[Full Paper]

Computer simulation of electrophoretic transport

II. Modified general procedure and an application to isoelectric focusing of proteins using immobilized pH gradient

Kazuo Shimao

SUMMARY

My computer simulation procedure was applied to isoelectric focusing of proteins using immobilized pH gradient formed by Immobilines. The results for small amount of proteins (micromole order of proteins on a medium 4cm long, 10cm wide and 0.1cm thick) agreed well with those of widely reported experiments, while simulations for very large amount (10 to 100 times the above amount) of proteins with slightly differing isoelectric points resulted in a pattern that could be expressed as zero-velocity isotachophoretic separation of proteins.

Key words: isoelectric focusing, computer simulation, protein, immobilized pH gradient.

INTRODUCTION

Isoelectric focusing (IEF) of proteins using immobilized pH gradient (IMPHG) formed by Immobilines (IMMOBs) has been extensively used since its publication by Bjellqvist *et al.*¹⁾ in 1982. In spite of many experimental applications of the procedure, computer simulation (CS) of focusing of proteins has not been reported. My CS procedure²⁾ was modified to be applicable to IEF of proteins using IMPHG and applied to IEF of albumin, transferrin- and (or) γ -globulin-like molecules. The results for IEF of small as well as large amount of proteins are reported in this paper. For analytical scale small amount of proteins, results of CS are in agreement with widely reported experiments. For IEF of large amount of proteins having slightly differing pI , the CS resulted in a sep-

aration pattern that may be expressed as a zero-velocity isotachophoretic separation, which is suggestive for preparative application of IEF using IMPHG to proteins.

METHODS**1. Basic assumptions**

Medium for electrophoresis is assumed to have unit cross-sectional area and only electrophoretic and diffusional transports are taken into account.

Basic equations for electrophoretic and diffusional transports and dynamic Gaussian model for electrophoretic behavior of protein molecule are the same as those used in the previous reports.^{3,4)}

All components in the system, i.e. IMMOBs, proteins, hydrogen ion and hydroxyl ion, have known physico-chemical constants such as numbers and pK s of dissociation groups, ionic mobilities,

島尾和男; Densitometer Research Laboratory.

Correspondence address: Kazuo Shimao, 4-22-29 Nukui, Nerima-ku, Tokyo 176, Japan.

Abbreviations: IEF, isoelectric focusing; CS, computer simulation; PG, potential gradient; IMPHG, immobilized pH gradient; IMMOB, Immobililine.

(Received April 14, 1994, Accepted June 10, 1994)

diffusion coefficients etc., and electric neutrality holds throughout the medium. Electric current is carried solely by proteins and hydrogen and hydroxyl ions. Anolyte and catholyte are not included because results of preliminary study ascertained that the existence of these components does not influence focusing pattern of protein molecules distant from electrodes.

Table 1 shows physico-chemical constants used for CS.

2. Description of the system

The system is assumed to be composed of acidic and basic ionization residues derived from IMMOBs of known concentrations and pK s fixed to the matrix of electrophoretic medium of unit cross-sectional area and three protein molecules. IMPHG having desired pH range is formed by appropriate combinations of linear concentration gradients of IMMOBs.^{1,5)} An electrophoretic medium, $L+dx$ cm long is divided into $N+1$ segments with equal length, dx , numbered from anodic to cathodic end as 0 to N so that $dx=L/N$.

The three protein molecules are numbered as 0, 1 and 2, and the concentration of the j -th molecule in the i -th segment at time t is expressed as $C(j, i, t)$.

t). Values of $C(j, i, 0)$ s ($j=1$ to 2, $i=0$ to N) are given as an initial condition.

3. An elementary step to get $C(j, i, t+dt)$ s from $C(j, i, t)$ s

An elementary step of the previous report²⁾ was modified in the following steps 3) and 8) to be applicable to IEF of proteins on IMPHG.

1) $pH(i, t)$ s ($i=0$ to N) of the i -th segment at time t are calculated by using electric neutrality condition of each segment.

2) Conductivities, $\kappa(i, t)$ s ($i=0$ to N) of the i -th segment at time t are calculated, and the total resistance of the system $R(t)$ is calculated by $R(t) = 1/(\sum_{i=0}^N(dx/\kappa(i, t)))$.

3) Determination of current density for maximum current, voltage and wattage settings of MC A, MV V and MP W, respectively, is performed as follows.

Current density $I(t)$ is tentatively set at $I = kMC/100$, starting from $k=1$ to 100. For each k

3-1) if $IR(t) \geq MV$ and $I^2R(t) \leq MP$, then $I(t) = MV/R(t)$,

3-2) if $IR(t) \leq MV$ and $I^2R(t) \geq MP$, then $I(t) = \sqrt{MP/R(t)}$,

3-3) if $IR(t) \leq MV$ and $I^2R(t) \leq MP$, then

Table 1. Physico-chemical input data of albumin- transferrin- and γ -globulin-like protein molecules, H^+ and OH^- ions.

Protein		Albumin 0	Albumin 1	Albumin 2	Transferrin	γ -Globulin
	pK					
Acidic residues	3.5	1	1	1	2	4
	4.0	50	49	48	70	48
	4.4	80	80	80	55	69
	8.0	4	4	4	0	32
	10.8	16	16	16	1	60
Total acidic residues		154	153	152	128	213
Basic residues	6.5	15	15	15	18	26
	8.0	1	1	1	4	4
	10.5	58	58	58	79	89
	12.5	24	24	24	26	45
Total basic residues		98	98	98	127	164
Mobility of monovalent cation ($10^{-6} \text{ cm}^2/\text{Vs}$)		2.5	2.5	2.5	2.4	2.0
Diffusion coefficient ($10^{-7} \text{ cm}^2/\text{s}$)		6.0	6.0	6.0	5.8	4.0
Isoelectric point		4.734	4.749	4.765	5.815	7.976
Mobility of hydrogen ion : 0.00361 cm^2/Vs , mobility of hydroxyl ion : -0.0019.92 cm^2/Vs						

try the next k ,

3-4) if $IR(t) \leq MV$ and $I^2R(t) \leq MP$ for all k , then $I(t) = MC$.

4) Potential gradients (PGs) at the i -th segment, $PG(i, t)$ s ($i=0$ to N) are calculated by $PG(i, t) = I(t)/\kappa(i, t)$.

5) Mobilities of the j -th molecule in the i -th segment, $u(j, i, t)$ s ($j=0$ to 2 , $i=0$ to N) are calculated from the respective Gaussian model and pH (i, t)s, and migration velocities of the j -th molecule in the i -th segment, $v(j, i, t)$ s are calculated by $v(j, i, t) = u(j, i, t)PG(i, t)$ ($j=0$ to 2 , $i=0$ to N).

6) The maximum value of absolute values of $v(j, i, t)$ s is expressed as V_m and tentative value of dt for CS at time t is calculated by $DT = 0.95 dx/V_m$.

7) $C(j, i, t+DT)$ s ($j=0$ to 2 , $i=1$ to $N-1$) are calculated by

$$\begin{aligned} C(j, i, t+DT) = & C(j, i, t) \left(1 - \frac{|v(j, i, t)DT|}{dx} \right) \\ & + C(j, i-sgn, t) \frac{(sgn)v(j, i-sgn, t)DT}{dx} \\ & + D_j DT (C(j, i+1, t) - 2C(j, i, t) \\ & + C(j, i-1, t)) / (dx)^2, \end{aligned}$$

where, $sgns$ are signs of $v(j, i, t)$ s and are equal to 1, 0 and -1 , for positive, zero and negative values of $v(j, i, t)$ s, respectively.

The first term of the second line of the above expression is the amount of the j -th component in the i -th segment at time t which still remains in the i -th segment at time $t+DT$, and the second line is the amount that flows in from the neighbouring segment during DT electrophoretically. Thus, the first and the second lines give the result of electrophoretic transport and the third and the fourth lines are well-known diffusional transport.

8) Determination of dt for CS

If DT is too large for diffusional transport, it happens that some of the $C(j, i, t+DT)$ are calculated to be negative. If this is the case, a new tentative value of dt , DT , is set at $0.8 DT$ and the calculation of the step 7) is repeated until all the $C(j, i, t+DT)$ s become larger than or equal to zero. The last value of DT is dt for that particular elementary step.

4. Computer program

My program in N₈₈Basic (NEC) was run with an NEC Model 9821 Ap/u 7 personal computer. Hard copies of patterns on NEC MultiSync display were taken by NEC PC-PR 1000/4 page printer to obtain graphic representations of the CS.

RESULTS AND DISCUSSION

Typical results of CS are shown in Fig. 1 to 3. In each figure, anode is to the left, upper panel A is a starting condition and lower panel B is a steady state of IEF after sufficiently many elementary steps of CS corresponding to sufficiently long time of electrophoresis. In each panel pH and PG profiles are on the top and protein concentrations are on the bottom. IMMOB concentrations are shown on the bottom of panel A.

Maximum current, voltage and wattage settings for Fig. 1 and 2 are 0.025 A, 1250 V and 2.5 W, those for Fig. 3 are 0.025 A, 2500 V and 5.0 W, respectively.

1. Analytical scale separation of three albumin-like molecules of similar isoelectric points on an IMPHG of pH 4.5-5

Starting from an initial state of Fig. 1 A, three albumin-like protein molecules slightly differing in pI by about 0.015 focused to the respective isoelectric region with complete separation after 500 elementary steps of CS (3393 s of electrophoresis) as shown in B. The existence of such a small amount of proteins in the system makes little influence on both pH and PG in the final steady state. IMMOB concentration ratios are taken from a part of the recipe for pH range 4-6 of reference,⁵⁾ but the absolute values are about 1/10. The resolving power shown in Fig. 1 A is in agreement with experimental values (e. g., reference¹⁾) and the fact certifies the correctness of the CS procedure.

2. Separation pattern of large amount of protein molecules

Application of large amount of proteins (300 times that in Fig. 1) to IMPHG of Fig. 1 causes remarkable change in both pH and PG patterns as shown in Fig. 2 A and B. After 6001 steps of CS (21031 s of electrophoresis), the three protein mol-

ecules form pH zones equal to their respective pI s and are located side by side in the order of their pI s as shown in B. A hill-like concentration pat-

tern of each protein in this steady state may be called separation of the three protein molecules by zero-velocity isotachopheresis rather than isoelectric

Fig. 1. CS of IEF of analytical amount of three albumin-like molecules of Table 1 on an IMPHG of pH 4.52-5.02. $dx=0.01$ cm, $L=4.01$ cm. 6, 4.8 and 3.84×10^{-6} moles of albumin 0, 1 and 2, respectively, per cm^2 cross-sectional area of medium, are in the system. One elementary step takes about 10s. See text for other details.

focusing. The result seems to correspond to those of experiment by Ek *et al.*⁶⁾ In this state, buffering capacity of IMPHG is too small to keep the pH gradient linear and the amount of protein at which

these states occur depends on and is proportional to IMMOB concentrations of IMPHG.

Fig. 2. CS of IEF of large amount of three albumin-link molecules of Table 1 on an IMPHG of pH 4.52-5.02. $dx=0.01$ cm, $L=4.01$ cm.

300 times the amounts of proteins for Fig. 1 are in the system. See text for other details.

Fig. 3. CS of IEF of analytical amount of albumin-, transferrin- and γ -globulin-line protein molecules of Table 1 on a non-linear IMPHG of pH 4.20-10.56. $dx=0.02cm$, $L=10.02cm$. $6, 4.8$ and 3.84×10^{-6} moles of albumin 0, transferrin and γ -globulin, respectively, per cm^2 cross-sectional area of medium, are in the system. IMMOB 0, 1, 2, 3, 4 and 5 are IMMOB pK 3.6, 4.6, 6.2, 7.0, 8.5 and 9.3, respectively.

See text for other details.

3. Separation on non-linear IMPHG of wide pH range

Non-linear wide pH range IMPHG (pH 4.12–10.56) is formed by linear concentration gradients of IMMOBs (Fig. 3 A). Wide range IMPHG can not be linear because of non-uniform distribution of p*K* values of IMMOBs available. Non-the-less, the degree of non-linearity of Fig. 3 A might be allowable on the basis of excellent reproducibility of IMPHG formed by IMMOBs and the fact that wide range IMPHG is useful as a first step of 2 D-electrophoretic analyses of complex mixtures.⁷⁾

Figure 3 B shows separation after 2,000 steps of CS (3539 s of electrophoresis) of albumin-, transferrin- and γ -globulin-like molecules. From the nature of IEF by using IMPHG, PG is much higher in neutral than in acidic or alkaline zone. This brings the order of sharpness of the bands as γ -globulin > transferrin > albumin.

4. Other pH range and protein molecules

My procedure for CS of IEF of proteins on IMPHG successfully simulates focusing process of other proteins in other pH ranges than those described above and is expected to be conveniently used for designs of IEF experiments using IMPHG (data not shown).

REFERENCES

- 1) Bjellqvist B, Ek K, Righetti PG, Gianazza E, Gorg A, Westermeier R, Postel W. Isoelectric focusing in immobilized pH gradients: Principle, methodology and some applications. *J Biochem Biophys Methods* 1982; 6: 317–39.
- 2) Shimao K. Computer simulation of electrophoretic transport I. General procedure and an application to isoelectric focusing of proteins using carrier ampholytes. *Jpn J Electroph* 1994; 38: 221–5.
- 3) Shimao K. Mathematical simulation of isotachopheresis boundary between protein and weak acid. *Electrophoresis* 1986; 7: 297–303.
- 4) Shimao K. Mathematical simulation of steady state isoelectric focusing of proteins using carrier ampholytes. *Electrophoresis* 1987; 8: 14–9.
- 5) Ek K, Bjellqvist B, Bishop, R. Recipes for the preparation of broad Immobiline pH gradients. LKB Application Note 322 1983.
- 6) Ek K, Bjellqvist B. Preparative isoelectric focusing in immobilized pH gradients. In: Hirai H editor. *Electrophoresis '83*. Berlin: Walter de Gruyter, 1983: 563–567.
- 7) Bjellqvist B, Pasquali C, Ravier F, Sanchez J-C, Hochstrasser D. A non-linear wide range immobilized pH gradient for two-dimensional electrophoresis and its definition in relevant pH scale. *Electrophoresis* 1993; 14: 1357–65.