February, 1987] © *1987 The Chemical Society of Japan* NOTES *Bull. Chem. Soc. Jpn.*, **60**, *759—760 (1987)*

Self Regulation in the Cooperative Binding of Small Molecules to Linear Polymers

Takayuki SANO,* Thoru NAKATSUKA, Hirotsugu TSURUTA, and Shoji HARADA
Department of Materials Science, Faculty of Science, Hiroshima University, Hiroshima 730
(Received September 26, 1986)

Synopsis. As for the cooperative binding of small molecules to the linear polymers, including not only synthetic polymers but also biopolymers, an interesting relationship, the so called "self regulation effect" between the isolated binding constant (intrinsic binding constant) and the cooperative binding constant, (binding constant of growth process) can be observed amongst the large amount of data so far reported. The biological significances as well as the possible origin for this "self regulation mechanism" will be discussed.

A number of investigations into the cooperative binding of small molecules to polymers have been undertaken,[1-15] and several theories based on the one dimensional Ising model have been advanced.[6,7,11] For cooperative binding, it has been pointed out that the cooperativity seen in the binding of small dye molecules to counter charged synthetic linear polymers arises from a stacking interaction between bound dyes on polymer.[10,13] However, no systematic investigation of the binding mechanism has been completed. We report here an interesting correlation found between the isolated binding constant and the cooperative interaction parameter obtained for a variety of systems, both synthetic and biological, and propose a "self-regulation" cooperative binding effect in these systems.

Since the theory on the cooperative binding phenomenon was developed, our knowledge of the binding mechanism has been considerably extended. As for the cooperative binding of small molecules to polymers, the following three equations have been independently derived by Scatchard,[1] Schwarz,[7] and McGhee and von Hippel[11] respectively:

$$r/C_f = K_o e^{-2\omega r}(n-r), \tag{1}$$

$$C_f = 1/K_{co} - \left\{\frac{C_f}{qK_{co}r(n-r)}\right\}^{1/2}(n-2r) \tag{2}$$

with

$$K_{co} = q\,K_o,$$

and

$$r/C_f = K_o(n-r)\left\{\frac{n-2r+R}{2(n-r)}\right\}^2 \tag{3}$$

with

$$R = \{(n-2r)^2 + 4qr(n-r)\}^{1/2},$$

where r is the ratio of the bound ligand to total polymer concentration, C_f is the concentration of free ligand, K_o is the intrinsic binding constant, ω is the electrostatic force between bound ligands, n is the number of binding sites per residue of polymer, K_{co} is the cooperative binding constant, and q is the cooperative interaction parameter. In these three theories the binding isotherms are described by three parameters, viz., the number of binding sites per residue of polymer, n; the intrinsic binding constant, K_o; and a cooperative interaction parameter, q or ω. The above three equations can be combined as follows through the experimentally measured apparent binding constant, K, determined by the conventional method of using the value of that at the isotherm midpoint;

$$K = \frac{1}{(C_f)_{r=1/2}} = K_o e^{-\omega n} = K_o q = K_{co} \tag{4}$$

The focus of our investigation was to examine the proposed theory and to clarify the origin of the cooperative interaction energy. From the investigations reported so far, it has been found in general that the

Table 1. The Binding Parameters of the Cooperative Binding in Various Systems

	System	T / °C	n	K_0 / M^{-1}	q	pH	c.f.	Ref.
1.	$(L\text{-}Glu)_n$[a)]+AO[b)]	25	1.24	5×10^4	12	7.5	at low P/D[c)] 1 mM Na_2HPO_4/NaH_2PO_4	8
2.		25	1.24	3.5×10^2	1700	7.5	at high P/D	
3.	$(L\text{-}Glu)_n$+PF[d)]	25	1.11	6.7×10^2	150	7.5	1 mM Na_2HPO_4/NaH_2PO_4	9
4.	$(L\text{-}Glu)_n$+TB[e)]	25	0.45	4.7×10^4	3	5	No added electrolyte	12
5.		25	1.1	$8.6\times10_4$	8	8		
6.	$(Acry)_n$[f)]+PF	25	0.37	1.1×10^4	90	7.9	high mol. wt. 1 mM Na_2HPO_4/NaH_2PO_4	10
7.		25	0.37	1.4×10^4	180	6.9	low mol. wt.	
8.	$(Acry)_n$+TB	20	1.1	2.4×10^4	20	6.5	No added electrolyte	14
9.		20	1.2	1.8×10^4	30	8		
10.		20	1.4	2.1×10^4	80	10		
11.	$(Phos)_n$[g)]+PF	25	0.8	6.4×10^2	700	6.9	1 mM $Na_2HPO_4/Na_2H_2PO_4$	10
12.	Poly(A)+PF	0	0.9	7×10^3	140	6.8	1 mM cacodylate buffer	13
13.	DNA+PF	25	1.0	4×10^3	20	6.8	1 mM cacodylate buffer	
14.	$(L\text{-}Lys)_n$[j)]+MO[j)]	25	1.3	9.2×10^4	2.4	7.0	No added electrolyte	15

a) $(L\text{-}Glu)_n$=poly(L-glutamic acid). b) AO=Acridine Orange. c) P/D=polymer to dye ratio. d) PF=Proflavin. e) TB=Toluidine Blue. f) $(Acry)_n$=poly(acrylic acid). g) $(Phos)_n$=poly(phosphoric acid). h) poly (A)=poly (adenylic acid). i) $(L\text{-}Lys)_n$=poly(L-lysine). j) MO=Methyl Organge. 1 M=1 mol dm^{-3}.

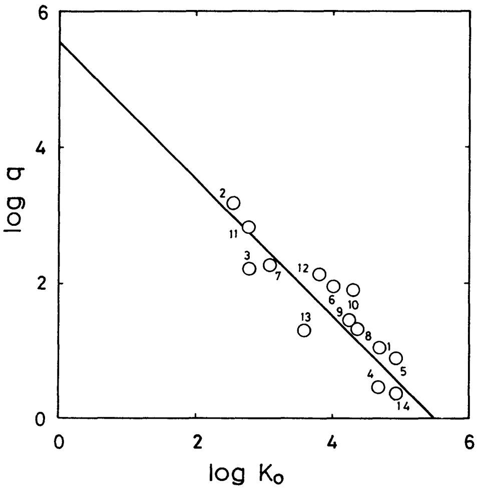

Fig. 1. The logarithmic plots of the cooperative interaction parameter, q against the isolate binding constant, K_o. The numbers on circles correspond to those in Table 1.

binding process of ionic polymers to ionic dye molecules is governed by an electrostatic interaction between bound molecules. The binding parameters obtained by independent workers on various systems at low ionic strength are listed in Table 1. As can be seen from the table there is a systematic trend in the values of q and K_o, i.e., the smaller K_o is, the larger is q. The logarithmic plot of K_o vs. q is shown in Fig. 1 where a general linear correlation was found having a slope equal to -1 regardless of such solution conditions as pH. This new finding between K_o and q has an important meaning with respect to the mechanism of the cooperative binding of small molecules to linear polymers. As described above, the parameter q can be related to K_o by the cooperative binding constant K_{co} or the binding strength K (see, e.g., Eqs. 1—4). Consequently, the obtained linear relationship between K_o and q implies that all the systems listed in Table 1 have essentially the same binding mechanism and also have a single cooperative binding constant, $K_{co}=(2.5\pm 0.5)\times 10^5\ M^{-1}$. In other words, the isolated binding is compensated in strength by the cooperative interaction effect so as to give the same binding strength regardless of the system. We call this effect "self-regulation" in the cooperative binding of small ions to polymers.

It has been reported that the binding mechanisms of the ionic polymer and dye molecules are dependent on the chemical properties of both molecules.[10,13] This is certainly true. However on the basis on the new finding of the linear correlation between K_o and q, it can be pointed out that there must exist a more specific factor in the binding mechanism of small ionic molecules to ionic polymers. At the present time there has still not been enough data reported to clarify what this specific factor is. If, for instance, the electrostatic binding interaction between counter-charged ionic species is the dominant factor in the binding of the systems listed in Table 1, then the state of hydration of the reacting species, especially of the ionic groups of the polymers, may be the most important factor in the "self-regulation" resulting from the cooperative binding interaction.

The "self-regulation" effect has been observed not only for the synthetic polymers but also for biopolymers such as DNA.[13] Therefore it is likely that the effect plays an important role in keeping the in vivo concentration of small ionic molecules at the optimum level. The self-regulation effect proposed here provides motivation for further investigation on the binding of small ligands to polymers, particularly on the role of the state of hydration of the reacting spacies.

References

1) G. Scatchard, Ann. N. W. *Acad. Sci.*, **51**, 660 (1949).

2) D. F. Bradley, and M. K. Wolf, *Proc Natl. Acad. Sci., U. S.*, **45**, 744 (1959).

3) G. G. Hammes, and C. D. Hubbard, *J. Phys. Chem.*, **70**, 165 (1966).

4) D. Crothers, *Biopolymers*, **6**, 575 (1968).

5) W. C. Galley, *Biopolymers*, **6**, 1279 (1968).

6) F. W. Schneider, C.L. Croman, and S.K. Podder, *J. Phys. Chem.*, **72**, 4563 (1968).

7) G. Schwarz, *Eur. J. Biochem.*, **12**, 442 (1970).

8) G. Schwarz, S. Klose, and W. Balthasar, *Eur. J. Biochem.*, **12**, 454 (1070).

9) G. Schwarz and W. Balthasar, *Eur. J. Biochem.*, **12**, 461 (1970).

10) G. Schwarz and S. Klose, *Eur. J. Biochem.*, **29**, 249 (1972).

11) J.D. McGhee and P. H. von Hippel, *J. Mol. Biol.*, **86**, 469 (1974).

12) H. Ushio, T. Yasunaga, T. Sano, and Y. Tsuji, *Biopolymers*, **15**, 187 (1976).

13) M. Dourlent and J. F. Hogrel, *Biopolymers*, **15**, 29 (1976).

14) H. Ushio, H. Nishimura, Y. Tsuji, T. Sano, and T. Yasunaga, *Polymer J.*, **9**, 489 (1977).

15) K. Murakami, T. Sano, K. Ishii, N. Kure, and T. Yasunaga, *Biopolymers*, **22**, 2035 (1983).