Sheet Structures in Alternating Poly(D,L-peptides)

F. Heitz,* G. Détriché, F. Vovelle, and G. Spach

Centre de Biophysique Moléculaire, CNRS, 45045 Orléans Cedex, France.
Received April 16, 1979; Revised Manuscript Received April 14, 1980

ABSTRACT: Sheet structures of synthetic alternating poly(D,L-peptides) containing the valine and alanine residues found in the natural linear peptide gramicidin A were studied by infrared and diffraction techniques. Some of these structures could be characterized by a chain repeat unit of about 0.56 nm, in agreement with a conformation of the polar pleated sheet type in which all carbonyl groups point in the same direction.

Introduction

Polypeptides with alternating D and L residues in the chain have received much attention since the discovery of the antibiotic and ionophore properties of linear gramicidin.

It appears now that these model synthetic poly(D,L-peptides) can be distributed into two structural classes, although some of them belong to both, depending on molecular weight, conditions of sample processing, and so on. First, there are those polypeptides which adopt various specific helical structures, single or double stranded. Some of them were predicted by Urry[1,2] and Veatch et al.[3] They include high molecular weight $(\gamma$-benzyl-D,L-glutamate$)_n$,[4,5] $(D\text{-Val-L-Val-D-Val-L-Ala})_n$,[6] and, when cast from dioxane, $(D\text{-Phe-L-Leu})_n$.[6] These conformations seem to play an important role in the ion-transfer properties[7] of gramicidin itself.

The second class includes poly(D,L-peptides) forming sheet structures. These are low molecular weight $(\gamma$-benzyl-D,L-glutamate$)_n$,[8] $(D\text{-Ala-L-Ala})_n$,[9] and several new polypeptides described in this paper which contain residues found in the N-terminal moiety of gramicidin, i.e., $(D\text{-Val-L-Ala})_n$, $(D\text{-Ala-L-Ala-D-Val-L-Ala})_n$, and $(D\text{-Ala-L-Val-D-Val-L-Ala})_n$, hereafter abbreviated respectively as $(VA)_n$, $(VA_3)_n$, and $(V_2A_2)_n$. Because sheet structures of alternating poly(D,L-peptides) have not yet received much attention, it seems useful to give a brief historical account of the present status of knowledge on this subject. A special interest will be taken in the parameter best describing sheet structures, i.e., the chain axis repeat distance, identical with the C_{α_1}–C_{α_3} fiber axis distance measured experimentally by electron or X-ray diffraction techniques on oriented fibers.

Historical Background

In 1951, Pauling and Corey[10] described a molecular model, originally called "pleated sheet", in which the successive residues in a chain were similarly oriented, directing their carbonyl groups in one direction. These authors pointed out that this conformation involved two kinds of residues for all optically active poly(α-amino acids) because each amide group may be described as obtained from the preceding one by the operation of a glide plane of symmetry. They found the C_{α_1}–C_{α_3} fiber axis distance between 0.614 and 0.664 nm, depending on some rotation of the amide groups from 0 to 20°. Later,[11] the same authors reconsidered this structure and stated that it should be ruled out for proteins, as the stable orientation around the N–C and C–C′ bonds actually leads to a residue length along the chain axis of 0.284 nm, i.e., a C_{α_1}–C_{α_3} length of 0.568 nm, shorter than originally postulated for the model and, in particular, shorter than that found in proteins. They finally christened this structure "polar pleated sheet" inasmuch as all the carbonyl groups are oriented in the same direction, saving the expression "pleated sheet" for its still accepted meaning. In terms of more recent nomenclature the polar pleated sheet structure can be described by a repeated dipeptide unit, the torsion angles ϕ and ψ of one residue being located in the right-handed α-helix region while the angles of the neighboring residue correspond to the left-handed region. The symmetry condition implies that $\phi_i = -\phi_{i+1}$ and $\psi_i = -\psi_{i+1}$.

In 1972, Hesselink and Scheraga,[12] following Miller et al.,[13] pointed out that a polypeptide chain with $(\phi,\psi)_i = (-\phi,-\psi)_{i+1} = (\phi,\psi)_{i+2}$ for any i adopts a helical conformation with zero pitch, i.e., a ring structure with n residues, the size of which depends on the values of ϕ and ψ. Actually this corresponds to cyclic peptides and therefore to integral values of n. But if a pair of these angles is located on the line ϕ equal about ψ shown on Figure 1, then the rings have infinite diameter, leading to an extended regular structure. De Santis et al.[14] reached similar conclusions. The curve for $n = \infty$ crosses within the sterically allowed conformations corresponding only to the right- and left-handed α-helical regions (the polar pleated sheet) and marginally the fully extended chain ($\phi = \psi = \pm 180$); the latter case was discussed by Ramachandran and Chandrasekaran.[15]

In Figure 1, we have represented the lines of constant C_{α_1}–C_{α_3} distances ranging from 0.46 to 0.72 nm. On the same map, the energy contour for stable conformations of the L-alanyl residue is also reported.[16] It can be seen that Figure 1 bears similarities, but should be identical with that given by Némethy.[17] Actually, the graph of Némethy is in error (Némethy, personal communication) whereas the proper one is given here.

From Figure 1, the limiting values of the repeat unit along the chain axis for the polar pleated sheet are 0.52 and 0.60–0.62 nm with a most favorable value around 0.56 nm, as already predicted by Pauling and Corey.[11] As pointed out by De Santis et al.[14] the glide symmetry imposes a rigid limitation on the monomer conformation. The allowed limits of the C_{α_1}–C_{α_3} distance depend on the energy contour considered and on the position of the L residue either in the right- or the left-handed α-helical region (and vice versa for the D residue).

No search has been made to determine the conditions for providing further extended structures with suitable hydrogen-bonding patterns between adjacent chains, either parallel or antiparallel, in the case where the glide symmetry is not perfectly adhered to, but it can be expected that the C_{α_1}–C_{α_3} distances would not be very different from the above value.

Thus for truly alternating poly(D,L-peptides) the polar pleated sheet structure appears as a most plausible model. As this structure is valid for all L-peptides, it may also apply to random poly(D,L-peptides), and the finding of a dipeptide length of about 0.56 nm can be taken as a good indication for the existence of this structure.

Experimental Part

All polypeptides used in this study were obtained by polycondensation of the corresponding tetrapeptide 2-hydroxyphenyl ester.[18] The characteristics of some intermediate products are given in Tables I–III and in ref 6. The average molecular weights

0024-9297/81/2214-0047$01.00/0

Table I[l]
Intermediate Products in the Synthesis of $(VA)_n$

peptide (mol wt)	method of synthesis	% yield	R_f value (argentometric titration)	mp, °C	$[\alpha]^{25}_{546}$ (c 1), deg		analyses C	H	N
Boc-L-Ala-D-Val-L-Ala-OPop $C_{30}H_{39}O_8N_3$ (569.65)	mixed anhydrides	95[a]	0.70[b] (—)	169–171	−63.3[c]	calcd	63.25	6.90	7.37
						found	62.60	6.89	7.19
HCl-L-Ala-D-Val-L-Ala-OPop $C_{25}H_{32}O_6N_3Cl$ (496.00)	HCl/AcOH	99[d]	0.46[e] (99.3)	184–186	−39.1[f]	calcd	59.34	6.37	8.30
						found	59.09	6.36	8.22
Boc(D-Val-L-Ala)$_2$-OPop $C_{35}H_{48}O_9N_4$ (668.79)	DCCI	31[g]	0.76[b] (—)	184–186	−37.8[c]	calcd	62.86	7.23	8.37
						found	62.70	7.30	8.33
Boc(D-Val-L-Ala)$_2$-OC$_6$H$_4$OH-*o* $C_{27}H_{42}O_8N_4$ (550.65)	Zn/90:10 AcOH-H_2O	87.6[h]	0.60[b] (—)	115–117	+14.7[c]	calcd	58.89	7.69	10.17
						found	58.15	7.59	10.04
HCl(D-Val-L-Ala)$_2$-OC$_6$H$_4$OH-*o* $C_{22}H_{35}O_6H_4Cl$ (477.00)	HCl/AcOH	85[i]	0.65[b] (99.8)	149–151	−56.8[f]	calcd	55.40	7.40	11.75
						found	53.59	7.29	11.28

[a] 2-Propanol–isopropyl ether. [b] Chloroform–methanol–acetic acid (85/10/5, v/v). [c] Chloroform. [d] Ether. [e] Butanol–acetic acid–water (5/1/1, v/v). [f] Acetic acid. [g] Isopropyl ether. [h] Ether–hexane. [i] Ethyl acetate–petroleum ether. [j] Cyclohexane. [k] Filtration on neutral alumina. [l] Abbreviations used: AcOH, acetic acid; Boc, *tert*-butyloxycarbonyl; DCCI, dicyclohexylcarbodiimide; Pop, phenacyloxyphenyl.

Table II[l]
Intermediate Products in the Synthesis of $(VA_3)_n$

peptide (mol wt)	method of synthesis	% yield	R_f value (argentometric titration)	mp, °C	$[\alpha]^{25}_{546}$ (c 1), deg		analyses C	H	N
Boc-D-Ala-L-Ala-D-Val-L-Ala-OPop $C_{33}H_{44}O_9N_4$ (640.73)	DCCI	67[g]	0.60[b] (—)	145–148	−33.4[c]	calcd	61.86	6.92	8.74
						found	61.05	7.03	8.63
Boc-D-Ala-L-Ala-D-Val-L-Ala-OC$_6$H$_4$OH-*o* $C_{25}H_{38}O_8N_4$ (522.60)	Zn/90:10 AcOH-H_2O	88[j]	0.50[b] (—)	99–102	+23.6[c]	calcd	57.46	7.33	10.72
						found	57.41	7.67	10.30
HCl-D-Ala-L-Ala-D-Val-L-Ala-OC$_6$H$_4$OH-*o* $C_{20}H_{31}N_4O_6Cl$ (458.94)	HCl/AcOH	84[i]	0.45[e] (99.9)	148–150	−41.2[f]	calcd	52.34	6.81	12.21
						found	49.99	7.06	11.67

[a-l] See Table I footnotes.

Table III[l]
Intermediate Products in the Synthesis of $(V_2A_2)_n$

peptide (mol wt)	method of synthesis	% yield	R_f value (argentometric titration)	mp, °C	$[\alpha]^{25}_{546}$ (c 1), deg		analyses C	H	N
Boc-D-Ala-L-Val-D-Val-L-Ala-OPop $C_{35}H_{48}N_4O_9$ (668.79)	DCCI	76[c]	0.64[b] (—)	179–180	−62.9[f]	calcd	62.86	7.23	8.37
						found	61.55	7.40	8.27
Boc-D-Ala-L-Val-D-Val-L-Ala-OC$_6$H$_4$OH-*o* $C_{27}H_{42}O_8N_4$ (550.65)	Zn/90:10 AcOH-H_2O	65[k]	0.52[b] (—)	163–166	−7.07[f]	calcd	58.89	7.68	10.17
						found	58.60	7.96	9.78
HCl-D-Ala-L-Val-D-Val-L-Ala-OC$_6$H$_4$OH-*o* $C_{22}H_{35}O_6N_4Cl$ (476.99)	HCl/AcOH	68[i]	0.45[e] (99.0)	155–158		calcd	55.40	7.40	11.75
						found	52.44	7.49	11.14

[a-l] See Table I footnotes.

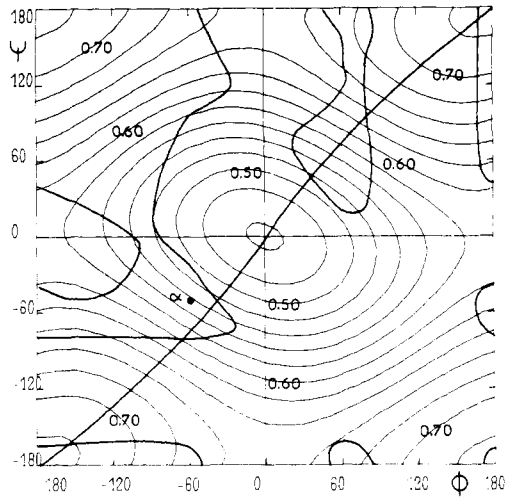

Figure 1. Lines of constant C_{α_1}–C_{α_3} distance (nm) in a polypeptide chain in the ϕ/ψ conformational map. The diagonal line near $\phi = \psi$ refers to linear chains in the glide symmetry condition. The superimposed energy contour at 4 kcal/mol of the alanyl residue[16] has been chosen to give the largest limits of the C_{α_1}–C_{α_3} distance in the allowed α regions. See text for a comparison with a similar graph in ref 17.

Table IV
Diffraction Data of the Various Poly(D,L-peptides) Containing Valine and Alanine

	$(VA)_n$		$(VA_3)_n$		$(V_2A_2)_n$	
hkl	d_{obsd}, nm	d_{calcd}, nm	d_{obsd}, nm	d_{calcd}, nm	d_{obsd}, nm	d_{calcd}, nm
200	1.470 vs	1.470	1.550 s	1.550	1.706 vs	1.706
400	0.735 s	0.735	0.775 w	0.775	0.853 s	0.853
500					0.681 w	0.682
220	0.521 w	0.520	0.521 w	0.519	0.519 w	0.522
002	0.475 vs	0.475	0.475 vs	0.475	0.475 vs	0.475
112	0.431 w	0.432	0.433 w	0.432	0.435 w	0.433
012		0.437		0.436		0.436
800	0.370 w	0.367				

were estimated to about 5000–10000. Oriented films for infrared spectroscopy and fibers for the diffraction investigation were prepared by stroking until dryness solutions in hexafluoroisopropyl alcohol.

The C_{α_1}–C_{α_3} distances were calculated on the basis of the atomic coordinates using 10° variations of ϕ and ψ. Determination of ϕ and ψ angles corresponding to linear chains, i.e., rings with an infinite number of residues, was made by a minimization procedure[19] for given values of the C_{α_1}–C_{α_3} distance.

Results

Diffraction Techniques. X-ray and electron diffraction patterns on $(VA)_n$, $(V_2A_2)_n$, and $(VA_3)_n$ (Table IV) are rather poor, but all reveal a strong meridional reflection corresponding to a spacing of 0.475 nm, which strongly suggests an interchain hydrogen bonding as usually found for cross-β-type structures.

For $(VA_3)_n$ and $(V_2A_2)_n$ all the observed reflections can be indexed on the basis of orthorhombic unit cells, especially the near-meridional one which lies on the second layer line indexed as 201 or 211. The dimensions of the cells are $a = 0.95$ nm (double interchain distance), $b = 3.10$ and 3.41 nm (intersheet distance) for $(VA_3)_n$ and $(V_2A_2)_n$, respectively, and $c = 1.10$ nm (chain repeat unit distance).

The densities (1.25 and 1.22 g cm^{-3}) measured by flotation in chloroform/hexane mixtures are in good agreement with those calculated from the parameters obtained by the diffraction technique (1.28 and 1.26 g cm^{-3}, respectively), assuming that $c = 1.10$ nm corresponds to a tetrapeptide unit.

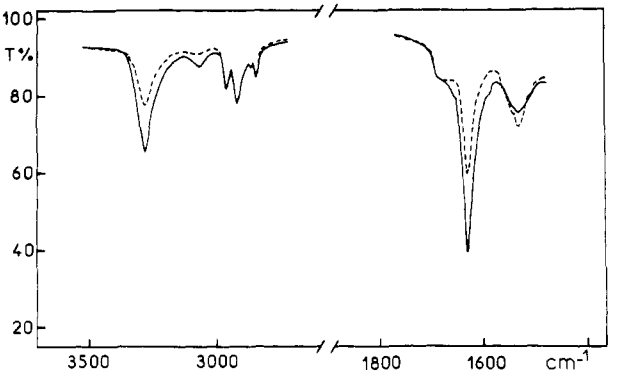

Figure 2. Infrared spectra of an oriented film of $(V_2A_2)_n$: (—) electric vector parallel to the direction of stroking; (- - -) electric vector perpendicular to the direction of stroking.

As for $(VA)_n$, although its diffraction pattern bears much similarity to those of $(VA_3)_n$ and $(V_2A_2)_n$ (all the reflections can also be indexed on the basis of an orthorhombic cell of parameters $a = 0.95$, $b = 2.94$, and $c = 1.11$ nm), the measured density (1.23 g cm^{-3}) is much lower than that calculated (1.30 g cm^{-3}). Better agreement on this latter point is obtained when indexing on the basis of a monoclinic cell with $\beta = 106°$. However, this could lead to a value of parameter $c = 1.26$ nm. It is worthwhile to mention here than Ascoli et al.[20] reported for (D-Ala-L-Val)$_n$ a pseudoorthorhombic cell with $a = 0.97$, $b = 1.47$, and $c = 0.62$ nm, the last value in poor agreement with our observations. This discrepancy and differing CD spectra in trifluoroethanol [ours (unpublished work) have the opposite sign of that given by Ascoli et al.[20]] are yet unexplained.

Infrared Spectroscopy. When cast from solutions in hexafluoroisopropyl alcohol, polypeptides $(VA)_n$, $(VA_3)_n$, and $(V_2A_2)_n$ show similar infrared spectra with amide A, I, and II bands around 3285, 1633, and 1630 cm^{-1}, respectively, and a shoulder at 1690 cm^{-1}. Further, the infrared linear dichroism (Figure 2) shows that the hydrogen bonds are oriented in the direction of stroking. The origin of an absorption band at 1730 cm^{-1} found on sample $(VA_3)_n$ is still unknown but seems to be a true property of the polymer. It must be mentioned that an unexplained absorption band at 1730 cm^{-1} was also found for some species of gramicidin A in solution.[3]

Discussion and Conclusion

The finding of a pure-meridional reflection of spacing 0.475 nm rules out any helical conformation for all three polypeptides and suggest rather an extended-type structure. The dimensions of the unit cells also rule out the possible existence either of structures similar to that proposed for (D-Ala-L-Ala)$_n$[9] or of the (Ala-Gly)$_n$[21] type ($c = 0.94$ nm for four residues).

Further, as pointed out by Ramachandran and Chandrasekaran[15] from examination of conformational maps, no sheet structure can be built up with chains containing both L and D residues having the same torsion angle located in the usual β region, except, as already seen, in the vicinity of the fully extended chain, which, however, is thought to be energetically not very favorable[15] and which requires a C_{α_1}–C_{α_3} distance of about 0.72 nm for two residues.

Therefore, as no up-to-date observed conformation can account for the diffraction characteristics of the poly(D,L-peptides) described in this paper, we propose that they adopt pleated sheet conformation which requires a C_{α_1}–C_{α_3} distance of about 0.55 nm (really 1.10 nm, as the chemical repeat unit is that of a tetrapeptide), in good agreement

with the data obtained from $(VA_3)_n$ and $(V_2A_2)_n$ and possibly $(VA)_n$.

This polar pleated sheet structure has already been described, however, without reference to the original work of Pauling and Corey, for a sheet structure called "βDL" obtained on a low molecular weight sample of (γ-benzyl-D,L-glutamate)$_n$.[8] This βDL conformation was suggested on the basis of a value of the chain repeat unit c between 0.52 and 0.56 nm estimated from X-ray and density measurements.

The proposal of the polar pleated sheet structure for $(VA_3)_n$, $(V_2A_2)_n$, and (γ-benzyl-D,L-glutamate)$_n$, however, raises questions concerning the positions of the infrared absorption bands. Indeed, the spectra are very similar to those found for the usual cross-β structures of the pleated sheet type with an antiparallel arrangement of the chains for all-L polypeptides, although the conformational states of the residues are very different in the two structures. Theoretical analysis may help to elucidate this question.

References and Notes

(1) Urry, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 672–6.
(2) Urry, D. W.; Goodall, M. C.; Glickson, J. D.; Mayers, D. F. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 1907–11.
(3) Veatch, W. R.; Fossel, E. T.; Blout, E. R. *Biochemistry* **1974**, *13*, 5249–56.
(4) Heitz, F.; Lotz, B.; Spach, G. *J. Mol. Biol.* **1975**, *92*, 1–13.
(5) Lotz, B.; Colonna-Cesari, F.; Heitz, F.; Spach, G. *J. Mol. Biol.* **1976**, *106*, 915–42.
(6) Heitz, F.; Lotz, B.; Détriché, G.; Spach, G. *Biochim. Biophys. Acta* **1980**, *596*, 137–40.
(7) Haydon, D. A.; Urban, B. W.; Heitz, F.; Spach, G. Proceedings of the International Symposium on Biomolecular Structures, Conformation, Function and Evolution, Madras, 1978, in press.
(8) Lotz, B.; Heitz, F.; Spach, G. *C. R. Hebd. Seances Acad. Sci., Ser. C* **1973**, *276*, 1715–8.
(9) Heitz, F.; Lotz, B.; Colonna-Cesari, F.; Spach, G. In ref 7.
(10) Pauling, L.; Corey, R. B. *Proc. Natl. Acad. Sci. U.S.A.* **1951**, *37*, 251–6.
(11) Pauling, L.; Corey, R. B. *Proc. Natl. Acad. Sci. U.S.A.* **1951**, *37*, 729–40.
(12) Hesselink, F. T.; Scheraga, H. A. *Macromolecules* **1972**, *5*, 455–63.
(13) Miller, W. G.; Brant, D. A.; Flory, P. J. *J. Mol. Biol.* **1967**, *23*, 67–80.
(14) De Santis, P.; Morosetti, S.; Rizzo, R. *Macromolecules* **1974**, *7*, 52–8.
(15) Ramachandran, G. N.; Chandrasekaran, R. *Indian J. Biochem. Biophys.* **1972**, *9*, 1–11.
(16) Scott, R. A.; Scheraga, H. A. *J. Chem. Phys.* **1966**, *45*, 2091–2101.
(17) Némethy, G. *Ann. N.Y. Acad. Sci.* **1969**, *155*, 492–506.
(18) Trudelle, Y. *J. Chem. Soc., Perkin Trans. 1* **1973**, 1001–5.
(19) Nelder, J. A.; Mead, R. *Comput. J.* **1965**, *7*, 308–13.
(20) Ascoli, F.; De Angelis, G.; Del Bianco, F.; De Santis, P. *Biopolymers* **1975**, *14*, 1109–14.
(21) Lotz, B.; Keith, H. D. *J. Mol. Biol.* **1971**, *61*, 201–15.

Expansion of Long Polyions in Simple Salt Solutions

D. M. Soumpasis* and K. H. Bennemann

Institute for Theoretical Physics, Freie Universität Berlin, 1000 Berlin 33, Arnimallee 3, West Germany. *Received August 1, 1980*

ABSTRACT: Using simple theoretical arguments, the results of Monte Carlo work on off-lattice chains with excluded volume, and published light scattering data of polyion expansion in the dilute regime, it is found that for degrees of polymerization $N \gtrsim 10^3$, the electrostatic expansion factor α_s obeys a general asymptotic relation of the form $\alpha_s = f(\xi,\chi^*)N^{g(\xi,\chi^*)}$, where ξ and χ^* are natural parameters describing the polyion charge density and salt-screening effects, respectively. In contradiction to existing theories that predict $g(\xi,\chi^*) = \text{const} \approx {}^1/_{10}$, the behavior of g and f is found to be very complex. There is evidence that for $\xi > 1$, both f and g become independent of ξ as may be predicted by Manning's limiting laws for polyelectrolytes.

Introduction

A rigorous ab initio treatment of the expansion of polyion chains in salt solution amounts to dealing with a system of N particles, interacting via screened electrostatic interactions and subject to the constraint of being connected into a chain. Due to these three aspects the problem is extremely difficult to handle and no satisfactory solution exists to date. Quite recently, the problem has been attacked by Richmond[1] and Bailey,[2] who used a Gaussian chain model and the self-consistent field (SCF) approach introduced by Edwards[3] and subsequently discussed, refined, and extended by de Gennes,[4] Reiss,[5] and Yamakawa,[6–8] as well as by Odijk and Houwaart[9] and Fixman and Skolnick,[10] who adopted a wormlike model for the polyion and studied the electrostatic excluded-volume effect, explicitly taking into consideration local chain stiffening. In this work we adopt a completely different approach. We avoid mathematical complexities as far as possible and try to understand the basic features of the problem, using a combination of general theoretical arguments, our present state of knowledge of the excluded-volume problem in general, and last but not least a rather extensive analysis of available experimental data of polyion expansion. It is our opinion that, due to the complexity of the problem at hand, real progress toward a quantitative theory cannot be achieved unless at least the qualitative aspects are understood and taken into consideration.

Apart from its attractiveness per se, the polyion expansion problem can serve as a particularly convenient model for the excluded-volume problem in general, since in this case the relevant monomer–monomer interactions are well-defined and may be generally described in terms of a few natural parameters. It is therefore hoped that its study will also contribute toward a better understanding of the excluded-volume effect of real polymers in solution.

Electrostatic Expansion Factor

Consider a chain of N monovalent charged sites joined by $N - 1$ bonds of length a immersed in a 1:1 electrolyte solution with n ions per unit volume and a dielectric constant ϵ at temperature T. Introducing the mean-square radius of gyration[7]

$$\langle S^2 \rangle = \frac{1}{2N^2} \sum_i \sum_j \langle R_{ij}^2 \rangle \tag{1}$$

where R_{ij} denotes the distance between sites i and j, we characterize the expansion of the chain due to the elec-

0024-9297/81/2214-0050$01.00/0