Journal of Chromatography, 359 (1986) 147–155
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 850

APPLICATION OF THE STOICHIOMETRIC DISPLACEMENT MODEL OF RETENTION TO ANION-EXCHANGE CHROMATOGRAPHY OF NUCLEIC ACIDS

R. R. DRAGER and F. E. REGNIER*
Department of Biochemistry, Purdue University, West Lafayette, IN 47907 (U.S.A.)

SUMMARY

The stoichiometric displacement model has been refined in its application to anion-exchange chromatography. The revised stoichiometric displacement model has been shown to be valid for anion-exchange chromatography with respect to all particulars of the model tested. It has been shown that the use of displacing agent activity is not a necessary condition for valid application of the stoichiometric displacement model to anion-exchange chromatography. While the cation of the displacing salt can influence anion-exchange chromatography, the data indicate that the displacing anion is of primary importance. It has been shown that solutes with three-dimensional structure have Z_n value to solute charge ratios less than unity, and that the stoichiometric displacement model may be useful as a probe of solute three-dimensional structure.

INTRODUCTION

Fractionation of oligonucleotides has been accomplished by conventional and high-performance liquid chromatography (HPLC) on sorbents ranging from anion-exchange[1–3] through mixed-mode[4,5] to reversed-phase[6,7] materials. However, high-performance anion-exchange chromatography (HPAEC) has emerged as the most generally useful chromatographic technique for oligonucleotide fractionation[3,8]. The anion-exchange process for oligonucleotides and other polyanions is poorly understood. For example, it is not understood why oligonucleotides of the same size but different sequence are resolved by anion-exchange chromatography (AEC) even in the presence of organic solvents[2,3] that eliminate differences in hydrophobicity.

Considerable work has been done to elucidate the ion-exchange process for small organic and inorganic ions[9–11]. These papers have clearly described relationships between ionic structure, mobile phase composition, and stationary phase composition for ion-exchange chromatography (IEC) of small ions. Mathematical models have been developed in these papers which relate analyte retention to mobile phase composition for given stationary phases. These models allow, to varying degrees, successful prediction of analyte retention and optimization of elution conditions for IEC of small ions. It is difficult, however, to extend these models to IEC of polymeric ions.

0021-9673/86/$03.50 © 1986 Elsevier Science Publishers B.V.

A model has been proposed for AEC of proteins[12,13]. The model is based on the following general equilibrium:

$$P \cdot C_i + ZD_b \leftrightarrows P_b + ZD_0 + ZC_i \qquad (1)$$

where $P \cdot C_i$ refers to protein in solution with accompanying counter-ion, C_i; P_b refers to protein bound to the stationary phase; D_b and D_0 refer to displacing ion (in AEC, the anion) bound to the stationary phase and free in solution, respectively; and Z refers to the number of ions required to displace the protein from the stationary phase. It is assumed here that the displacing salt is monovalent (*e.g.* sodium chloride). Beginning with this equilibrium, an expression was derived which relates solute retention, as the capacity factor (k'), to the molarity of the displacing salt in the mobile phase:

$$k' = I/[\mathrm{NaCl}]^{2Z} \qquad (2)$$

where I is a constant, proportional to the formation constant for equilibrium 1. Eqn. 2 can be linearized by taking the logarithm of both sides:

$$\ln k' = 2Z \ln (1/[\mathrm{NaCl}]) + \ln I \qquad (3)$$

The value of Z is derived from the slope of the line obtained by plotting $\ln k'$ *versus* $\ln (1/[\mathrm{NaCl}])$, and I is the antilog of the Y-intercept of the same line. The values of $\ln k'$ and $\ln 1/[\mathrm{NaCl}])$ are obtained from a series of isocratic elutions of the analyte protein. This model applies strictly to the analytical mode of chromatography, where it is valid to assume that the amount of protein bound to the stationary phase is negligible relative to the total binding capacity of the stationary phase for the protein.

This model of protein AEC has been successful in that the predicted linearity between $\ln k'$ and $\ln (1/[\mathrm{salt}])$ has been observed repeatedly, with correlation coefficients $r \geqslant 0.97$, for all proteins tested. However, the structural complexity of proteins has made it difficult to validate the particulars of the model.

This model of protein retention in AEC is quite general. It implies a fundamental stoichiometric relationship between solute, mobile phase, and stationary phase in AEC. As such, it is expected that the model will apply to AEC of a broad range of solutes. The purpose of this paper is to validate and refine this stoichiometric displacement model (SDM) of AEC by applying it to AEC of nucleotides, oligonucleotides, and transfer RNA (tRNA).

MATERIALS AND METHODS

Nucleic acids and reagents

Nucleotides were obtained from Sigma (St. Louis, MO, U.S.A.). Oligonucleotides were purchased from P. L. Biochemicals (Milwaukee, WI, U.S.A.). Phenylalanine specific tRNA from *Escherichia coli* was purchased from Boehringer-Mannheim (Indianapolis, IN, U.S.A.). HPLC grade acetonitrile was obtained from Mallinckrodt (Paris, KY, U.S.A.). Inorganic reagents were of AR grade or comparable quality.

All nucleic acid samples were dissolved in 0.01 M Tris (pH 8.0). Nucleotide concentrations were 10 mM. Oligonucleotides were used at 0.01 A_{254} units/μl. tRNA was used at a concentration of 1.0 mg/ml.

Chromatography

Chromatography was performed with a Varian 5000 pumping system equipped with a UV-100 detector (Walnut Creek, CA, U.S.A.) and a Model C6U injector with a 100-μl injection loop (Valco, Houston, TX, U.S.A.). Nucleic acids were monitored at 257 nm. Eluent conductance was monitored with an AN400 ion chromatograph (Anspec, Ann Arbor, MI, U.S.A.). Data were collected on a Kipp & Zonen dual-pen chart recorder.

All samples were first analyzed by gradient elution with a binary gradient. The gradient time was 40 min. Buffer A was 0.01 *M* Tris, buffer B was a mixture of 0.01 *M* Tris and 1.0 *M* salt. Salts used in isocratic analyses were sodium chloride, sodium sulphate, magnesium chloride and magnesium sulphate. The pH of the buffer was adjusted with either hydrochloric or sulphuric acid, depending on the displacing salt used. In certain experiments, acetonitrile was added to Buffers A and B so as to achieve the same percentage acetonitrile in both buffers and to obtain the appropriate salt and Tris concentrations.

Approximation of the isocratic elution conditions required for the collection of retention data was achieved with gradient elution. Isocratic analyses were then performed at salt concentrations above and below the elution concentration determined in gradient chromatography. Each sample was analyzed in duplicate. Between five and seven salt concentrations were used in the analysis of each sample, so as to produce k' values varying evenly from *ca.* 1.0 to 15.0. The consistency of isocratic salt concentrations was maintained from analysis to analysis by monitoring eluent conductivity. Sample injection volumes were 10–20 μl for nucleotides (volume was constant for any one analysis) and 15 μl for all tRNA analyses. Recorded peak sizes were held approximately constant by varying detector response to facilitate accurate measurement of retention time (t_R). For each analysis, the elution time for zero ionic retention (t_0) was obtained by eluting at two salt concentrations high enough so that the elution time no longer decreased. All analyses were performed on a "Mono-Q" strong anion-exchange column 5.0 × 0.5 cm I.D.) (Pharmacia, Uppsala, Sweden).

Calculations and statistical analysis

Retention times were converted to k' values according to:

$$k' = (t_R - t_0)/t_0 \tag{4}$$

Symbols are as described above.

Two methods of plotting the data were used. In Method 1, ln k' values were plotted against ln (1/[salt]). In Method 2, ln k' values were plotted against ln (1/[D_0]) (see Results and discussion). The Z values obtained by both methods {slope of ln k' *vs.* ln (1/[salt]) or ln (1/[D_0])} are taken to be the number of ions required for displacement. A more useful value, for purposes of comparison and validation, is the number of charge interactions between solute and stationary phase. By Method 2, the number of charge interactions between solute and stationary phase (Z_n) is calculated as follows:

$$Z_n = eZ \tag{5}$$

where e is the valence of the displacing anion and Z_n and Z are as described above. The procedure of Rounds and Regnier[11] was used to calculate Z_n when Method 1 was employed. By Method 1, Z_n was calculated by multiplying Z by 2 when sodium chloride was used, 2/3 when magnesium chloride or sodium sulphate was used, and 1 when magnesium sulphate was used. These factors are a consequence of valence and the inclusion of cation concentration in Method 1. Z_n values are reported exclusively here.

By either Method 1 or 2, for each sample, ln k' and ln (1/[salt]) or ln (1/[D_O]) values were fit to a straight line by a least squares calculator program. Data were then fit to a straight line by an iterative linear least squares program, run on an Apple IIe computer. Values for Z and I obtained from the calculator program were used as estimates of the constants (Z = slope, ln I = Y-intercept), for the iterative program. Data were next subjected to residuals analysis by standard methods[14]. Data lying outside two standard errors of the estimate in the residuals analysis were eliminated. Data sets (ln k' and ln (1/[D_O]) values for a given sample) that showed systematic errors in the residuals analysis were eliminated and the appropriate experiments were repeated. Data for some samples were analyzed by an iterative, non-linear, least squares program, fitting eqn. 2, on an Apple IIe computer.

RESULTS AND DISCUSSION

The data presented here were obtained by fitting the raw data {ln k' and ln (1/[NaCl]) or ln (1/[D_O]) values} to straight lines (except those in Table I). We wanted to avoid the possibility that linear analysis, of logarithmic plots would suppress error and provide unreliable Z_n and I values. Therefore, we analyzed randomly selected data sets by non-linear (eqn. 2) and linear curve fitting, and compared the resultant Z_n and I values. A representative comparison is given in Table I. The data in Table I were obtained by using 1/[D_O] for the non-linear curve fit, and ln (1/[D_O]) for the linear curve fitting (Method 2, see Materials and methods, and the discussion below). The data from linear and non-linear curve fitting in Table I are considered to agree within experimental limits for cyclic adenine monophosphate (cAMP) and pentadeoxythymidylate [$(dT)_5$]. As the curve-fitting methods were equally good, the more time-efficient linear method was used to obtain the results presented here.

The correlation coefficients for the linear analyses of the data presented here averaged 0.994. The lowest correlation coefficient obtained was 0.990.

The question of whether to use the molarity or the activity of displacing agents

TABLE I

COMPARISON OF Z_n AND I VALUES FOR cAMP IN SODIUM CHLORIDE AND $(dT)_5$ IN MAGNESIUM CHLORIDE BY LINEAR AND NON-LINEAR CURVE FITTING

Solute	*Linear*		*Non-linear*	
	Z_n	I	Z_n	I
cAMP	1.2	0.087	1.2	0.091
$(dT)_5$	5.3	$2.3 \cdot 10^{-4}$	5.2	$3.3 \cdot 10^{-4}$

TABLE II

Z_n VALUES FOR SOME NUCLEOTIDES IN VARIOUS SALTS BY METHOD 1

Solute	*NaCl*	*$MgCl_2$*	*Na_2SO_4*	*$MgSO_4$*
cAMP	0.60	0.33	0.66	0.45
5'-AMP	0.95	0.60	1.5	1.2
$(dT)_5$	2.7	1.9	3.5	3.0

in these analyses was addressed. Values of Z_n and I previously obtained for proteins by using molarity of sodium chloride, were compared with Z_n and I values obtained by repeating the analyses and using literature values of activity[15] for sodium chloride in place of the corresponding molarities. The correlation coefficients obtained when activity was used were slightly improved. However, within experimental limits, the Z_n and I values agreed with those obtained when molarity was used (data not shown). The applicability of displacing agent activity in these analyses was put into question by the observation that a protein or nucleic acid is eluted earlier by magnesium sulphate than by an equal concentration of sodium chloride, despite the fact that sodium chloride has a higher activity than magnesium sulphate at the same concentration[15]. It is also difficult to know if the changes in the activity of a salt in solution that occur when the concentration is changed parallel the changes in the activity of the salt at an ion-exchange surface. The observations and reasoning above led to the conclusion that it was most appropriate to use the molarity of the displacing agent in these analyses.

Z_n values for cAMP, 5'-adenine monophosphate (5'-AMP) and $(dT)_5$ obtained by Method 1 (see methods) are given in Table II. The expected Z_n values are simply the total number of anionic charges in each molecule. Thus the number of charge interactions (Z_n values) expected in AEC for cAMP, 5'-AMP, and $(dT)_5$ are 1, 2, and 6, respectively. It is expected that the Z_n values for a given solute will not change with the displacing agent used, provided the displacing agent does not alter solute structure. The data in Table II indicate that Method 1 fails in this respect. Still, the plots from which the Z_n values were derived were linear.

The results in Table II led to a revised approach to the derivation of the equations relating solute retention to mobile phase composition. The revised approach was begun by divorcing the equilibrium between solute and stationary phase from that between solute and counter-ion. The pertinent equilibrium is then as follows:

$$S_0 + ZD_b \rightleftarrows S_b + ZD_0 \quad (6)$$

where S_0 is solute free in solution. Equilibrium 6 is in accord with the traditional notion that the anion of the displacing salt is of primary importance in AEC. While cations or anions are equally capable of disrupting charge interactions, it is useful to note that at an anion-exchange surface a cation is much less likely to disrupt a charge interaction than an anion, as a consequence of charge repulsion between the surface and the cation.

By a derivation strictly analogous to that described in the Introduction, the following equation is obtained:

$$k' = I/[D_0]^Z \quad (7)$$

TABLE III

Z_n VALUES FOR SOME NUCLEOTIDES IN VARIOUS SALTS BY METHOD 2

Solute	*NaCl*	*$MgCl_2$*	*Na_2SO_4*	*$MgSO_4$*
cAMP	1.2	1.0	1.0	0.9
5'-AMP	1.9	2.2	1.8	2.3
$(dT)_5$	5.4	5.3	5.6	6.0

Eqn. 7 can be linearized by taking the logarithm of both sides giving:

$$\ln k' = Z \ln (1/[D_0]) + \ln I \quad (8)$$

Z values are obtained directly as the slope of the $\ln k'$ *versus* $\ln (1/[D_0])$ plots, and I values are the antilog of the Y-intercepts of such plots. For monovalent anions (*e.g.* Cl^-), Z_n is equal to Z. For divalent anions (*e.g.* SO_4^{2-}), Z_n values are twice the Z values. Z_n is then the number of charge interactions between solute and stationary phase, and Z (the slope of the $\ln k'$ *versus* $\ln (1/[D_0])$ plot) is the number of anions required to displace the solute from the stationary phase. This derivation again applies to chromatography in the analytical mode, as described for the previous derivation. Analyses based on molarity of displacing anion are referred to as being by Method 2 (see Materials and methods).

Z_n values for cAMP, 5'-AMP and $(dT)_5$ by Method 2 are given in Table III. For each solute in each displacing salt, the Z_n values in Table III very closely approximate the expected values for these solutes, and the plots from which they were derived were again linear. The data in Table III are taken as validation of the applicability of the revised SDM (Method 2) to AEC.

The SDM was applied to AEC so as to provide a mathematical description of the retention behavior of large polyanions in AEC. Z_n values for mononucleotides, oligodeoxyadenylates [$(dA)_n$], and oligodeoxythymidylates [$(dT)_n$], obtained in sodium chloride by Method 2 are given in Table IV. The expected Z_n values for the oligodeoxynucleotides are greater by 1 than the number of bases in the oligomer, *e.g.* for $(dA)_{11}$ the expected Z_n value is 12. For oligodeoxyadenylates up to ten bases in length the Z_n values in Table IV closely approximate those expected. For $(dA)_{11}$ and $(dA)_{14}$, the Z_n values in Table IV are significantly less than expected. For oligodeoxythymidylates up to $(dT)_{11}$ the Z_n values closely approximate the expected values

TABLE IV

Z_n VALUES FOR ADENINE AND THYMIDINE CONTAINING NUCLEOTIDES IN SODIUM CHLORIDE

Solute	*Z_n*	*Solute*	*Z_n*
cAMP	1.2		
5'-AMP	1.9		
$(dA)_5$	5.3	$(dT)_5$	5.4
$(dA)_{10}$	9.5	$(dT)_{10}$	10.0
$(dA)_{11}$	8.6	$(dT)_{11}$	10.9
$(dA)_{14}$	9.8	$(dT)_{14}$	12.5

TABLE V

Z_n VALUES FOR $tRNA^{Phe}$ IN SODIUM CHLORIDE WITH VARIED ACETONITRILE CONCENTRATIONS

% Acetonitrile	Z_n
0	18.8
5	19.6
10	21.9
15	29.0

(all within 10% of the expected value). Only for $(dT)_{14}$ does the ratio of Z_n to the number of anionic charges on the solute fall significantly from unity.

There are several possible explanations for the observed drop in the ratio of Z_n to the number of anionic charges for $(dA)_{11}$, $(dA)_{14}$ and $(dT)_{14}$. The most likely is considered to be the occurrence of three-dimensional structure in the longer oligomers. It is known for oligoadenylates (oligonucleotides containing 2′-hydroxyl groups) that single-stranded coils occur as a consequence of base stacking interactions[16]. It is probable that such structures occur in oligodeoxynucleotides as well. If this were the case, when a coil formed in the oligomer, all of the anionic sites could no longer interact with the AEC stationary phase simultaneously. The consequence of this would be a reduction in the ratio of Z_n to the number of solute anionic sites. Further, such a coil forming oligomer would be expected to form a longer coiled region as the length of the oligomer increased. If the number of monomeric units involved in a single coil were three, an increase in oligomer length of three monomeric units would increase the Z_n value by 1. Such a structural scenario could explain the change in Z_n in going from $(dA)_{11}$ to $(dA)_{14}$, seen in Table IV. The fact that Z_n values for oligodeoxythymidylates do not drop off as soon as the Z_n values for oligodeoxyadenylates could be explained in terms of the size and hydrophobicity of the pyrimidine base (T) relative to the purine base (A). The smaller and less hydrophobic pyrimidine is less likely to enter a base stacking interaction than the larger, more hydrophobic purine.

Another possible explanation for the data in Table IV would be that, as the oligomer increases in length, the termini could become more flexible. In that event, when the oligomer is bound to a surface, the terminal monomeric units might spend a smaller fraction of time bound to the surface. The Z_n value would then be a measure of a distribution function for all bound states of the oligomer. In such a case, one might expect the oligopyrimidine (T) to be more flexible than the oligopurine (A) and yield lower Z_n values for steric reasons.

TABLE VI

Z_n VALUES FOR SOME NUCLEOTIDES WITH AND WITHOUT ACETONITRILE

Solute	*No Acetonitrile*	*15% Acetonitrile*
cAMP	1.2	1.2
$(dA)_5$	5.3	5.8
$(dT)_5$	5.4	5.5

To examine more closely the effect of solute three-dimensional structure in the SDM analysis, phenylalanine-specific tRNA ($tRNA^{Phe}$) was analyzed by Method 2 in sodium chloride, in the absence and presence of the denaturant acetonitrile. Z_n values obtained for $tRNA^{Phe}$ in this analysis are given in Table V. The crystal and solution structure of $tRNA^{Phe}$ is well documented[17]. It is expected that for solutes with three-dimensional structure that the Z_n values for AEC will be significantly less than the total number of anionic sites in the solute. Further, when a denaturant is added to the mobile phase it is expected that the Z_n value will increase as the solute unfolds, taking on a more extended conformation and allowing more solute anionic sites to interact simultaneously with the anion-exchange surface. The data in Table V fit expected behavior quite well. Z_n for $tRNA^{Phe}$ in the absence of acetonitrile is *ca.* 19. This value is one-fourth of the total number of anionic sites in $tRNA^{Phe}$ (76 bases). Z_n values increase with increasing acetonitrile in the mobile phase to 29 at 15% acetonitrile. More than one-third of the total number of anionic sites in the solute interact with the stationary phase in the presence of acetonitrile.

It is known that in ion-exchange systems, in addition to ionic interactions, hydrophobic interactions can contribute to solute retention. To estimate the effect of hydrophobic interactions on the SDM analysis of AEC with the "Mono Q" column, Z_n and I values for cAMP, $(dA)_5$ and $(dT)_5$ were determined in sodium chloride, by Method 2, with and without 15% acetonitrile in the mobile phase. In the presence of 15% acetonitrile, hydrophobic contributions to retention will be minimal[3]. It should be noted that eluent conductivity was not altered by the addition of acetonitrile. Hence, acetonitrile did not influence retention by altering displacing ion activity. Since these solutes have no three-dimensional structure, acetonitrile will not influence Z_n as a result of denaturation. The presence of acetonitrile in the mobile phase had no influence on Z_n values. Z_n values for solutes (Table VI) in the presence and absence of acetonitrile agree within experimental limits, suggesting that hydrohobic interactions do not contribute to the ionic stoichiometry of solute–stationary phase interactions in the system used here. However, retention times were reduced for each of these solutes when acetonitrile was added to the mobile phase. This appears to be a consequence of a shift in the basic equilibrium between solute and stationary phase, toward dissociation, since the I values were reduced in the presence of acetonitrile by a factor of 5 for cAMP, by a factor of 3 for $(dA)_5$, and by a factor of 2 for $(dT)_5$. Since the I term is directly proportional to the association constant in the SDM derivation, these results are essentially as one would predict if hydro-

TABLE VII

I VALUES FOR ADENINE AND THYMIDINE CONTAINING NUCLEOTIDES IN SODIUM CHLORIDE

Solute	*I*	*Solute*	*I*
cAMP	0.087		
5'-AMP	0.017		
$(dA)_5$	$1.6 \cdot 10^{-3}$	$(dT)_5$	$2.0 \cdot 10^{-3}$
$(dA)_{10}$	$4.5 \cdot 10^{-5}$	$(dT)_{10}$	$1.3 \cdot 10^{-4}$
$(dA)_{11}$	$4.5 \cdot 10^{-4}$	$(dT)_{11}$	$5.6 \cdot 10^{-5}$
$(dA)_{14}$	$2.6 \cdot 10^{-4}$	$(dT)_{14}$	$3.9 \cdot 10^{-5}$

phobic interactions were contributing to solute retention in this system. The Z_n value reflects the ionic stoichoimetry of the solute–stationary phase interaction, and is independent of hydrophobic interctions in the system used here. However, the I value is influenced by both ionic and hydrophobic interactions.

I values for mononucleotides, oligodeoxyadenylates, and oligodeoxythymidylates obtained in sodium chloride by Method 2 are given in Table VII. The data clearly represent a trend. I values are reduced as oligomer size increases. One anomaly is observed in going from $(dA)_{10}$ to $(dA)_{11}$. This anomaly may reflect the occurence of three-dimensional structure in oligomers of this size.

ACKNOWLEDGEMENT

This work was supported by NIH Grant GM 25431. This is Journal Paper No. 10605 from the Purdue University Agricultural Experiment Station.

REFERENCES

1 G. T. Asteriadis, M. A. Armbruster and P. T. Gilham, *Anal. Biochem.*, 70 (1976) 47.
2 R. R. Drager and F. E. Regnier, *Anal. Biochem.*, 145 (1985) 47.
3 T. G. Lawson, F. E. Regnier and H. L. Weith, *Anal. Biochem.*, 133 (1983) 85.
4 R. Bischoff and L. W. McLaughlin, *J. Chromatogr.*, 270 (1983) 117.
5 R. D. Wells, S. C. Haldies, G. T. Horn, B. Klein, J. E. Larson, S. K. Nevendorf, N. Panayotatos and P. K. Patient, *Methods Enzymol.*, 65 (I) (1980) 2367.
6 D. A. Usher, *Nucleic Acids Res.*, 6 (1979) 2289.
7 G. D. McFarland and P. N. Borer, *Nucleic Acids Res.*, 7 (1979) 1067.
8 W. Haupt and A. Pingoud, *J. Chromatogr.*, 260 (1983) 419.
9 D. R. Jenke, *Anal. Chem.*, 56 (1984) 2674.
10 F. Murakami, *J. Chromatogr.*, 198 (1980) 241.
11 P. Jandera, M. Janderová and J. Churáček, *J. Chromatogr.*, 148 (1978) 79.
12 W. Kopaciewicz, M. A. Rounds, J. Fausnaugh and F. E. Regnier, *J. Chromatogr.*, 266 (1983) 3.
13 M. A. Rounds and F. E. Regnier, *J. Chromatogr.*, 283 (1984) 37.
14 J. Neter, W. Wasserman and G. A. Whitmore, *Applied Statistics*, Allyn and Bacon, Boston, 1978.
15 R. C. Weast (Editor), *CRC Handbook of Chemistry and Physics*, CRC Press, West Palm Beach, 1977.
16 J. Brahms, A. M. Michelson and K. E. VanHolde, *J. Mol. Biol.*, 15 (1966) 467.
17 P. R. Schimmel, D. Soll and J. N. Abelson (Editors), *Transfer RNA, Structure, Properties, and Recognition*, Cold Spring Harbor Laboratory, Cold Spring Harbor, 1979.