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Isoelectric Characteristics and the Secondary Structure of Some Nucleic Acids

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Abstract. The isoelectric characteristics of some nucleic acid preparations from rat liver have been examined. 10*S* and 4*S* RNA species and SV-DNA were found to have isoelectric points of 5.2, 6.0–6.7, and 4.35 respectively. The molecular charge ratios (net negative charge/nucleotide) were calculated. Using SV-DNA as a standard, these isoelectric characteristics and charge ratios have been interpreted as indicating that the 10*S* and 4*S* RNAs have 35 and 56% of the molecules involved in secondary structure.

Key words: Nucleic acids – Isoelectric characteristics – Secondary structure

Introduction

Biological macromolecules assume specific spatial configurations which are compatible with the state of thermodynamic equilibrium. The three dimensional organisation and the degree of secondary structure involved may be deduced by physical methods such as X-ray diffraction, optical rotatory dispersion etc. The spatial organisation of macromolecules in their native state is determined by their primary structure. A consideration of the primary structure of nucleic acids and proteins and the distribution of ionogenic groups along the molecule would suggest that these macromolecules are amenable to characterisation by isoelectric equilibrium method. The use of isoelectric data as a means of characterisation is a fairly new approach and first used by Sherbet et al. (1972) and Sherbet and Lakshmi (1973) in the characterisation of cell surfaces by treating them as analogous to multivalent ions. In this paper, we examine the isoelectric characteristics of some nucleic acid preparations in relation to their secondary organisation.

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Materials and Methods

a Preparation of RNAs

The RNAs were isolated from male albino rat livers. Livers were removed from animals starved overnight, washed, and homogenised in (w/v) 2.5 volumes of 0.25 M sucrose in TKM (50 mM *Tris*-HCl, pH 7.8, 25 mM KCl, 5 mM $MgSO_4$) at 4° C and a post-mitochondrial supernatant (PMS) was prepared. Six milliliters of the PMS was treated with deoxycholate (1.3% final concentration), layered over 4 ml of 2 M sucrose in TKM and centrifuged for 2 h at 221,000 *g* to obtain a polysome pellet.

The polysomes (20 mg RNA) were resuspended in 3 ml of TK buffer and 3 ml of EDTA 0.06 M in TK and washed at 37° C for 2 min to remove aggregates. One milliliter of the sample was layered on a 12–30% (w/v) linear sucrose gradient in TK buffer and centrifuged for 17 h at 95,000 *g*. Fractions containing the ribosomal subunits were collected, diluted 1:1 in TKM buffer and sedimented for 3 h at 108,000 *g*. Fractions sedimenting around 20*S* were pooled (approx. 10 ml) and the RNA was precipitated overnight at −20° C by the addition of three volumes of absolute ethanol. After sedimenting the precipitate at 12,000 *g* for 15 min, the RNA was resuspended in SDS buffer and layered over a 15–30% (w/v) linear sucrose gradient in SDS buffer and centrifuged for 21 h at 95,000 *g*. Fractions of 10*S* and 4*S* material were collected, the RNAs precipitated, dried and dissolved in 1 ml TKM and stored at −70° C. Simian virus DNA (SV-DNA) was obtained from Dr. T. E. Sensky.

b Isoelectric Characterisation

The isoelectric points (pI) were determined using an LKB-8101 column. A 3–10-pH gradient was generated using ampholines (1%, LKB). The pH gradient was supported by a 15–50% (w/v) linear sucrose gradient. After the pH gradient was formed, the material to be investigated was introduced into the column and equilibrated for 30 min. The optical density (260 nm) profile was recorded using a Unicam SP800 spectrophotometer. Two-milliliter fractions were collected and their pH was measured. Details of methods for generating pH gradient and post-pH introduction of material have been described elsewhere (Sherbet 1978; Sherbet and Lakshmi 1973; Sherbet et al. 1972).

c Calculation of Molecular Charge

The molecular charge was calculated from the isoelectric data using an equation devised by Sherbet (1978) and given in a modified form previously by Sherbet and Lakshmi (1973) as applicable to large particles. The equation for potential P_1 on a spherical particle of radius r bearing negative charge Q is

$$P_1 = \frac{-Q}{Dr}, \tag{1}$$

where D is the dielectric constant of water (78.54 at 25° C). Since this particle will be bounded by an electrical layer of unlike charges, the potential P_2 due to this outer sphere of charges will be

$$P_2 = \frac{Q}{D(r + d)}, \tag{2}$$

where d is the thickness of the electrical double layer. The resultant net potential P is provided by a summation of Eq. (1) and (2) and can be written as

$$P = \frac{-Q}{Dr} \times \frac{d}{(r + d)}, \tag{3}$$

i.e.,

$$-Q = P\,Dr \times \frac{(r + d)}{d}. \tag{4}$$

Since $d = {}^1/K$ where K is the Debye Hückel function [$0.327 \times 10^8 I^{1/2}$ at 25° C; I is the ionic concentration which, in the context of isoelectric focusing, is due to ampholines (0.01 M)]; Eq. (4) can be rewritten as follows:

$$-Q = P\,Dr\,(1 + Kr). \tag{5}$$

Now the net surface potential P is measured by isoelectric focusing and the relationship between P and pI is written as follows (Sherbet 1978).

$$P = (7 - \text{pI})\ 2.303\ RT/F,$$

where pI is the isoelectric point of the molecule, R the molar gas constant (8.315 J/° C), T the absolute temperature, and F the Faraday (96,500 Coulombs). The value of $2.303\ RT/F = 0.0592$ at 25° C. Since P is measured in volts ($V = 3.33 \times 10^{-3}$ e. s. u. and e, the electronic charge, $= 4.8 \times 10^{-10}$ e. s. u.), Eq. (5) assumes its final form, which is as follows:

$$Q = P\,Dr\,(1 + Kr) \times 3.3 \cdot 10^{-3}/e. \tag{6}$$

(The negative sign for net charge Q is omitted by design, since this quantity is described in the text as the net negative charge).

The radius r of the molecules is 1.489×10^{-6}, 3.02×10^{-7}, and 1.229×10^{-7} cm for DNA, 10S and 4S RNAs respectively, a spherical shape being assumed for the sake of simplification of charge calculation.

The charge ratio is defined as the number of electric charges per nucleotide. On approximation of molecular weight of the 10S and 4S RNAs to 0.4×10^6 and 0.27×10^5 daltons, it is estimated that they contain 1,230 and 80 nucleotides. For SV-DNA a molecular weight of 3×10^6 daltons was assumed which is equivalent to approximately 4,615 base pairs.

Results and Discussion

The present experiments indicate that the isoelectric points of the various samples range from 4.4 to 6.7 (Table 1). The values are higher than expected, but they are compatible with the pK'_a value of the primary phosphoryl groups and those of protonated bases (see Tables 2 and 3). The deprotonated (anionic) bases possess pK'_a in the range 9–10, but on account of their weakly acidic nature they hardly exert effects on the isoelectric characteristics when the highly acidic primary phosphoryl groups are present (Sherbet 1978). Drysdale and Righetti (1972) and Drysdale and Shafritz (1975) also found that nucleic acids focused in

Table 1. Isoelectric characteristics of rat liver RNAs

Preparation	pI	Charge ratio	% Secondary structure
SV–DNA	4.35	0.08	100
10*S* RNA	5.2	0.028	35
4*S* RNA	6.0–6.7	0.045	56

Table 2. pK'_a values of ionogenic groups of nucleic acids

Ionogenic groups	pK'_a	pK'_a of[a] deoxyribo-nucleoside	pK'_a of[a] ribo-nucleoside	Reference
Primary phosphoryl	1–2			
Uracil	9.5	9.3	9.25	Wempen et al. (1961)
Guanine	–	9.33	9.22	Clauwaert and Stockx (1968)
Thymine	9.9	9.8	9.68	Shugar and Fox (1952) Fox and Shugar (1952) Fox et al. (1958)
Adenine	4.1	3.8	3.6	Clauwaert and Stockx (1968)
Cytosine	4.46	4.3	4.1	Wempen et al. (1961)

[a] The pK'_a values of ionisation of the bases in the nucleoside show appreciable increase in nucleotide, being a result of the influence of the phosphate group (Clauwaert and Stockx 1968)

Table 3. The isoelectric points and ionogenic groups of rat liver nucleic acids

Ionogenic groups	pK'_a	Isoelectric point of nucleic acid	
		Calculated[a]	Observed
Primary phosphoryl	1–2		
Deprotonated bases	9–10	4.5–6.0	4.35–6.7
Amino groups	3.5–6		

[a] Calculated using the equation $pI = \log\left[\frac{\Sigma\, ka \cdot kw}{\Sigma\, kb}\right]^{1/2}$ where ka and kb are dissociation constants of the most acidic and most basic groups respectively, kw the dissociation constant of water

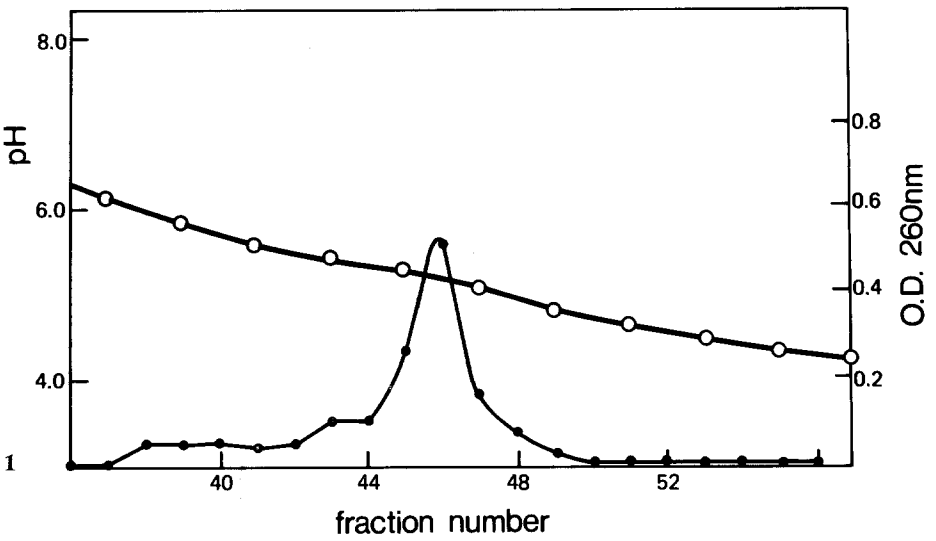
pH range of 3–6. The high pI values which we have seen may indicate that some basic protein may be associated with the RNAs or that the nucleic acids may have formed complexes with Mg^{2+}. Drysdale and Righetti (1972) found that the pI values of certain RNA molecules were lower when focusing was carried out in higher concentration of ampholines or in the presence of urea. It may be pointed out, however, that increasing ampholine concentration may have the effect of raising the pK values of amino groups which will be reflected in a reduction of the pI values. Also, it would appear possible that in the focusing of the nucleic acids in polyacrylamide gels of the concentrations employed by Drysdale and colleagues, molecular sieving may affect true focusing.

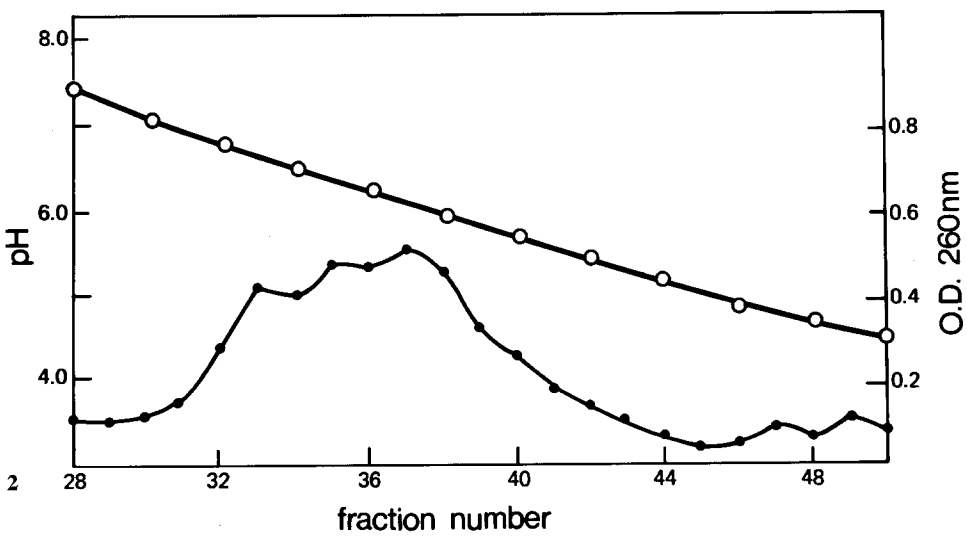
Hobart (1975) proposed the use of isoelectric focusing as a means in the investigation of proteins made up of non-covalently associated subunits. Using discontinuous urea gradients, Hobart (1975) showed how denaturation accompanied by change in pI could provide clues to the structure of proteins. Earlier Zeigler et al. (1972) had reported that the electrophoretic mobility of microbial DNAs was related to their $G + C$ content. They found that the greater the $G + C$ content of the nucleic acid, the greater was the electrophoretic mobility. Since base composition is known to affect secondary structure and since the stability of helical DNA is known to correlate with $G + C$ content, Ziegler et al. (1972) suggested that the process of electrophoresis may be discriminating between differences in secondary structure. In the present paper, the isoelectric characteristics of some nucleic acids have been interpreted in terms of the degree of secondary structure possessed by these macromolecules.

The two RNA species with 10*S* and 4*S* sedimentation characteristics were found to be isoelectric at pH 5.2 and a median pH value of 6.35 respectively (Figs. 1 and 2). From these data, it may be shown that the 10*S* molecule has on average 35 net negative charges distributed along the molecule, while the 4*S* RNA may be shown to possess, on average, four net negative charges per molecule. Now, if molecular weights of 0.4×10^6 and 0.27×10^5 daltons compatible with the occurrence of about 1,230 and 80 nucleotides respectively are assumed, it would appear that the distribution of the negative charges would occur at the rate of about one charge per 36 nucleotides (charge ratio = 0.028) in the 10*S* molecule, and one for approximately every 22 nucleotides (charge ratio 0.045) in the 4*S* molecule (Table 1).

The molecular charge ratio of SV-DNA was 0.08, which is equivalent to 3.2 PO_4^- groups per 20 base pairs. This value is in close agreement with the data of Clementi (1983), which suggest that there are about three phosphate groups per 20 base pairs that can participate in cation binding.

The calculation of the molecular charge has used the pI of the peak of absorption as the dominant isoelectric characteristic. This appears to be a reasonable basis because the peak broadening phenomenon is not a reflection of gross heterogeneity of the molecular species, although it cannot be denied that there could exist microheterogeneity in the species, as is apparent in our 4*S* RNA preparations (Fig. 2). Also, the isoelectric or electrophoretic behaviour is dependent upon the net charge densities, and within a macromolecular species it should not depend upon molecular size, unless an intramolecular heterogeneity exists in the form of domains composed of different ionisable species. Such





Figs. 1 and 2. Isoelectric profiles of 10*S* and 4*S* RNAs (○——○) pH curve; (●——●) optical density

intramolecular heterogeneity does not occur in nucleic acids. Therefore, within a given molecular species the banding of the macromolecules in isoelectric focusing merely reflects a pattern of normal continuous distribution.

In single-stranded nucleic acid molecules, stability is achieved by the apolar stacking forces and in double-stranded molecules by stacking as well as by hydrogen bonding between the bases which further restricts the movement of the bases, thereby contributing to the stability of the molecule. In single-stranded RNA, the stereochemistry is dependent upon a balance between the degree of

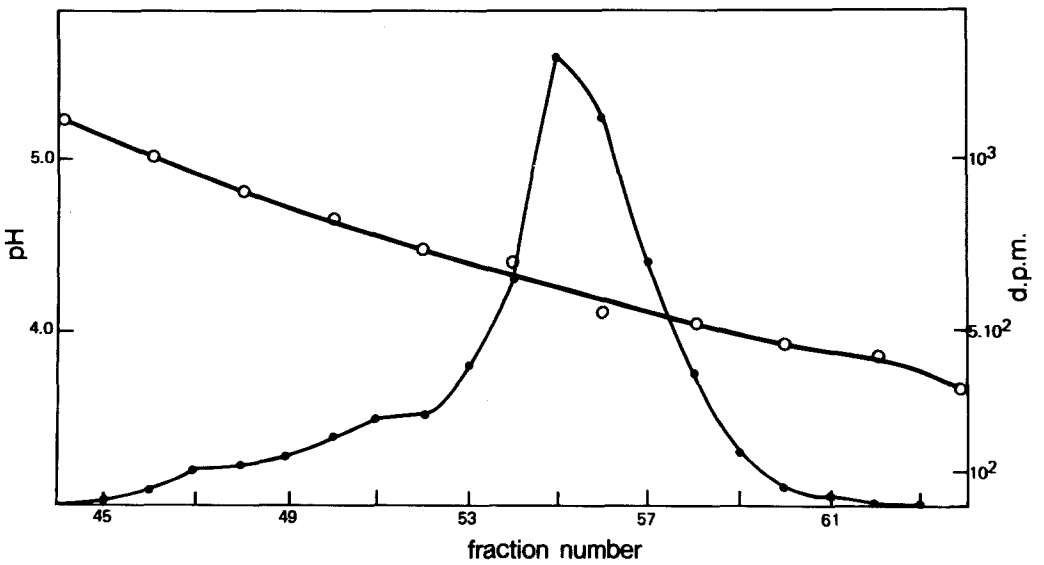


Fig. 3. Isoelectric distribution pattern of ^{14}C-SVDNA. (○———○) pH curve; (●———●) radioactivity

ionisation of the phosphate groups and of the bases. A complete ionisation of the latter will obviously alter this balance and cause a movement of the bases relative to one another. Since the Van der Waals' forces vary as an inverse function of the distance to the sixth power, even a small change will lead to considerable instability. It is thermodynamically unfavourable for the molecule to offer a vast area to the aqueous phase and hence the need for it to assume a secondary structure which may involve hydrogen bonding between complementary base pairs. However, if non-complementary residues occurred in the molecule, the conformation achieved would tend to sequester the bases that are not involved in complementary base pairing. Since deprotonation of the bases of uridine, guanosine, and thymidine would require high pH values, it may be expected that positive charges will be predominantly sequestered and this will have the effect of increasing the net negative charge of the molecule. In other words, the greater the degree of secondary structure achieved, the higher will be the effective net negative charge of the molecule.

The differences in the charge characteristics of the 10*S* and 4*S* RNAs may therefore reflect differences in the degrees of secondary structure achieved by these molecules. An estimation of the degree of secondary structure involved has been attempted using SV-DNA as a reference molecule. The major part (~ 83%) of SV-DNA had a pI of 4.35 (Fig. 3) and a charge ratio of 0.08 was calculated for this component.

If the SV-DNA with charge ratio of 0.08 is used as a reference standard molecule which has a perfect secondary structure, the 10*S* and 4*S* RNAs with charge ratios of 0.028 and 0.045 may be described as having 35 and 56% respectively of the molecule involved in secondary structure. RNAs do form secondary structures very much in the manner of DNA, but with imperfect

helices in 30–70% of their nucleotides. The secondary structure of several transfer RNAs has been deduced. It appears that approximately 60% of the residues occur as paired bases and on account of this, the molecule assumes a three dimensional configuration (Quigley et al. 1975; Rich and Rajbhandary 1976; Robertus et al. 1974; Simoncsits et al. 1977). The estimate of 56% secondary structure for 4*S* RNA deduced from isoelectric studies, therefore, appears to be reasonable.

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