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Interaction between Serum Albumin and Mercaptoundecahydrododecaborate Ion (An Agent for Boron-Neutron Capture Therapy of Brain Tumor). II. Proposal of Two Interaction Models

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Two models have been proposed for the reaction between mercaptoundecahydrododecaborate ions and protein molecules. Both models assume two-step reactions, the first step being different between the two models; random- and monogamous-pairing models. Measuring the equilibrium concentrations of protein-ligand complex for many combinations of the borate and protein concentrations, and applying the modified simplex method to the data obtained, one can find out a set of parameter values by which the experimental results are best explained. These parameters include the number of active sulfhydryl groups, α, the number of active disulfide groups, β, per intact protein molecule, and the equilibrium constants, K_1 and K_2, for the first and second steps.

As described in Part I, mercaptoundecahydrododecaborate ions $B_{12}H_{11}SH^{2-}$ (abbreviated as BSH) can bind to serum albumin quite probably through the formation of disulfide linkage. In the albumin molecule, there are both disulfide groups and sulfhydryl groups. Some of these groups will not participate in the binding reaction for some reasons such as steric inaccessibility to the ions. The active disulfide and sulfhydryl groups that participate in the reaction will, henceforth, be expressed by PS-SP and PSH, respectively. Their reactions with BSH will be discussed by using two reaction models.

Random-Pairing Model

Provided all PS-SP groups are equivalent to each other and the same is true also for PSH, the reaction equilibrium can be formulated as

$$\text{PS-SP} + \text{BSH} \overset{K_1}{\rightleftarrows} \text{PS-SB} + \text{PSH} \tag{1}$$

$$\text{PS-SB} + \text{BSH} \overset{K_2}{\rightleftarrows} \text{BS-SB} + \text{PSH} \tag{2}$$

where PS-SB stands for the protein-ligand linkage and BS-SB the dimerized oxidation product, *i.e.* $B_{12}H_{11}S\text{–}SH_{11}B_{12}^{4-}$ ion. It should be noted that the equivalency of all PS-SB groups is assumed additionally.

The equilibrium concentrations can be obtained by solving the simultaneous equations

$$K_1 = \frac{[\text{PS-SB}][\text{PSH}]}{[\text{PS-SP}][\text{BSH}]} \tag{3}$$

$$K_2 = \frac{[\text{BS-SB}][\text{PSH}]}{[\text{PS-SB}][\text{BSH}]} \tag{4}$$

under the conditions of

$$[\text{BSH}] + [\text{PS-SB}] + 2[\text{BS-SB}] = [\text{B}] \tag{5}$$

$$[\text{PS-SP}] + [\text{PS-SB}] + [\text{BS-SB}] = \beta[\text{P}] \tag{6}$$

$$[\text{BSH}] + [\text{PSH}] = \alpha[\text{P}] + [\text{B}] \tag{7}$$

where [P] and [B] are respectively the total protein and total borate concentrations, and α and β are respectively the numbers of active sulfhydryl groups and of active disulfide groups per

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intact protein molecule. The concentrations, [P] and [B], are specified by experimental conditions, while the parameters, α and β, depend on the species of protein sample. The latters have definite values as far as the same sample of albumin is used. Our experimental technique at the present stage can determine only the concentration of PS-SB; those of BSH, BS-SB, PS-SP, and PSH cannot be measured individually. Therefore, the authors have tried to express [PS-SB] as a function of [P] and [B], and attained equation (8) through the derivation course shown in Appendix.

$$\begin{aligned}&(4K_2-K_1)(K_1K_2-K_1+1)x^3 + K_1[(4K_2-K_1)t + (4K_2-K_1)(\alpha+2\beta)\\&\quad + (K_1-2)(2K_2-1)\beta]x^2 + K_1[K_1K_2t^2+K_1\{\alpha-(2K_2-3)\beta\}t\\&\quad + (\alpha+2\beta)(\alpha+K_1\beta)]x - K_1^2\beta(\alpha+2\beta)t = 0 \qquad (8)\end{aligned}$$

where

$$x = [\text{PS-SB}]/[\text{P}] \qquad (9)$$

$$t = [\text{B}]/[\text{P}] \qquad (10)$$

Assuming a set of values for K_1, K_2, α, and β, and substituting the t value specified by the experimental conditions, one can obtain the theoretical x, *i.e.* x_{theo}, by solving equation (8) algebraically or by means of successive approximation.

Consider that one has carried out n experiments with different combinations of [P] and [B] to observe n experimental values of x; $(x_{\text{obs}})_i$ with $i=1$—n. Let $(x_{\text{theo}})_i$ be the corresponding theoretical x calculated with a set of (K_1, K_2, α, β) assumed provisionally. Then, the sum of squared relative errors is expressed by

$$S = \sum_{i=1}^{n}\left(\frac{(x_{\text{obs}})_i-(x_{\text{theo}})_i}{(x_{\text{theo}})_i}\right)^2 \qquad (11)$$

The set of parameters (K_1, K_2, α, β) that gives the smallest S is regarded as the best-fit set. The modified simplex method proposed by Deming and Morgan[2] has been applied with

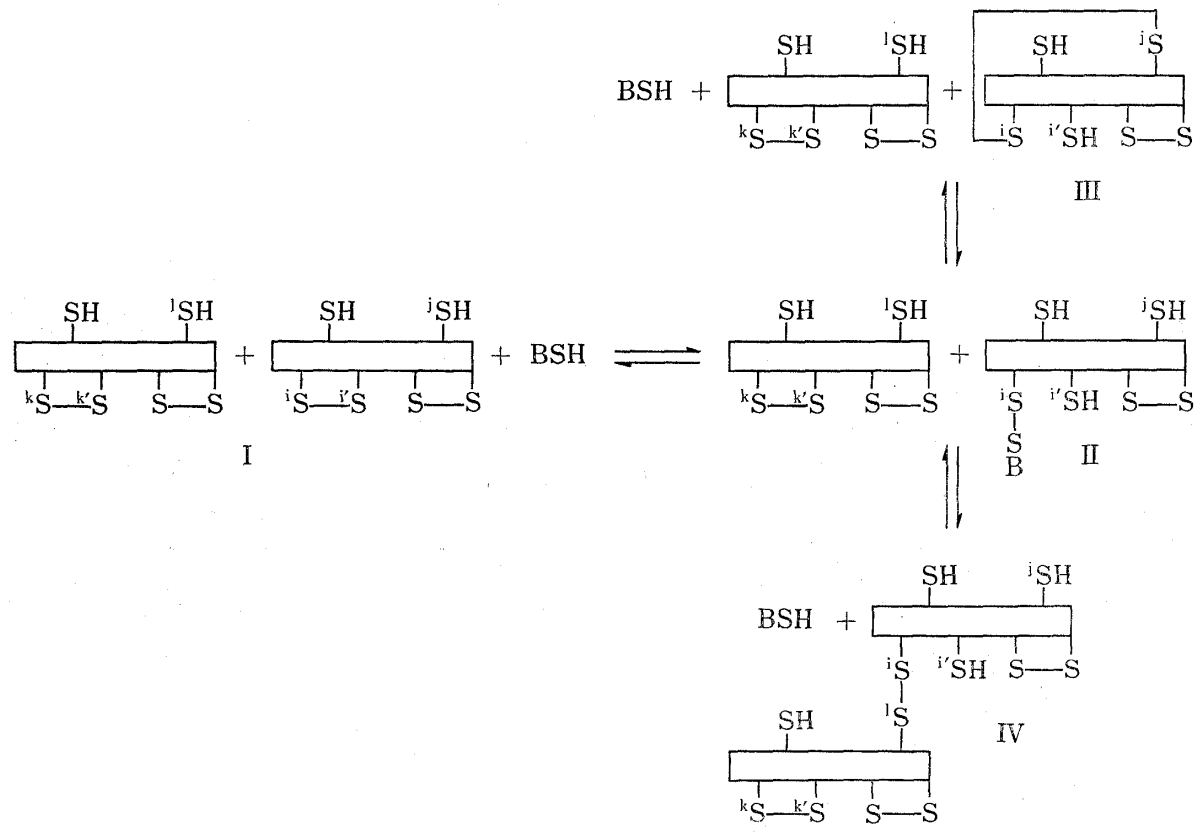

Fig. 1. Reaction Equilibrium between PS-SP and BSH

2) S.N. Deming and S.L. Morgan, *Anal. Chem.*, **45**, 278A (1973).

success to find the best-fit set in the succeeding paper, while the original simplex method without. The mode of modification is described briefly in Appendix, leaving the general discussion on the simplex method to reference 2).

In the above-mentioned "random-pairing model," the equivalency of all groups has been assumed with respect to PS-SP, PS-SB, and PSH. To further our understanding, Fig. 1 is presented. Consider that a protein molecule (I) has reacted with a BSH ion to produce a protein-ligand complex (II). Setting free a BSH ion, II may return to I, may change to III with an intramolecular disulfide linkage, or may change to IV with an intermolecular disulfide linkage. The random-pairing model postulates that these transformations occur with equal probabilities, because $^{i'}$SH, ^{j}SH, and ^{i}SH are equivalent and furthermore ^{i}S–$^{i'}$S, ^{i}S–^{j}S, and ^{i}S–^{i}S are equivalent. The equal probabilities are hardly supposed to reflect actual situations. We do not know, however, how to assign reasonable probabilities to these and many other conceivable transformations. Even when the evaluation of the probabilities were possible, the mathematical treatment for such a complicated system would become formidably difficult, yet with little practical benefit.

Monogamous-Pairing Model

Another model of practical significance is the one that allows only the returning of II to I, prohibiting the transformations II→III and II→IV. Any PS–SP linkage appearing at any stage of the reaction must be the one that was present at the start of the reaction; ^{i}S can combine only with $^{i'}$S to make a PS–SP linkage, and ^{k}S only with $^{k'}$S. This model may be called "monogamous-pairing model."

Inspecting Fig. 1, one might feel that in this model any sulphur atom that formed a sulfhydryl group at the start of the reaction, *e.g.* ^{i}SH, can never bind with an SB to produce a PS–SB linkage. This is not the case, because an exchange reaction such as shown in Fig. 2

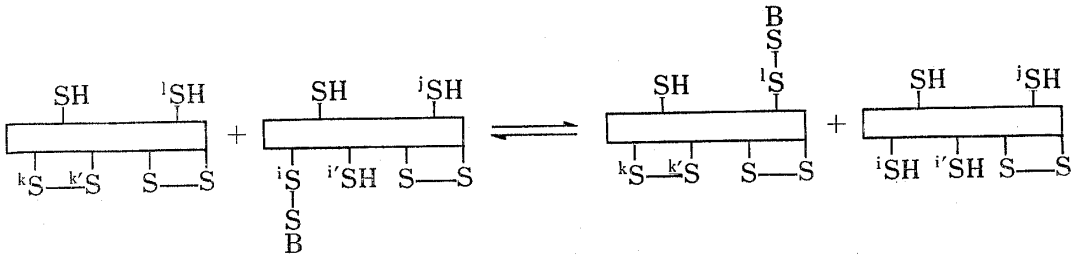

Fig. 2. An Example of Exchange Reaction

is not prohibited, and also because PS–SB can be formed by the backward reaction in formula (2). The $P^{i}S$–SB thus produced cannot react with a PSH to make a PS–SP linkage. For the sake of later convenience, sulphur atoms in the protein are classified into two categories, i-type S and j-type S. The former (hereafter expressed by ^{i}S) is a sulphur atom that formed a PS–SP linkage at the start of the reaction. It has a partner sulphur atom ($^{i'}$S) with which it can form a PS—SP linkage probably (but not necessarily) intramolecular. The latter (^{j}S) is a sulphur atom that formed a PSH group at the start. It has no partner. The monogamous-pairing model adopts the following assumptions. 1) All ^{i}S atoms are equivalent. 2) All ^{j}S atoms are equivalent. 3) All PS–SP linkages are equivalent; only $P^{i}S—^{i'}SP$ is allowed. 4) $P^{i}S—SB$ and $P^{j}S—SB$ are not equivalent with respect to the formation of PS—SP linkage; only $P^{i}S—SB+P^{i'}SH \rightleftharpoons P^{i}S—^{i'}SP+BSH$ is allowed. In all other respects, they are equivalent. 5) $P^{i}SH$ and $P^{j}SH$ are not equivalent with respect to the formation of PS—SP linkage; only $P^{i}SH+P^{i'}S—SB \rightleftharpoons P^{i}S—^{i'}SP+BSH$ is allowed. In all other respects, they are equivalent.

The assumptions postulate that

$$P^{i}S\text{-}SB + BSH \rightleftarrows P^{i}SH + BS\text{-}SB \quad (12)$$

$$P^{j}S\text{-}SB + BSH \rightleftarrows P^{j}SH + BS\text{-}SB \quad (13)$$

have the same equilibrium constant. Thus,

$$\frac{[P^iSH][BS\text{-}SB]}{[P^iS\text{-}SB][BSH]} = \frac{[P^jSH][BS\text{-}SB]}{[P^jS\text{-}SB][BSH]} = \frac{\{[P^iSH]+[P^jSH]\}[BS\text{-}SB]}{\{[P^iS\text{-}SB]+[P^jS\text{-}SB]\}[BSH]}$$
$$= \frac{[PSH][BS\text{-}SB]}{[PS\text{-}SB][BSH]} \quad (14)$$

$$\therefore \frac{[P^iS\text{-}SB]}{[P^iSH]} = \frac{[P^jS\text{-}SB]}{[P^jSH]} = \frac{[PS\text{-}SB]}{[PSH]} \quad (15)$$

Comparing (14) with (4), one knows that (2) and (4) are valid also for the monogamous-pairing model.

On the other hand, the reaction equilibrium corresponding to (1) may be written as

$$P^iS\text{-}^{i'}SP + BSH \overset{K_1}{\rightleftarrows} P^iS\text{-}SB + P^{i'}SH \quad (16)$$

This formulation needs a detailed explanation, because it is different from an ordinary reaction scheme. The sulphur atoms ^iS and $^{i'}S$ constitute a pair. The concentration of such pairs is given by $\beta[P]$, where β and $[P]$ have the same definitions as previously described. Every pair takes one of the five states i)—v) with probabilities p_1—p_5 as shown in Table I. From

Table I. States of ^iS—$^{i'}S$ Pairs

State	Form i	Form i′	Probability	Concn. of pairs
i)	PSH	PSH	p_1	$p_1\beta[P]$
ii)	PSH	PS–SB	p_2	$p_2\beta[P]$
iii)	PS–SB	PSH	p_3	$p_3\beta[P]$
iv)	PS–SB	PS–SB	p_4	$p_4\beta[P]$
v)	PS–SP	PS–SP	p_5	$p_5\beta[P]$=[PS-SP]

the definition of p's and the equivalency of ^iS and $^{i'}S$, one gets

$$p_1 + p_2 + p_3 + p_4 + p_5 = 1 \quad (17)$$
$$p_2 = p_3 \quad (18)$$
$$p_5 = [PS\text{-}SP]/\beta[P] \quad (19)$$

The reaction of BSH with a pair in state v) breaks the PS—SP linkage, resulting in a decrease in [PS—SP].

$$-\frac{d[PS\text{-}SP]}{dt} = k_1 p_5 \beta[P][BSH] \quad (20)$$

where k_1 is an appropriate rate constant. Setting free a BSH ion, a pair in state ii) or iii) can form a PS—SP linkage.

$$\frac{d[PS\text{-}SP]}{dt} = k_{-1}(p_2+p_3)\beta[P] = 2k_{-1}p_2\beta[P] \quad (21)$$

where k_{-1} is an appropriate rate constant. At equilibrium, (20) and (21) must be equal to each other. Thus, one obtains

$$K_1 = \frac{k_1}{k_{-1}} = \frac{2p_2}{p_5}\frac{1}{[BSH]} = 2p_2\frac{\beta[P]}{[PS\text{-}SP][BSH]} \quad (22)$$

The following comment may be worthy of mention, because (22) might seem strange at a glance. In the reaction of (16), an increase in $[P^iS—SB]$ causes the same amount of increase in $[P^{i'}SH]$. However, an individual $P^iS—SB$ has only one reaction parter $P^{i'}SH$ whose "concentration" is, of course, invariable. It is unreasonable and meaningless to write down

$$K_1 = \frac{[P^iS\text{-}SB][P^{i'}SH]}{[P^iS\text{-}^{i'}SP][BSH]}$$

in simple analogy to an ordinary reaction scheme.

Now, we shall consider about the probabilities p_1—p_5. When an ^{i}S atom is not in the form of PS–SP, it must be in the form of PSH or in the form of PS—SB. The probability that the ^{i}S atom is in the form of PSH may vary somewhat depending on whether the partner $^{i'}$S atom is in the form of PSH or in the form of PS—SB. Assume, however, the same probability ρ in both cases as an approximation.

$$p_1 = (1-p_5)\,\rho^2 \tag{23}$$

$$p_2 = (1-p_5)\,\rho(1-\rho) \tag{24}$$

$$p_3 = (1-p_5)\,\rho(1-\rho) \tag{25}$$

$$p_4 = (1-p_5)(1-\rho)^2 \tag{26}$$

Then, hold apparently; $(1-p_5)$ is contributed from the condition that the ^{i}S atom is not in the form of PS–SP.

Inserting (23)—(26) in the relation

$$\frac{p_3+p_4}{p_1+p_2}\left(=\frac{p_2+p_4}{p_1+p_3}\right) = \frac{[\text{PS-SB}]}{[\text{PSH}]} \tag{27}$$

which is obtained from (15), one gets $(1-\rho)/\rho=[\text{PS—SB}]/[\text{PSH}]$, and from it, $\rho=[\text{PSH}]/\{[\text{PSH}]+[\text{PS—SB}]\}$ and $1-\rho=[\text{PS—SB}]/\{[\text{PSH}]+[\text{PS—SB}]\}$. Substitution of the last two relations and (19) into (24) gives.

$$p_2 = \frac{\{\beta[\text{P}]-[\text{PS-SP}]\}}{\beta[\text{P}]}\,\frac{[\text{PSH}][\text{PS-SB}]}{\{[\text{PSH}]+[\text{PS-SB}]\}^2}$$

which is used to derive

$$K_1 = \frac{2\{\beta[\text{P}]-[\text{PS-SP}]\}}{\{[\text{PSH}]+[\text{PS-SB}]\}^2}\,\frac{[\text{PS-SB}][\text{PSH}]}{[\text{PS-SP}][\text{BSH}]} \tag{28}$$

from (22).

In short, the equilibrium concentrations for the monogamous-pairing model can be obtained by solving the simultaneous equations (28) and (4), instead of (3) and (4) for the random-pairing model, under the conditions of (5), (6), and (7). Note that the dimension of K_1 is different between the two models; (concentration)$^{-1}$ in (28) and dimensionless in (3).

Through a lengthy course of derivation, the authors have arrived at

$$C_5x^5 + C_4x^4 + C_3x^3 + C_2x^2 + C_1x + C_0 = 0 \tag{29}$$

where $x=[\text{PS—SB}]/[\text{P}]$ as already defined by (9). The coefficients C_0 through C_5 are complicated functions of K_1, K_2, α, β, [B], and [P] as shown in Appendix. Since (29) cannot be solved algebraically, another technique to obtain x_{theo} has been devised. This technique makes use of

$$[\text{BS-SB}] = \tfrac{1}{4}\{(2K_2+1)[\text{PS-SB}]+\alpha[\text{P}]\} \times [\sqrt{1-8K_2[\text{PS-SB}]\{[\text{PS-SB}]-[\text{B}]\}/\{(2K_2+1)[\text{PS-SB}]+\alpha[\text{P}]\}^2}-1] \tag{30}$$

the derivation of which being outlined in Appendix.

For a set of (K_1, K_2, α, β, [B], [P]), suppose a provisional value of [PS—SB], and calculate [BS—SB] by (30), [BSH] by (5), [PS—SP] by (6), and [PSH] by (7). Substitute these values into (28), and compare the result with K_1. Adjust the value of [PS—SB] and repeat the above calculations until a satisfactory agreement is attained. The method of the adjustment is described in Appendix. Using $x_{\text{theo}}(=[\text{PS—SB}]/[\text{P}])$ thus obtained, one can find out the best-fit set of parameters (K_1, K_2, α, β) by means of the modified simplex method.

Appendix

Derivation of Equations (8) and (30)——Elimination of [PSH] and [BSH] from (4) by the use of (5) and (7) gives

$$K_2 = \frac{[\text{BS-SB}]\{[\text{PS-SB}]+2[\text{BS-SB}]+\alpha[\text{P}]\}}{[\text{PS-SB}]\{[\text{B}]-[\text{PS-SB}]-2[\text{BS-SB}]\}}$$

from which one gets

$$2[\text{BS–SB}]^2 + \{(2K_1+1)[\text{PS–SB}]+\alpha[\text{P}]\}[\text{BS–SB}] + K_2[\text{PS–SB}]\{[\text{PS–SB}]-[\text{B}]\} = 0 \quad (31)$$

applicable both to the random- and monogamous-pairing models. Equation (30) is obtained as the proper solution of (31). For the random-pairing model,

$$K_1[\text{BS–SB}]^2 + K_1\{[\text{PS–SB}] - \beta[\text{P}]\}[\text{BS–SB}] + K_2[\text{PS–SB}]^2 = 0 \quad (32)$$

is easily derived by eliminating [PS–SP] from (2), (3), and (6). The calculation of $\{K_1 \times (31) - 2 \times (32)\}$ gives [BS–SB] as a function of K_1, K_2, α, β, [B], [P], and [PS–SB]. Inserting this expression into (32), and making use of (9) and (10), one attains to (8).

Explicit Formulae for the Coefficients C_0—C_5 in Equation (29)——Although (29) has not been used to determine x_{theo}, the comparison between (8) and (29) is useful to contrast some mathematical features between the random- and monogamous-pairing models. For this reason, the explicit formulae for C_0—C_5 are given here without describing their derivation.

$$C_0 = 4K_2mn - (2K_2+1)K_2nr - \alpha(K_2mr+nq) - K_2^2r^2t$$

$$C_1 = 2K_2(2ln+m^2) - (2K_2+1)(K_2mr+nq) - \alpha(K_2lr+mq+np) + K_2^2r^2 - 2K_2qrt$$

$$C_2 = 4K_2(kn+lm) - (2K_2+1)(K_2lr+mq+np) - \alpha(K_2kr+lq+mp) + 2K_2qr - (2K_2pr+q^2)t$$

$$C_3 = 2K_2(2km+l^2) - (2K_2+1)(K_2kr+lq+mp) - \alpha(kq+lp) + (2K_2pr+q^2) - 2pqt$$

$$C_4 = 4K_2kl - (2K_2+1)(kq+lp) - \alpha kp + 2pq - p^2t$$

$$C_5 = 2K_2k^2 - (2K_2+1)kp + p^2$$

where

$$k = 4K_1[\text{P}](4K_2^2-10K_2+7)$$

$$l = 4\{-4-2K_1[\text{P}](3\beta-\alpha-2\beta K_2) + K_1[\text{P}](12K_2-7-4K_2^2)t\}$$

$$m = 8K_1[\text{P}]\{(3\beta-\alpha-2\beta K_2-K_2t)t-\alpha\beta\}$$

$$n = 8\alpha\beta K_1[\text{P}]t$$

$$p = 4K_1[\text{P}](8K_2^3-20K_2^2+14K_2-1)$$

$$q = 4\{(2\beta+\alpha)K_1[\text{P}]-4K_2-4(3\beta+\alpha)K_1[\text{P}]K_2+4(2\beta+\alpha)K_1[\text{P}]K_2^2 + 8K_1[\text{P}]K_2(K_2-1)t\}$$

$$r = 16\beta K_1[\text{P}]t$$

$$t = [\text{B}]/[\text{P}]$$

Figure 3 compares x—t curves expected for the random- and monogamous-pairing models. In the former model, only one x—t curve is obtained regardless of the value of total protein concentration [P]. Its slope at the origin $(dx/dt)_{t=0}$ is smaller than unity. In the latter model, different x—t curves are obtained for different [P]. Their slopes at the origin are all equal to unity. It is easy to show from equations (5), (6), (7), and (30) that in the case of random-pairing model each of [PS–SP]/[P], [PSH]/[P], [BS–SB]/[P], and [BSH]/[P] is a function of t only.

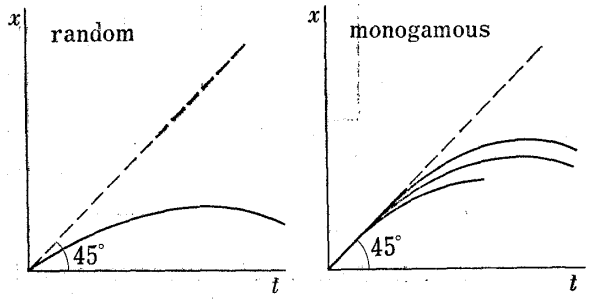

Fig. 3. Comparison of x—t Curves between Random and Monogamous-pairing Models

Method for Obtaining x_{theo} in the Case of Monogamous-Pairing Model——a) Take two memory fields, HIGH and LOW, in a computer. b) Compare [B] and β[P], and put the smaller one in HIGH. c) Put zero in LOW. d) Equate [PS-SB] to (HIGH+LOW)/2. e) Calculate [BS-SB], [BSH], [PS–SP], and [PSH] by (30), (5), (6), and (7) in this order. f) If any one of them is negative, put [PS–SB] in HIGH, and recycle to d). g) If all of them are positive, calculate the right-hand side of (28). h) According as the result is larger or smaller than K_1, put [PS–SB] in HIGH or LOW, and then recycle to d). i) If the result is equal to K_1, divide [PS–SB] by [P] to obtain x_{theo}. j) After twenty recyclings, divide LOW by [P] to obtain sufficiently accurate x_{theo}.

Modified Simplex Method——"Simplex" is defined as a geometric figure which has $n+1$ apexes in an n-dimensional hyperspace. A simplex in the present case has five apexes in the four-dimensional hyperspace whose coordinates stand for K_1, K_2, α, and β; each apex represents a set of parameters (K_1, K_2, α, β). The S-values at the five apexes are calculated by (11). The apex that gives the smallest S-value is regarded as

the best apex, and the one that gives the second smallest S-value is regarded as the second best. Thus, the five apexes may be denoted by B (best), SB (second best), M (middle), SW (second worst), and W (worst); their positions being expressed by vectors P_B, P_{SB}, P_M, P_{SW}, and P_W.

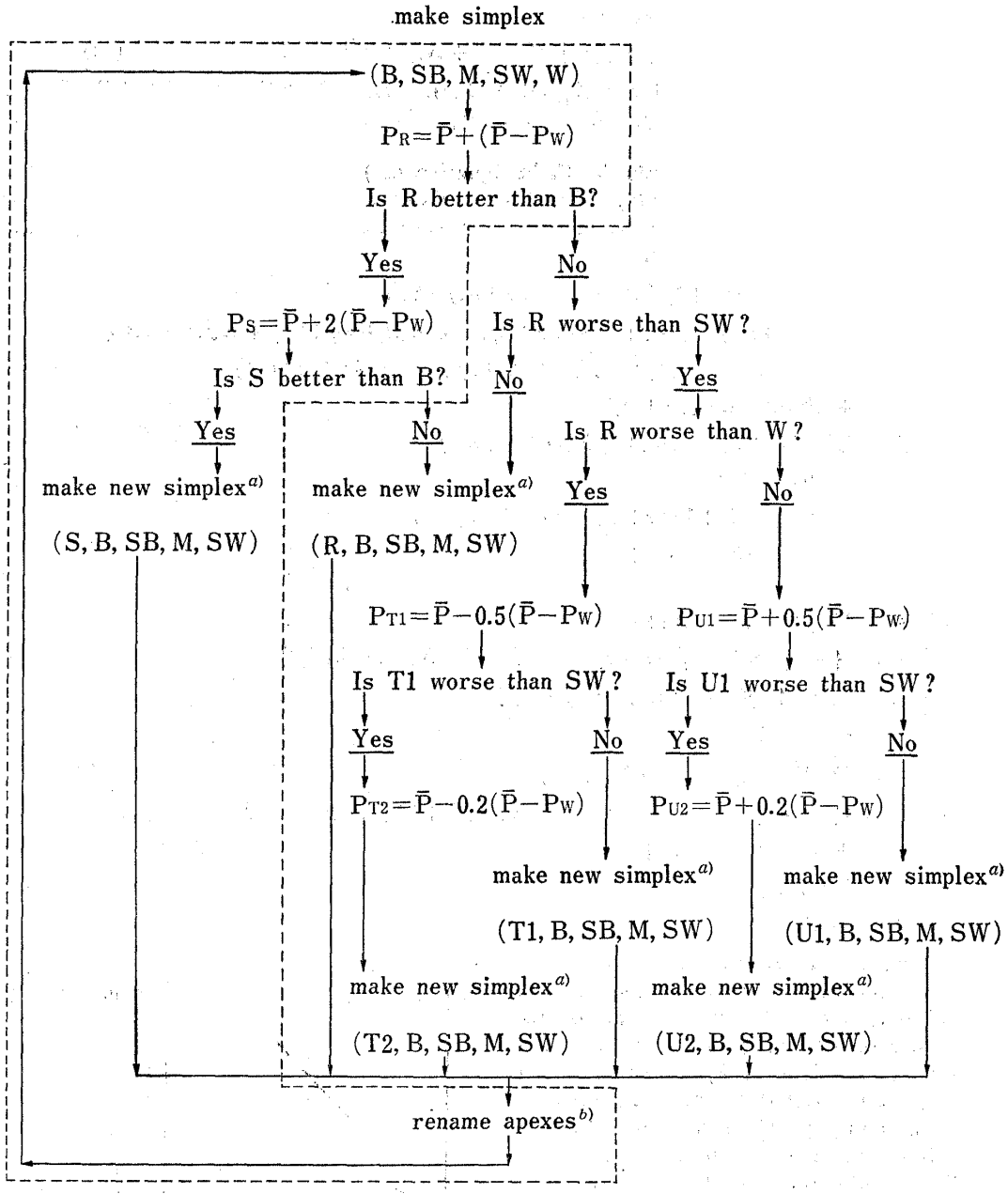

Fig. 4. Modified Simplex Method employed in the Present Study

a) Every time a new simplex is made, the position vector and S-value of the first apex in the parentheses are written out.

b) Rule of renaming: SW of the preceding simplex is always renamed as W of the new simplex, regardless whatever its S-value is. The other four apexes of the new simplex are renamed as B, SB, M, and SW in accordance with the order of their S-values. Sometimes, the S-value of W is smaller than that of SW in contradiction to the etymological meanings of symbols W and SW.

The calculation procedure adopted in this study is shown in Fig. 4, but only the part enclosed by dotted lines is explained below, since the other part is inferable by analogy. Consider a point R whose position is given by $P_R=\bar{P}+(\bar{P}-P_W)$, where $\bar{P}=\frac{1}{4}(P_B+P_{SB}+P_M+P_{SW})$. If the S-value at R is smaller than that at B, R is regarded to be better than B. Consider, then, a point S whose position is given by $P_S=\bar{P}+2(\bar{P}-P_W)$. If S is better than B, (S, B, SB, M, SW) is taken as a new simplex. The apexes of this new simplex should be renamed as shown by arrows; S→B, B→SB, SB→M, M→SW, SW→W. At the stage where series of position-vectors and S-values written out successively show no significant variations, the calculation is stopped after printing (K_1, K_2, α, β) and S-value at B of the last simplex.