Protein–Nucleic Acid Interactions. III. Cation Effect on Binding Strength and Specificity*

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ABSTRACT: Cations were shown to affect the extent and specificity of binding of individual members of the series (L-Lys)$_n$-ε-N-DNP-L-lysine (n = 3, 4, 5, 6, or 7) to synthetic polynucleotides, poly (A + U) or poly (I + C).

Interpreting this effect as the result of competition between the cation and the oligolysine for the polynucleotide phosphates, it was possible to group cations into those with a binding preference for poly (A + U) [$(CH_3)_4N^+ > Li^+ > Na^+$; Mg^{2+}, Ca^{2+}, Mn^{2+}]; those with little specificity for either polynucleotide [$LysH^+$, K^+, $TrisH^+$, NH_4^+]; and those with a binding preference for poly (I + C) [$ArgH^+$, $HisH^+$]. Cation specificity for poly (A + U) correlated with cation ability to organize water structure and with volume increase on cation neutralization of polyphosphates, suggesting that specificity in protein–nucleic acid interactions might be directed by the solvent structure surrounding the interacting species. This grouping according to solvent structure promotion also held regarding cation effect on messenger stimulated binding of tRNA to ribosomes, K^+, $TrisH^+$, and NH_4^+ promoting this binding and $(CH_3)_4N^+$, Li^+, and Na^+ inhibiting it, suggesting a connection between some specific cation effects in biological systems and cation binding to phosphate.

In the previous paper, equilibrium dialysis measurements were used to evaluate the strength, specificity, and stoichiometry of ε-DNP-oligolysine binding to poly (I + C) and poly (A + U).[1] The binding strength was shown to be strongly dependent on the NaCl concentration of the buffer used, and the data were interpreted by a theory which assumed the NaCl effect to be largely due to competition of the Na^+ with the oligolysine for binding sites on the polynucleotide. In this paper, a striking influence of other cations on the strength and specificity of this interaction is shown. Correlation of the cation effects with cation solvation suggests an important role of water of hydration in the control of nucleic acid–protein interactions.

Materials and Methods

These were generally those described in the accompanying paper (Latt and Sober, 1967). Polynucleotides used were made from poly A (Miles 110638), poly U (Miles 48646), poly I (Schwarz 6701), and poly C (Miles 27622).

TMACl was purchased from Baker Chemical Co. Amino acids and amino acid esters were either gifts from Dr. T. Otani or purchases from Nutritional Biochemicals Corp., Cleveland, Ohio, or Mann Biochemicals, New York, N. Y.

Curves of ε-DNP-oligolysine binding to polynucleotide demonstrating the effects of monovalent inorganic salts, TMACl, lysine-HCl, histidine-HCl, and arginine-HCl were obtained as described previously (Latt and Sober, 1967). Other experiments with amino acids, their derivatives, and divalent cations were designed only to compare the relative effects of the various salts on the binding. These latter experiments were done in paired sets of tubes containing identical amounts of the same ε-DNP-oligolysine and identical concentrations of poly (I + C) or poly (A + U), with each pair containing buffer of a different salt composition.

Results

Figures 1 and 2 show the results of binding experiments done in 0.25 M solutions of NaCl, KCl, LiCl, NH_4Cl, Tris-HCl, Lys-HCl, and TMACl. A pH of 7.0 was buffered by 0.01 M sodium cacodylate–cacodylic acid except for the solution of Tris-HCl to which was added approximately 0.02 M Tris. For all but one of the curves shown, the oligolysine used was ε-DNP-Lys(Lys)$_5$. In the case of 0.25 M TMACl, the binding was so strong that meaningful data could be obtained only for shorter oligomers. Therefore, measurements of ε-DNP-Lys(Lys)$_5$ binding were made in 1.0 M TMACl and are included in Figure 2.

The magnitude of the oligolysine–polynucleotide

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[1] Abbreviations used: ε-DNP-Lys(Lys)$_n$, L-(Lys)$_n$-ε-N-DNP-L-lysine; poly (A + U) and poly (I + C) represent association between polyadenylic and polyuridylic acid chains and between polyinosinic and polycytidylic acid chains, respectively; TMACl, tetramethylammonium chloride.

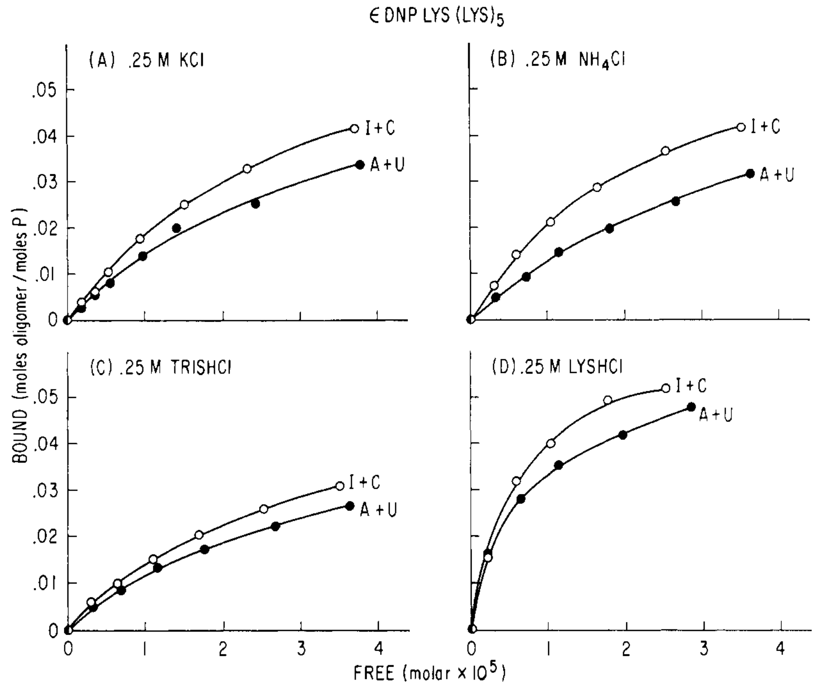

FIGURE 1: Effect of cations in ϵ-DNP-Lys(Lys)$_5$ binding to poly (I + C) and poly (A + U). Solutions were buffered at pH 7.0 by sodium cacodylate–cacodylic acid except in the case of Tris-HCl which was buffered by adding approximately 0.02 M Tris. Temperature was 4°. Coordinates determined by methods of previous paper (Latt and Sober, 1967). Polynucleotide concentrations 1.50 mM P.

binding in the cases shown is greatest in TMACl (note scale difference in Figure 2D) and weakest in NaCl. Ordering of the intermediate cases depends on the polynucleotide considered. Using the observed specificity or preference of the oligolysine for poly (I + C) over poly (A + U), the salts can be ordered TMACl > LiCl > NaCl > NH_4Cl ≈ KCl ≈ Lys-HCl ≈ Tris-HCl, the specificity in the last four cases being very small. Binding constants of ϵ-DNP-oligolysine to poly (I + C) and poly (A + U), K_{IC} and K_{AU} (K in M^{-1}), for the above curves and several others were determined by methods discussed in the previous paper, and the results are given in Tables I and II.

Figure 3 further illustrates the very large effect of TMACl on the observed specificity. Binding of ϵ-DNP-Lys(Lys)$_4$ to poly (I + C), poly (A + U), and poly (A + 2U) in 1.0 M TMACl is compared with that in 0.15 M NaCl. While binding to poly (I + C) is about the same in both salt solutions, binding to poly (A + U) and poly (A + 2U) is considerably depressed in 1 M TMACl. From the results with poly (A + 2U), it appears that the interaction between TMA^+ and polynucleotide is not blocked by the additional poly U strand in poly (A + 2U).

Basic Amino Acids. Competition of small cations with ϵ-DNP-oligolysine for poly (I + C) and poly (A + U) was used to examine the binding specificity of amino acids, their esters, and divalent metal ions. Since any appreciable effect of amino acids on binding was only obtained with the basic amino acids, the observed specificities are interpreted as largely due to the positively charged side chain and thus representative of the effects such amino acids would have as internal residues in a polypeptide.

Binding curves of ϵ-DNP-Lys(Lys)$_5$ to poly (I + C) and poly (A + U) in 0.25 M Arg-HCl (pH 7.0) and 0.19 M His-HCl, 0.03 M His, 0.03 M KCl (pH 5.6)[2] are compared with those in 0.25 M Lys-HCl (pH 7.0) in Figure 4. The reversal of the observed oligolysine specificity in the Arg-HCl and His-HCl solutions is striking. Since the binding of the oligolysine reflects the reverse of the binding of the monovalent cations (which compete with the oligolysine), these curves indicate a relative preference of binding of $ArgH^+$ and $HisH^+$ for poly (I + C). In addition the binding strength of $ArgH^+$ and $HisH^+$ to both polynucleotides exceeds that of $LysH^+$.

Results of single point experiments with paired tubes, for poly (I + C) or poly (A + U), in buffers containing $LysH^+$, $ArgH^+$, or $HisH^+$ are given in Table III. KCl was chosen as a reference salt since little difference in oligolysine binding to either polynucleo-

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[2] The His-HCl was partially neutralized by KOH to raise the solution pH from about 4.8 to 5.6, where changes in polybase–poly (I + C) interactions have been reported (Huang and Felsenfeld, 1960). Separate experiments in 0.25 M KCl have shown very little effect of pH on oligolysine binding specificity in the pH range of 5.4–7.0.

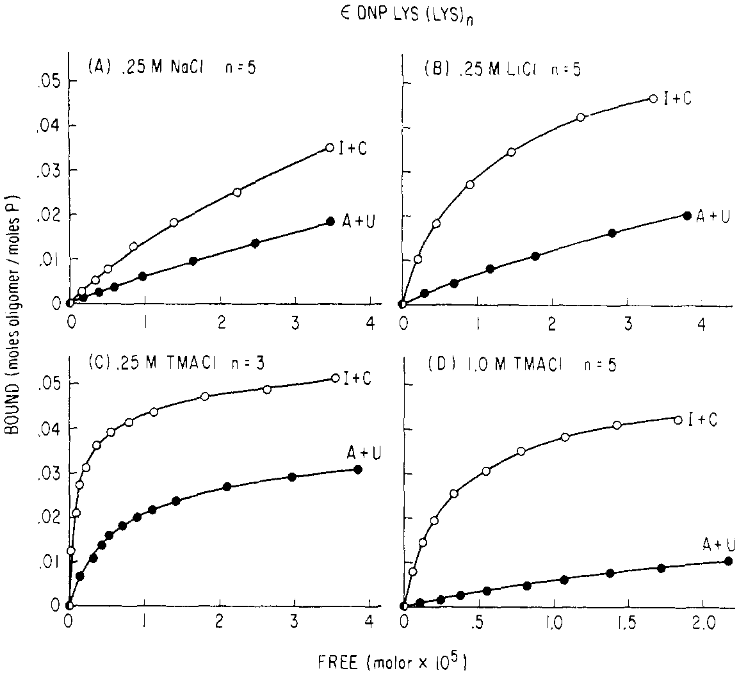

FIGURE 2: Effect of additional cations on ε-DNP-oligolysine binding to poly (I + C) and poly (A + U). pH buffered at 7.0 as above. Polynucleotide concentrations: (A and B) 1.50 mM P, (C) 1.33 mM P, and (D) 0.95 mM P.

TABLE I: ε-DNP-Lys(Lys)$_n$-Polynucleotide Binding Constants.[a,b]

	K_{IC}			K_{AU}			$K_{IC}:K_{AU}$		
NaCl	0.06 M	0.25 M	0.39 M	0.06 M	0.25 M	0.39 M	0.06 M	0.25 M	0.39 M
$n = 4$	4.97	2.85		4.63	2.39		0.34	0.46	
$n = 5$		3.22			2.79			0.43	
$n = 6$		3.70	2.56		3.15	2.13		0.55	0.43
$n = 7$		4.10			3.55			0.55	
KCl									
$n = 4$		2.95			2.82			0.13	
$n = 5$		3.36			3.26			0.10	
$n = 6$		3.80			3.59			0.21	
$n = 7$		4.11			3.96			0.15	
Lys-HCl									
$n = 4$		3.55			3.37			0.18	
$n = 5$		3.95			3.87			0.08	
$n = 6$		4.15			3.98			0.17	
LiCl									
$n = 4$	4.65			4.51			0.14		
$n = 5$		3.73			2.93			0.80	
$n = 6$			3.03			2.36			0.67
TMACl		0.25 M	1.0 M		0.25 M	1.0 M		0.25 M	1.0 M
$n = 3$		4.60	3.72		3.72	2.57		0.88	1.15
$n = 4$		4.78	4.12		4.21	2.64		0.57	1.48
$n = 5$			4.24			2.81			1.43

[a] Values are expressed as logs. Measured and equilibrated at 4°. Binding constants determined by methods of previous paper (Latt and Sober, 1967). [b] All buffers also contained 0.01 M sodium cacodylate-cacodylic acid (pH 7.0).

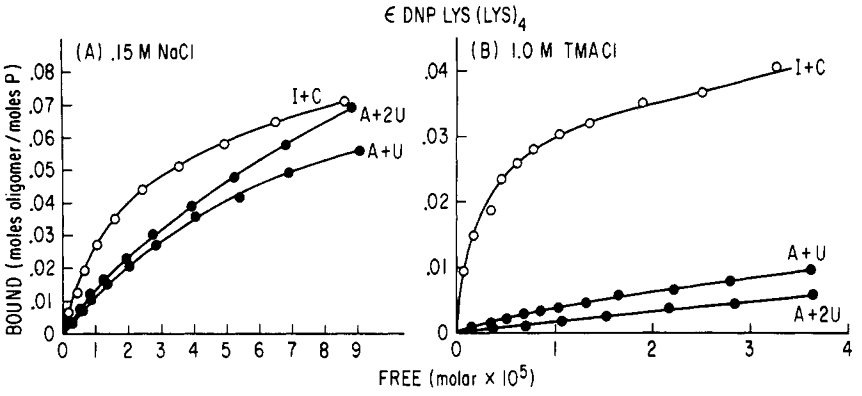

FIGURE 3: Effect of NaCl and $(CH_3)_4NCl$ on ε-DNP-Lys(Lys)$_4$ binding to poly (I + C), poly (A + U), and poly (A + 2U). Conditions as above. Polynucleotide concentrations: (A) Poly (I + C) 2.16 mM P, poly (A + U) 2.16 mM P, and poly (A + 2U) 2.12 mM P; (B) poly (I + C) 0.95 mM P, poly (A + U) 0.95 mM P, and poly (A + 2U) 0.97 mM P.

tide is seen in KCl solution. Compared with $LysH^+$, both $ArgH^+$ and $HisH^+$ again show a greater absolute binding affinity for both polynucleotides and a relative preference for poly (I + C), the latter result consistent with those of Leng and Felsenfeld (1966) showing that polyarginine preferentially precipitated out deoxyribonucleotides rich in (G·C) base pairs. Our results with His-HCl would predict a similar (G·C) preference for polyhistidine in Leng and Felsenfeld's system.

Divalent Metal Ions. The effect of Mg^{2+}, Ca^{2+}, and Mn^{2+} in oligolysine–polynucleotide binding is shown in Table IV. All three divalent cations show a preference (relative to K^+) for poly (A + U), since their addition to KCl solutions increased the oligolysine binding to poly (I + C), compared with that to poly (A + U). Mn^{2+} binds more strongly to the polynucleotides than do Mg^{2+} or Ca^{2+}.

TABLE II: ε-DNP-Lys(Lys)$_5$–Polynucleotide Binding Constants.[a]

Salt (M)[b]	K_{IC}	K_{AU}	K_{IC}:K_{AU}
NaCl (0.25)	3.22	2.79	0.43
LiCl (0.25)	3.73	1.93	0.80
TMACl (1.0)[c]	4.24	2.81	1.43
KCl (0.25)	3.36	3.26	0.10
NH_4Cl (0.25)	3.45	3.23	0.22
Tris-HCl (0.25)[b]	3.26	3.19	0.07
Lys-HCl (0.25)	3.95	3.87	0.08
Arg-HCl (0.25)	2.93	3.24	−0.31
His-HCl (0.19)[b]	3.37	3.67	−0.30

[a] Values are expressed as logs. [b] All solutions also contained 0.01 M sodium cacodylate–cacodylic acid except the Tris-HCl which was approximately 0.02 M Tris and the His-HCl which was 0.03 M His (pH 5.6). [c] TMACl (1.0 M) was used because ε-DNP-Lys(Lys)$_5$ binding in 0.25 M TMACl was too strong to obtain points for a binding curve.

Amino Acid Esters. Neutral amino acids, even in amounts approaching their solubility limits, had very little effect on oligolysine–polynucleotide binding. Salts of aspartic and glutamic acids had effects expected of the cation (Na^+ or K^+) used for neutralization. However, positively charged methyl or ethyl esters of the amino acids did bind to poly (I + C) and poly (A + U). While preliminary results indicate that doubly charged His, Lys, and Arg esters showed the same relative specificities as the basic amino acids themselves, most of the other amino acid esters, especially those of the aromatic and long-chain aliphatic amino acids, showed a relative preference for poly (A + U).

tRNA Binding. The effects of some of the monovalent cations used in our dialysis experiments were examined in the mRNA-induced binding of tRNA to ribosomes measured by the Millipore filter method of Nirenberg and Leder (1964). Na^+ and Li^+ inhibit the binding while K^+ and $NH_4{}^+$ enhance it. While $TrisH^+$ has, under some conditions, been shown to give less efficient binding than K^+ or $NH_4{}^+$ (Pestka and Nirenberg, 1966), it will, when present as the dominant monovalent cation, allow this binding to take place. Results of such experiments, measuring the binding to *Escherichia coli* ribosomes of [^{14}C]Phe-tRNA with poly U and of [^{14}C]Pro-tRNA with poly C, are shown in Table V. Also demonstrated was an inhibitory effect of TMACl on this binding (similar to that of Na^+ and Li^+), predicted by the TMA^+ specificity for poly (A + U) over poly (I + C) observed in our dialysis experiments.

Discussion

Many of the cations studied here have been shown to bind to polyphosphates and nucleic acids (Strauss and Ross, 1959; Strauss and Leung, 1965; Crutchfield and Irani, 1965). The data given here, interpreted in terms of a competition between solvent cations and oligolysine for the polynucleotide phosphates, indicate

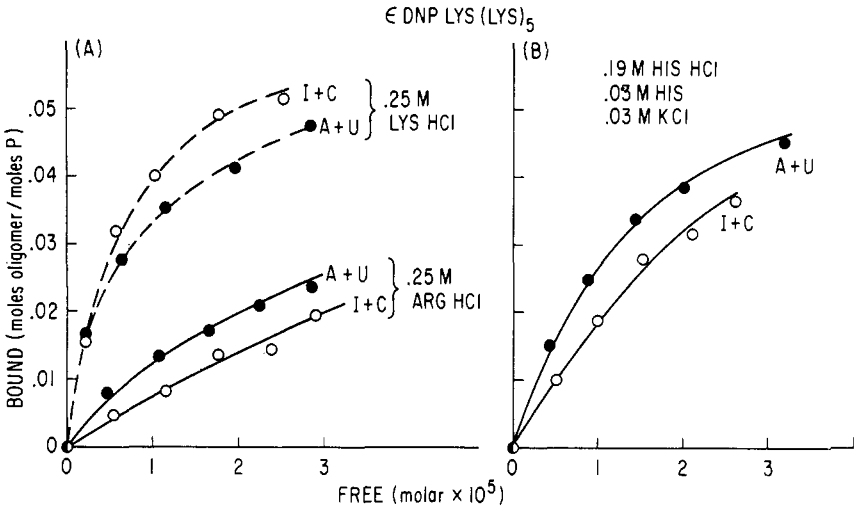

FIGURE 4: ε-DNP-Lys(Lys)$_5$ binding to poly (I + C) and poly (A + U) in the presence of salts of arginine, histidine, and lysine. Final pH 7.0 for Lys-HCl and Arg-HCl. Final pH of His-HCl solution, 5.6. Polynucleotide concentrations: (A) Lys-HCl, 1.29 μM P/ml; Arg HCl, 1.50 μM P/ml; (B) His HCl, 1.50 μM P/ml.

that the base composition of the polynucleotide somehow modulates the cation–phosphate interaction. Weaker oligolysine binding is interpreted as due to stronger salt-cation binding.[3]

From the binding data presented it appears that the oligolysine–polynucleotide system would be useful in detecting the strength and specificity of weak cation binding. For example, in a dialysis experiment with 1 μmole of P/ml of polynucleotide in the dialysis sack, free cation concentrations much greater than 10^{-3} M would make direct measurement of bound cation over this background level very difficult. Attempts to directly measure amino acid binding to nucleic acids made by Zubay and Doty (1958) were thwarted by this problem. However, the binding of a cation *via* competition with a much more strongly bound polycation, ε-DNP-oligolysine, is easily measurable.

The data presented demonstrate that even simple cations may exhibit specificity in their interaction with polynucleotides. The individual binding experiments reveal only the *relative* preference of a given cation for either poly (A + U) or poly (I + C) compared with other cations. One can estimate the *absolute* specificities of the cations using the results of binding experiments in Lys-HCl and NaCl solutions, respectively, and the assumption that the binding *specificity* of *LysH*$^+$ is the same as that of the *side chain* of a single residue of an oligolysine. Then, since the residue increase in ε-DNP-oligolysine specificity for poly (I + C) in NaCl solutions is about the same as the specificity difference per residue between NaCl and Lys-HCl solutions, one can calculate that the absolute specificity of lysine itself is very small. *The oligolysine binding preference seen in NaCl solutions appears to be largely a consequence of the reverse specificity of the Na*$^+$. This can also be shown to account for the absence of significant variation in $R = K_{IC}/K_{AU}$ over the range of NaCl concentrations of 0.15–0.39 M.

Using the result that LysH$^+$ has very little absolute specificity, one can group the cations examined as possessing specificity for poly (A + U) [TMA$^+$ > Li$^+$ > Na$^+$; Mg^{2+}, Ca^{2+}, Mn^{2+}], possessing essentially no specificity [K$^+$, LysH$^+$, NH$_4$$^+$, TrisH$^+$], or possessing specificity for poly (I + C) [ArgH$^+$, HisH$^+$].

One common denominator of the cations exhibiting a specificity for poly (A + U) is an organizing effect these cations have on the water structure around them. Evidence for this comes from thermodynamic, viscometric, and spectroscopic methods (Nightingale, 1966; Muirhead-Gould and Laidler, 1966; Von Hippel and Schleich, 1968). These same studies show that K$^+$ and NH$_4$$^+$, which exhibit nonspecific polynucleotide binding in our experiments, do not structure water. Finally, there is evidence that the guanidino group, the arginine side chain, with a specificity for poly (I + C), disrupts interactions directed by water structure (Wetlaufer *et al.*, 1964). In addition, the relative poly (A + U) specificity shown by Li$^+$, Na$^+$, and K$^+$ parallel the solution volume increase observed when these ions bind to phosphate groups (Strauss and Leung, 1965), the volume increase being greatest for Li$^+$. Ca^{2+} and Mg^{2+} binding was also shown to result in a volume increase.

No correlation can be made between cation specificity and cation binding strength (suggested by Barber

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[3] The apparent inversion of the strength of Na$^+$ and Li$^+$ binding to phosphate found here is probably connected with the high (0.25 M) concentrations used. The literature data indicating stronger Li$^+$ binding (Ross and Scruggs, 1964) were obtained at lower cation concentrations and an inversion of the Na$^+$ and Li$^+$ binding strength is indeed found at higher concentrations (Ross and Strauss, 1960). Also, experiments done with our system at 0.06 M cation show stronger binding to polynucleotides of Li$^+$ than Na$^+$.

TABLE III: Basic Amino Acid Competition Experiments.

Buffer (M)[b]	$\left(\frac{\text{OD}_{360}\text{ bound}/\mu\text{mole of P}^{a}}{\text{OD}_{360}\text{ free}}\right)_{\text{IC}}$ *A*	$\left(\frac{\text{OD}_{360}\text{ bound}/\mu\text{mole of P}}{\text{OD}_{360}\text{ free}}\right)_{\text{AU}}$ *B*	*A*:*B*	ε-DNP-Lys(Lys)$_n$ Indicator
KCl (0.12)	3.76	2.95	1.27	$n = 4$
Lys-HCl (0.25)	1.59	1.24	1.29	
Arg-HCl (0.25)	0.47	0.57	0.82	
His-HCl (0.25)	0.29	0.27	1.07	
KCl (0.12)	4.92	3.44	1.43	$n = 4$
Lys-HCl (0.25)	2.27	1.76	1.29	
Arg-HCl (0.25)	0.51	0.61	0.83	
His-HCl (0.25)	0.33	0.33	1.00	
Lys-HCl (0.25)	4.16	3.33	1.25	$n = 5$
Arg-HCl (0.25)	0.90	1.24	0.73	
Lys-HCl (0.25)	4.53	3.61	1.23	$n = 6$
Arg-HCl (0.25)	1.82	1.94	0.94	

[a] All tubes within each group contain the same total amount of ε-DNP-oligolysine. [b] Concentrations refer to those in the 10-ml solutions (originally containing 0.01 M sodium cacodylate–cacodylic acid, pH 7.0) in which the sack containing 1 ml of polynucleotide (in 0.12 M KCl) was suspended. The final pH of the Lys-HCl and Arg-HCl buffers was 6.7 while that of the His-HCl buffers was 4.8.

TABLE IV: Divalent Cation Competition Experiments.

Buffer (M)[a]	Addition (M)	$\left(\frac{\text{OD}_{360}\text{ bound}/\mu\text{mole of P}}{\text{OD}_{360}\text{ free}}\right)_{\text{IC}}$ *A*	$\left(\frac{\text{OD}_{360}\text{ bound}/\mu\text{mole of P}}{\text{OD}_{360}\text{ free}}\right)_{\text{AU}}$ *B*	*A*:*B*	ε-DNP-Lys(Lys)$_n$ Indicator
KCl (0.12)	None	4.92	3.44	1.43	$n = 4$
	$MgCl_2$ (0.001)	2.74	1.60	1.71	
	$CaCl_2$ (0.001)	2.50	1.31	1.91	
	$CaCl_2$ (0.002)	1.67	0.84	2.00	
KCl (0.12)	None	6.40	4.49	1.43	$n = 4$
	$MgCl_2$ (0.001)	3.08	1.60	1.92	
	$CaCl_2$ (0.001)	2.90	1.61	1.80	
	$MnCl_2$ (0.001)	2.70	1.59	1.70	
KCl (0.20)	None	6.77	5.92	1.14	$n = 6$
	$MgCl_2$ (0.001)	5.78	3.65	1.58	
	$CaCl_2$ (0.001)	5.36	3.44	1.56	
	$MnCl_2$ (0.001)	4.27	2.91	1.47	

[a] Buffer composition and additions again refer to 10 ml of original outside solution. Polynucleotide solutions as in previous table. Total ε-DNP-oligolysine amount again held constant only within groups of tubes.

and Noble, 1966), unhydrated cation radius (Nightingale, 1966), or cation stabilization of polynucleotide structure as manifested by hypochromism at room temperature. In addition, the cation specificities seen here do not correspond to cation position in the Hofmeister "lyotropic" series (Von Hippel and Wong, 1964) which correlates well with the effects of high ion concentrations (several molar) on macromolecular structure disruption.

However, the parallel between cation specificity and the tendency to structure water and the release of water on neutralization of polyphosphates suggests

TABLE V: Cation Effects on Polynucleotide-Stimulated Binding of RNA to *E. coli* Ribosomes.[a]

	Buffer[b]		Cpm Bound	
TMACl (M)	Tris-Acetate (M)	KCl (M)	Poly U Added	No Poly U
A. Poly U Stimulation of $[^{14}\mathrm{C}]$Phe-tRNA[c] Binding				
0	0.10	0.05	1650	189
0	0.05	0.05	1772	266
0	0.05	0	1384	229
0	0.05	0	1405	251
0.40	0.05	0	794	27[d]
0.80	0.05	0	240	27
0.40	0.10	0.10	971	40
			Poly C Added	No Poly C
B. Poly C Stimulation of $[^{14}\mathrm{C}]$Pro-tRNA[c] Binding				
0	0.05	0	357	38
0.40	0.05	0	33	34
0.80	0.05	0	34	27
0.40	0.10	0.10	41	28
0.80	0.10	0.10	42	25

[a] Method of Nirenberg and Leder (1964). Final volume (0.050 ml) 1.5×10^{-8} μmole of P of polynucleotide added at pH 7.2. [b] Buffers also were 0.02 M in magnesium acetate. [b] Counts per minute added, $[^{14}\mathrm{C}]$Phe-tRNA 2000, $[^{14}\mathrm{C}]$-Pro-tRNA 4000. [d] Note TMACl suppression of background binding.

that water of hydration may somehow direct the specificity of cation–polynucleotide interactions. An A·U base pair seems to direct more strongly the binding to its phosphates of those cations which organize the water around them than does an I·C base pair.[4] If this occurs with the A·U base pair because of its greater hydration, then the driving force for this specificity may resemble that postulated by Scheraga (1963) for the hydrophobic interactions influencing the structure of nucleic acids and proteins, namely the release of organized water by the participating species.[5]

It cannot be excluded that the observed cation specificities are due to differences between the poly (I + C) and poly (A + U) sugar–phosphate backbones rather than their different base compositions. However, Leng and Felsenfeld (1966), studying DNA precipitation, observed an A·T preference of TMA^+ and a G·C preference of polyarginine, results consistent with ours, under conditions where base composition but not sugar–phosphate backbone structure varied.

The correlation of the monovalent cation specificities observed in our dialysis experiments with cation effect on the mRNA-induced binding of tRNA to ribosomes suggests that both sets of results may be manifestations of some general cation properties, important in biological systems, possibly involving cation interaction with nucleic acid phosphate groups.

In interpreting the interaction of basic proteins with nucleic acids, it is clear from our studies that one must consider the effects due to monovalent and divalent cations competing with basic groups of the protein for nucleic acid phosphates. It seems reasonable in general, and was shown for ε-DNP-oligolysines in particular, that cations compete only with the positively charged groups of the protein. If one assumes that cation specificities for phosphates of (I·C) and (A·U) base pairs in synthetic polynucleotides reflect similar specificities for (G·C) and (A·U) or (A·T) base pairs in nucleic acids, then one can use the data from the dialysis experiments to make predictions about nucleic acid–protein interactions. For example, in situations where K^+ is the dominant cation, since K^+ itself exhibits very little binding specificity, the specificity of a basic protein would depend on the balance between arginine and histidine residues with a preference for I·C (and presumably G·C) base pairs and uncharged residues which seem to possess a specificity for A·U (and presumably A·T) base pairs. Na^+ and divalent alkaline earth cations, with A·U (or A·T) specificity, would

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[4] The cation effects observed here occurred at about one-tenth the cation concentrations needed for appreciable effects due to *bulk* solvent structuring (Von Hippel and Schleich, 1968). However, cation attraction by the polynucleotide phosphates could increase the effective cation concentration around the polynucleotide with resulting cation effects on the local solvent structure and large localized changes following cation phosphate site binding.

[5] Such a solvent effect may be connected with the correlations noted by Woese *et al.* (1966) between amino acid side-chain structure (hydrophobic or polar) and coding triplets.

tend to displace the basic protein to I·C- (or G·C-) rich regions.[6] It should be emphasized that these statements refer to binding. As demonstrated in the previous paper (Latt and Sober, 1967) cooperative effects or aggregation, occurring after binding has reached a certain level, could cause a reversal of apparent specificity if the more weakly binding regions of the nucleic acid have a greater tendency to aggregate (analogous to oligolysine–poly (A + U) complexes).

The interplay between hydrophobic and structure-breaking groups on proteins as well as effects of solvent cations can certainly result in significant specificity in nucleic acid–protein interactions. The difference in binding strength and specificity between Na^+ and K^+ may well play a key role in the regulation of nucleic acid–protein interactions in the cell. The different $Na^+:K^+$ ratios found in the nucleus, cytoplasm, and extracellular fluid (Siebert, 1966) may be important in such a control mechanism.

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[6] It is interesting in this respect that Pogo *et al.* (1967) have recently observed that the addition of Mn^{2+} to preparations of nuclei resulted in increased RNA transcription from A·T-rich regions of DNA (relative to G·C-rich regions). This is consistent with the results of our binding studies which would predict that Mn^{2+} would preferentially compete for and displace basic proteins from A·T-rich regions of DNA, presumably then allowing transcription from these regions.