction with formaldehyde of the CD and absorbance are characteristic of a structure with substantial base pairings at 20ºC. The presence of Cotton effects in one of the major ultraviolet absorption bands indicates a highly ordered secondary structure. The UV hyperchromism on thermal denaturation is consistent with a value of 58% double helical content.

## INTRODUCTION

While considerable progress has been attained in the understanding of the structure of several nucleic acids, we still know little about that of informational polyribonucleotides.

Viral RNAs were shown to be predominantly hydrogen bonded whereas synthetic polynucleotides were susceptible of in vitro translation only when lacking hydrogen bonds (1). Of all "natural" messengers, that is those messengers normally utilized by uninfected cells, only ovalbumin (2), procollagen (3) and hemoglobin mRNA (4-7) have been studied.

Earlier reports on the nature of the secondary structure in HbmRNA suffer in two respects. First, the extent of contamination of the mRNA with 18S rRNA, for example, is hard to judge. One report of an exceptionally high helical content (70%) for HbmRNA may be due in part to such contamination, as may also be the striking similarity in denaturation parameters between 18S rRNA and HbmRNA found by Holder & Lingrel (7). Second, a valid quantitative evaluation of the degree of secondary structure in high molecular weight RNA and the nature of the

ABBREVIATIONS

SDS, sodium dodecyl sulfate; ORD, optical rotatory dispersion; CD, circular dichroism.

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forces contributing to this structure is still lacking.

Because forces, besides hydrogen bonds, have also been definitely implicated in the stabilization of the secondary structure of polynucleotides (8) we have examined the secondary structure of HbmRNA by optical rotatory dispersion (ORD), circular dichroism (CD) and ultraviolet spectrophotometry, under conditions in which the solvent contribution can be evaluated as well as that from hydrogen bonding.

## MATERIALS AND METHODS

Cellular fractionation and HbmRNA purification and characterization. The preparation of rabbit reticulocyte ribosomes, RNA extraction by a cold chloroform-phenol method and HbmRNA purification by two consecutive chromatographies through oligo(dT)-cellulose have already been described (3,9). Purity of the HbmRNA fraction was established through polyacrylamide gel electrophoresis (10).

For the purposes of the present paper, HbmRNA is defined as a 9S RNA species recovered from reticulocyte ribosomes which can be translated in vitro by a wheat germ cell-free system into polypeptides which co-migrate with carrier hemoglobin in SDS-polyacrylamide gel electrophoresis (Santos, D'Abronzo & Brentani, unpublished data).

Optical rotatory dispersion (ORD) and circular dichroism (CD). Measurements were carried out in a 1 cm path length jacketed cell with a Cary 60 spectropolarimeter, with a COLORA liquid circulator for thermostatic control. The temperature was monitored by using a Metler TM 15 telethermometer with a bimetallic probe.

The optical rotatory dispersion is expressed in terms of $|m'|$, the reduced mean residue rotation (11) defined as

$$|m'| = |\alpha| \cdot MRW/100 \cdot \frac{3}{n^2 + 2}$$

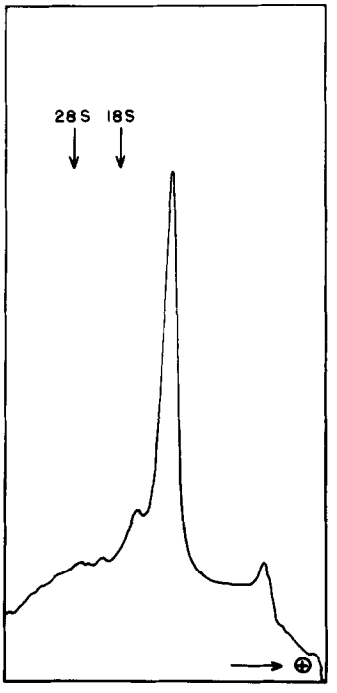
where $|m'|$ is the specific rotation at wavelength $\lambda$, MRW is the mean residue weight, i.e., molarity of residues, calculated from base composition values published for HbmRNA (4) and n is the refractive index of the solvent (12).

Circular dichroism measurements were converted into extinction per g atom of P by assuming a molar extinction of 8000 $\ell x g\ atom^{-1} x cm^{-1}$ for HbmRNA (13).

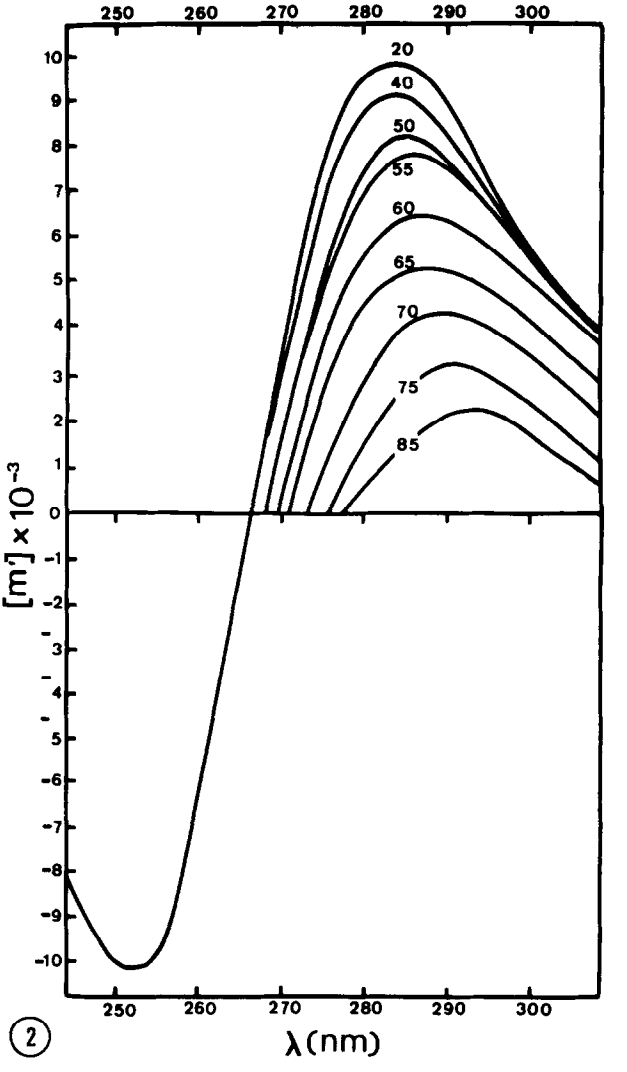
The value for the molecular ($\varepsilon p$) residue extinction coefficient was obtained by alkaline hydrolysis (14).

Ultraviolet absorbance - temperature profiles. RNA samples were dissolved in 10mM sodium phosphate buffer, pH 6.8, containing 0,1M NaCl unless otherwise specified. Absorbance measurements were made with a Zeiss DMR 21 recording spectrophotometer.

The temperature inside the sample cell was calibrated using a thermocouple inside the reference cuvette. All temperature studies were corrected for thermal expansion of the solvent. Absorbance temperature profiles were obtained by raising the temperature about 5º at a time and waiting 15 minutes before measuring the absorbance. Because the experiments took 3 hr, the cells containing both solutions and solvent were weighed before and after each experiment to check for




(1)




(2)

Fig 1. Polyacrylamide gel electrophoresis of HbmRNA.

Fig 2. Optical rotatory dispersion of HbmRNA as a function of temperature. HbmRNA (40µg) in 0,1M sodium phosphate buffer, pH6.8. Path length, 1cm. |m'| is the reduced mean residue rotation.

loss due to evaporation. Using stoppered quartz cells, less than 0.3% loss was observed in any experiment.

## RESULTS

Fig 1 shows the profile of HbmRNA as revealed by acrylamide gel

electrophoresis. The RNA fraction appears as a single peak with a mobility corresponding to 9S (4).

The optical rotatory dispersion curve of HbmRNA, at 20ºC shows a positive Cotton effect with a peak at 285nm; $|m'|$ =10,362±245; a cross-over point (zero rotation) at 267 nm and a trough at 253 nm; $|m'|_{253}$=-12,000 (Fig 2). Upon raising the temperature the amplitude of the 285nm peak slowly decreases with a concomitant shift of the peak and the cross-over point to longer wavelengths. At 85ºC the peak is at 292 nm and $|m'|_{292}$=2,442±276. The decrease in the Cotton effect can be taken to indicate a loss of asymmetry or secondary structure and most probably indicates a conformational helix-random chain transition (15). The value of $|m'|_{85º}$, however is greater than the predicted value of $|m'|$=2,122 calculated from the mole ratio of the monomers for a totally random non-interacting chain of HbmRNA. (The monomer rotations of CMP-5', AMP-5' and GMP-5' are taken from Fasman, Lindblow and Seaman (16)). This would indicate a small amount of residual structure or local base interaction.

After cooling to 20ºC the original spectra were restored: differences between the spectra obtained before and after heating were less than 5%.

The possible contribution of forces other than hydrogen bonds to HbmRNA conformational stability was further investigated by measuring $|m'|$ in the presence of ethylene glycol, a solvent that has been asserted to disrupt hydrophobic interactions (21), and after reaction with formaldehyde at 90ºC, which blocks amino groups capable of hydrogen bonding (17). Ethylene glycol causes a slight reduction in $|m'|$ ; after reaction with formaldehyde, $|m'|$ is greatly reduced (table I).

A comparison of the CD spectrum of HbmRNA before and after reaction with formaldehyde for 15 minutes at 90ºC (Fig 3) indicates that hydrogen bonds contribute to the overall structure, because of the reduction in ellipticity at 260 nm after reaction. The small negative peak near 300 nm has been attributed to a $n \rightarrow \pi^*$ transition (15).

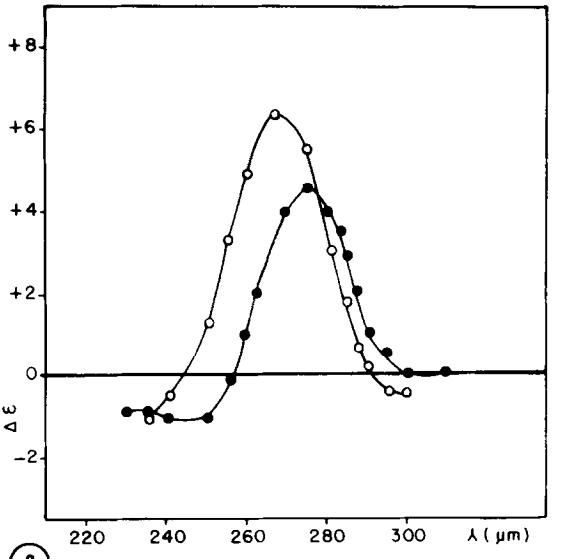
The U.V. absorption spectrum of HbmRNA is plotted at 255 nm. At this wavelength, Boedtker (19) has proposed the criterion that a 100 per cent double-stranded structure will have a hypochromicity of 0.3. Our experimental results for HbmRNA indicate a hypochromicity of 0.175 which would correspond to 58% double helical structure. Evidence for

TABLE I

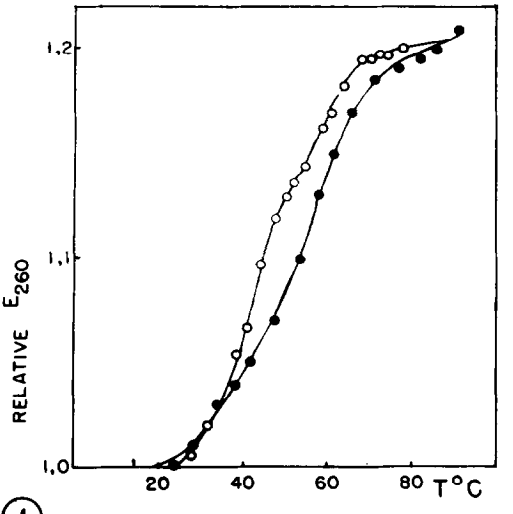
Values of $|m'|^{+}$ of HbmRNA under varying conditions in sodium phosphate buffer 0.1M, pH6.8

| Conditions | Peak (nm) | $|m'|^{\S}$ in degrees |
|---|---|---|
| SC*, 20ºC | 280 | 10,362 |
| SC, 85ºC | 292 | 2,442 |
| SC, 20ºC recooled | 280 | 10,124 |
| SC+90% ethylene glycol (20ºC) | 290 | 8,341 |
| SC+1% HCHO preheated, 20ºC | 290 | 4,920 |

+ mean reduced residue rotation
§ $|m'|$ values are ± 250
*SC= standard conditions, namely sodium phosphate buffer, 0.1M, pH6.8.




(3)




(4)

Fig 3. Circular dichroic spectra of HbmRNA . HbmRNA (40µg) in 0.1M sodium phosphate buffer, pH 6.8 before (-0——0-) and after reaction with formaldehyde (-●——●-).

Fig 4. Denaturation spectrum of HbmRNA in sodium phosphate buffer, pH 6.8. (-0-), 0.01M and (-●-), 0.1M buffer.

the presence of at least two distinct species of helical segments differing in $f_{AU}/f_{GC}$ is given in figure 4, which shows the relative values of $E_{260}$ in the range 25º - 85º. The biphasic nature of the

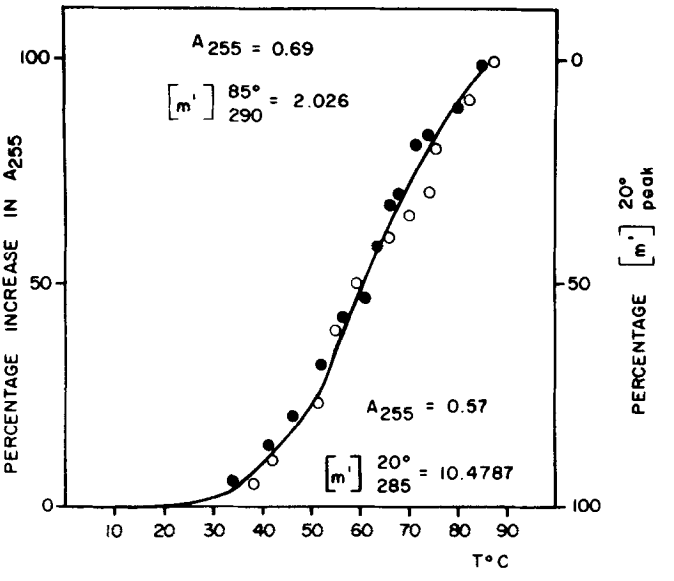



Fig 5. Correlation between percent change in optical activity and percent change in absorbance of HbmRNA upon heating from 20 to 90°C. Percent change in |m'| (-●-); Percent change in absorbance (-0-).

transition from helical to unfolded forms is evident at low sodium phosphate concentrations.

It is also evident from Figure 4, that Tm of the melting transition is dependent upon salt concentration, suggesting that changes in double-helical secondary structure make a large contribution to ΔE.

Fig. 5 shows that the percent change in |m'| and in absorbance as functions of temperature agree closely. Both methods thus measure a common structural transition.

## DISCUSSION

The optical rotatory dispersion studies presented here (Fig 2 and Table I) strongly indicate that HbmRNA exists in an asymmetric structure, probably a helical conformation. The decrease in the Cotton effect with peak at 280 nm is indicative of a helix random coil transition (16). The slight effect of ethylene glycol (20, 21) in reducing |m'| (Table I), minimizes the role of stacking interactions in maintenance of the native helical conformation of Hb mRNA. On the

other hand, the strong effect of formaldehyde upon |m'| (Table I) implicates hydrogen bonds as playing the major role in stabilization of the helical structure. Because the nature of the chromophore responsible for the Cotton effects has been altered on treating with formaldehyde, one cannot compare the size of the Cotton effects in the helical structure (16).

Further indication that the contribution of base stacking is negligible is given by the close agreement between ORD measurements and absorbance-temperature profiles (Fig. 5) since the optical activity of single stranded structures is large, whereas their hypochromicity is small (19, 22). These two methods, while generally reflecting the same change in structure, are capable of distinguishing minor differences in conformational stabilities (16).

It is clear from Fig. 4 that hydrogen bonds are very important to the maintenance of secondary structure because of the effect of ionic strength on the thermal denaturation profile. At high ionic strengths, the repulsion between negatively charged phosphate residues located on opposite strands of the double helix is neutralised (23).

The insignificant contribution of base-base stacking to the structure of HbmRNA is in striking contrast with that reported for rRNA and tRNA (24).

## ACKNOWLEDGMENTS

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