

Computerized Scheme for the Reaction of Hemoglobin with Ligands

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A scheme for the reaction of hemoglobin with ligands is described, which postulates the functional heterogeneity of the chains, considers all possible combinations of the distribution of the ligand on the four chains of hemoglobin, and does not require simplifying assumptions about the hemoglobin reactivity. Ten tetrameric species are considered, together with 16 reactions between these species, each with an "on" and an "off" rate constant. The dissociation of hemoglobin tetramers into dimers is also considered, with four "on" and four "off" rate constants for the reactions between dimers, and ten equilibrium constants for the reactions between tetramers and dimers. Moreover, some side reactions, such as the "trapping" of ligands by a hemoglobin competitor, are included. A FORTRAN program, suitable for microcomputers, is described for handling this scheme, with some examples showing its advantages.

KEY WORDS: hemoglobin; computer simulation; finite-element approximation; kinetic simulation.

1. INTRODUCTION

Vertebrate hemoglobin (Hb) is probably the best example of an allosteric protein. The presence of two α and two β chains with structural and functional differences and the property that the functionality of each chain depends on the quaternary structure of the whole molecule lead to great complexity when defining a model suitable for all its reactions. Since the pioneering work of Hill (1910) and Adair (1925), several models have been proposed to explain the Hb function (Monod *et al.*, 1965; Perutz, 1970; Szabo and Karplus, 1972; Herzfeld and Stanley, 1975). All of them are simplification, because their aim is not to represent the Hb reactions with ligands, but to explain the hemoglobin function by a few basic laws. Most attempts to fit experimental data to the proposed theoretical models have yielded unsatisfactory results (see, for example, Seaton and Lloyd, 1974; El-Yassin and Fell, 1982), implying that at least some of the proposed models are inadequate.

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The generalized mass-action scheme (Olson, 1981) might thus seem to be the best way to describe the reaction of Hb with ligands, but it has the drawback that no easily implementable procedures to solve this scheme have been provided. Furthermore, we will show that this scheme, too, is inadequate, especially when dealing with dilute hemoglobin solutions, because it does not consider the incidence of the dimerization of tetramers and the subsequent randomized reassociation of dimers. In this work we introduce a computer program to describe a more complete scheme, not a model, for the reactions of hemoglobin, which is not intended to explain the hemoglobin submolecular mechanisms, but only to provide a tool for developing theoretical simplification models.

2. THEORY

2.1. The Hemoglobin Scheme

The mass-action scheme for the reactions of Hb with ligands (L) is shown in Fig. 1. The scheme includes 16 "on" and 16 "off" reactions at the tetramer level, four "on" and four "off" reactions at the dimer level, and ten dimerization and association reactions. We assume that dimers do not dissociate into monomers, and that there are no specific steric hindrances for the ligand (or, if there are, they are included in the kinetic constants referring to the combination of a particular ligand to a particular hemoglobin species).

All species in Fig. 1 are univocally identified by a two-digit code to facilitate input, handling, and output of data. Each digit of the code refers to the dimers that assemble the tetramer, following the arbitrary coding in Table I. Free dimers are coded by "5" plus its specific code. The two digits are interchangeable and each code is a value in the range 1-5 inclusive.

The reactions in the model are coded by a three-digit code, each digit being an integer in the range 1-5 inclusive. The digits are, respectively, the code of the dimer that did not react in that reaction, the code of the dimer that reacted, and the code of the dimer that was formed in the reaction. For example, the "on" reaction $B(2,1) \rightarrow B(2,2)$ [corresponding to $(\alpha\beta)(\alpha\beta^L) \rightarrow (\alpha\beta^L)(\alpha\beta^L)$] is coded as (2,1,2). In this way, all the reactions in the model are univocally identified, including the dimerization and association reactions.

2.2. Description of the Programs

The FORTRAN program (HBKIN) is divided into three overlaid sections, which handle, respectively, data input, computation, and output. To provide

Table I. Codification of the Dimers

Dimer	Code
$\alpha\beta$	1
$\alpha\beta^L$	2
$\alpha^L\beta$	3
$\alpha^L\beta^L$	4

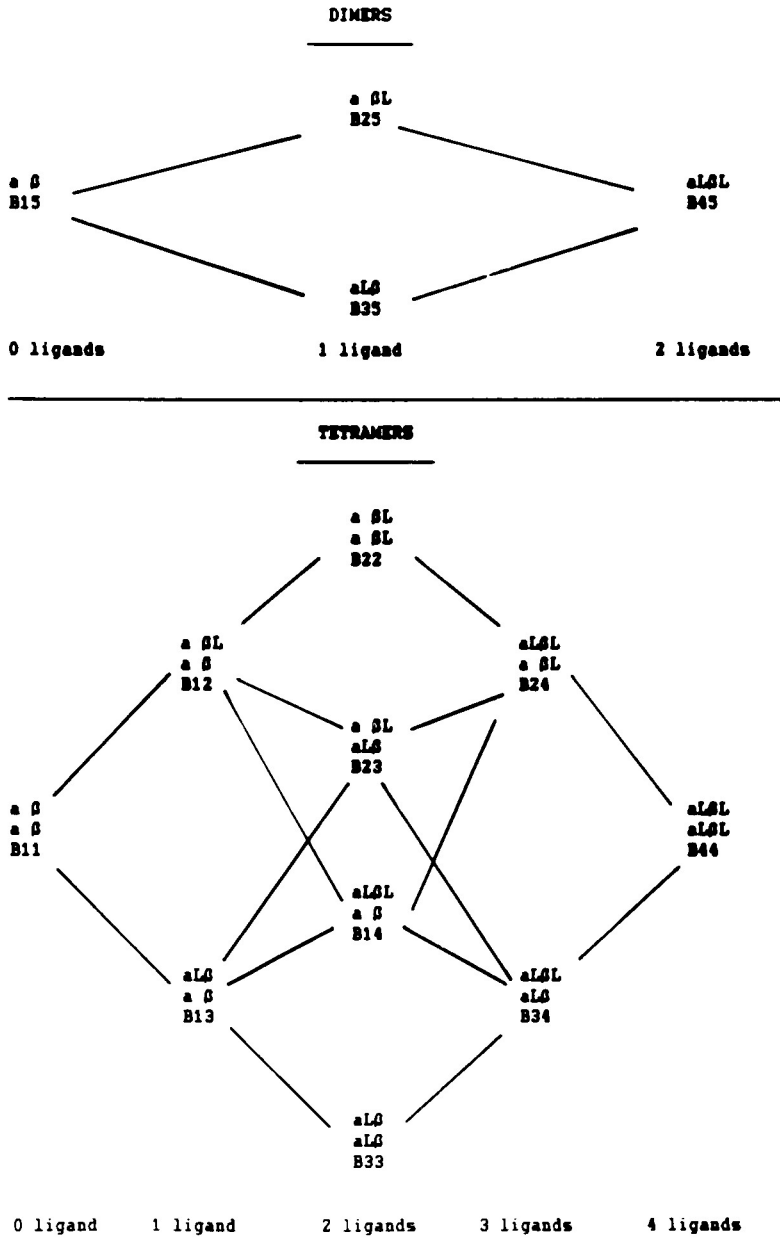


Fig. 1. Scheme for the reaction of hemoglobin with ligands (L). The tetramers and dimers (bottom and top, respectively) are reported with their codes. The dimerization reactions between the two levels are not shown.

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SUBROUTINE REAC
COMMON B(5,5),K(5,5,5),IRT(5,5),DT,LIG
REAL B,K,DT,LIG
INTEGER IRT
DATA IRT/0, 1, 1, 0,-2,
1      -1, 0, 0, 1,-2,
2      -1, 0, 0, 1,-2,
3       0,-1,-1, 0,-2,
4       2, 2, 2, 2, 0/

C
DO 100 N=1,5
DO 100 M=1,4
DO 100 J=1,4
IF(IRT(J,M).EQ.-2) THEN           ! Dimerization reaction
  D = B(N,M) * K(N,M,J) * DT
  B(N,M) = B(N,M) - D
  B(M,M) = B(M,M) + D
  B(N,J) = B(N,J) + D
  B(J,N) = B(N,J)
  B(M,J) = B(M,J) + D
  B(J,M) = B(M,J)
ELSE IF(IRT(J,M).EQ.-1) THEN      ! "Off" reaction
  D = B(N,M) * K(N,M,J) * DT
  B(N,M) = B(N,M) - D
  B(M,M) = B(M,M)
  B(N,J) = B(N,J) + D
  B(J,N) = B(N,J)
  LIG = LIG + D
ELSE IF(IRT(J,M).EQ.0) GOTO 100 ! No reaction here
ELSE IF(IRT(J,M).EQ.1) THEN       ! "On" reaction
  D = B(N,M) * K(N,M,J) * DT * LIG
  B(N,M) = B(N,M) - D
  B(M,M) = B(M,M)
  B(N,J) = B(N,J) + D
  B(J,N) = B(N,J)
  LIG = LIG - D
ELSE IF(IRT(J,M).EQ.2) THEN       ! Association reaction
  D = B(N,M) * B(M,J) * K(N,M,J) * DT
  B(N,M) = B(N,M) - D
  B(M,M) = B(M,M)
  B(N,J) = B(N,J) + D
  B(J,N) = B(N,J)
  B(M,J) = B(M,J) - D
  B(J,M) = B(M,J)
ENDIF
100  CONTINUE
RETURN
END

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Fig. 2. Subroutine REAC, which computes all the reactions of the Hb scheme in one loop by Euler's method.

maximum flexibility to the program, input data are divided, in turn, into three different files, which store, respectively, the concentrations of the species at zero time, the values for the kinetic constants (both files simply contain the code of the compound or of the constant, followed by their value; unused codes are omitted),

and the values for the control parameters (i.e., tolerance, life span of the simulated reaction, switches to include the ligand trapping reaction and/or the dimerization of tetramers, etc.). In a batch environment, the second subprogram reads sequentially from an accessory file the names of the three input data files, executes them, and outputs the concentrations of all species, the repartition of ligand among free, Hb-bound, and trap-bound, the current time increment for the iteration, and the total number of loops required. The third subprogram reads the last file and outputs graphs and/or printouts at convenience. The program is written in FORTRAN-77 language for a Digital Professional 350 computer.

A computer loop (Fig. 2) handles in one only passage all the reactions of the scheme. The IRT table identifies, for each combination of N , M , and J , the reaction type and if the reaction had a meaning. To compute the kinetics, Euler's method of numerical integration is applied over each interval of time Dt , in the form

$$[B]_{(N,M)} = [B]_{(N,M)} + \{d[B]_{(N,M)}/dt\}Dt \quad (1)$$

The time intervals Dt are selected by the algorithm shown in Fig. 3, which is an intermediate between the flux-tolerance (Barshop *et al.*, 1983) and the predictor-corrector algorithms (Gear, 1971). Briefly, the program continuously adjusts the value for Dt in such a way that the largest element change in the system for each iteration is less than twice a preset value (tolerance). If this change exceeds twice the tolerance, the values calculated during the current iteration are rejected and the iteration is recalculated with a smaller Dt value. If, on the contrary, the largest element change is less than one-half the tolerance, the next iteration is performed with a larger value for Dt .

A tool is added to compute the "trapping" of ligands by an Hb competitor, such as dithionite for oxygen or microperoxidase for carbon monoxide. The distribution of the ligand among free ligand and ligand bound either to Hb or to the trap is calculated at each step by the same integration technique described above, using the values of the "on" and "off" rate constants for the trapping reaction and the concentrations of the ligand and of the "trap."

The calculation of the dimerization and association reactions, although easily performed by the same procedure described for the "on"/"off" reactions, considerably slows down the whole process, since the kinetic values for the association reactions are usually several orders of magnitude larger than those of the fastest "on" reactions. Therefore, the time increments are set to unacceptably low values and the system is slow. An approximate but accurate solution is achieved by calculating the dimerization and association reactions not in a kinetic fashion, but by a succession of rapid equilibrium updates. The distributions of the species at equilibrium are calculated at a frequency selected by test and trial.

2.3. Testing the System

We selected two reactions that can be computed both by HBKIN and by ordinary differential equations. One is the two-step removal of carbon monoxide

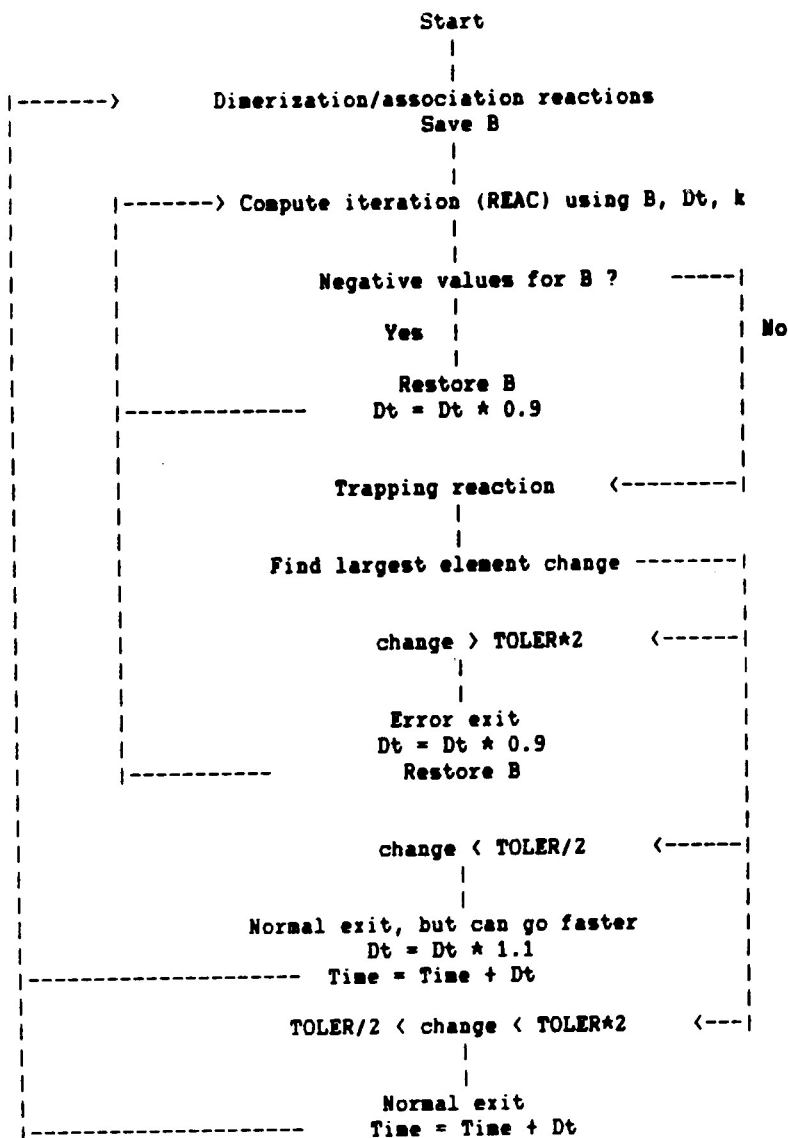
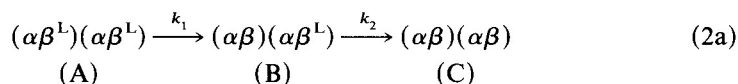
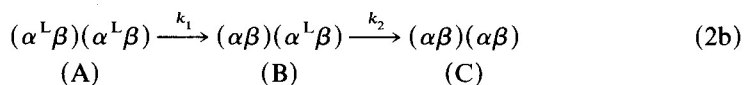


Fig. 3. Flowchart to adjust Dt values at each iteration. Dt is initially set to $(1/\text{maximal } k \text{ value})/100$. The concentrations of the species and the values for the kinetic constants are stored in arrays B and K, respectively.

(CO) from B(2,2) or B(3,3), i.e. $(\alpha\beta^L)_2$ or $(\alpha^L\beta)_2$, following the scheme



or



The differential equations used to compute these reactions are

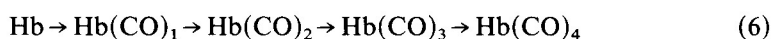
$$[A] = [A]_0 \exp(-k_1 T) \quad (3)$$

$$[B] = [A]_0 k_1 [\exp(-k_1 T) - \exp(-k_2 T)] / (k_2 - k_1) + [B]_0 \exp(-k_2 T) \quad (4)$$

$$[C] = [A]_0 + [B]_0 + [C]_0 - [A] - [B] \quad (5)$$

where T is time, and $[A]_0$, $[B]_0$, and $[C]_0$ are the concentrations of A, B, and C at zero time.

The other test was the four-step reaction of deoxygenated Hb with CO, following Adair's scheme and assuming that CO is in large excess (pseudomonomolecular process), and that α and β chains are functionally equivalent:



The differential equations used for this reaction are given by Olson (1981).

3. RESULTS

The largest HBKIN program section requires about 23,000 words core pool memory. The reliability of the described reaction scheme depends mainly on the selection of suitable values for tolerance (the maximum allowable variation of an element of the system along the fastest reaction during an iteration step), which in turn depends on the amplitude of the difference between the largest and the smallest kinetic constants. The computer time required to perform 1000 loops is about 200 sec, regardless of the number of reactions in a particular case.

Figures 4 and 5, top, report the time courses of the reactions (2) and (6), respectively, where the two solutions (ordinary differential equations and HBKIN) are represented by squares and by lines, respectively. In the latter case, two different tolerance values were selected (see legends). Currently available rate constant values were used (Gibson, 1973; Sharma *et al.*, 1976). The central panels (difference between the differential equation solution and the HBKIN curves) show the dependence of the accuracy on the value selected for the tolerance, assuming that the curves generated by the differential equations yield correct data. The bottom panels report the total number of loops required for the reaction when Dt is automatically corrected within the program by the mechanism shown in Fig. 3. The time increment, however, was never allowed to exceed a preselected value (usually 1/1000th of the total

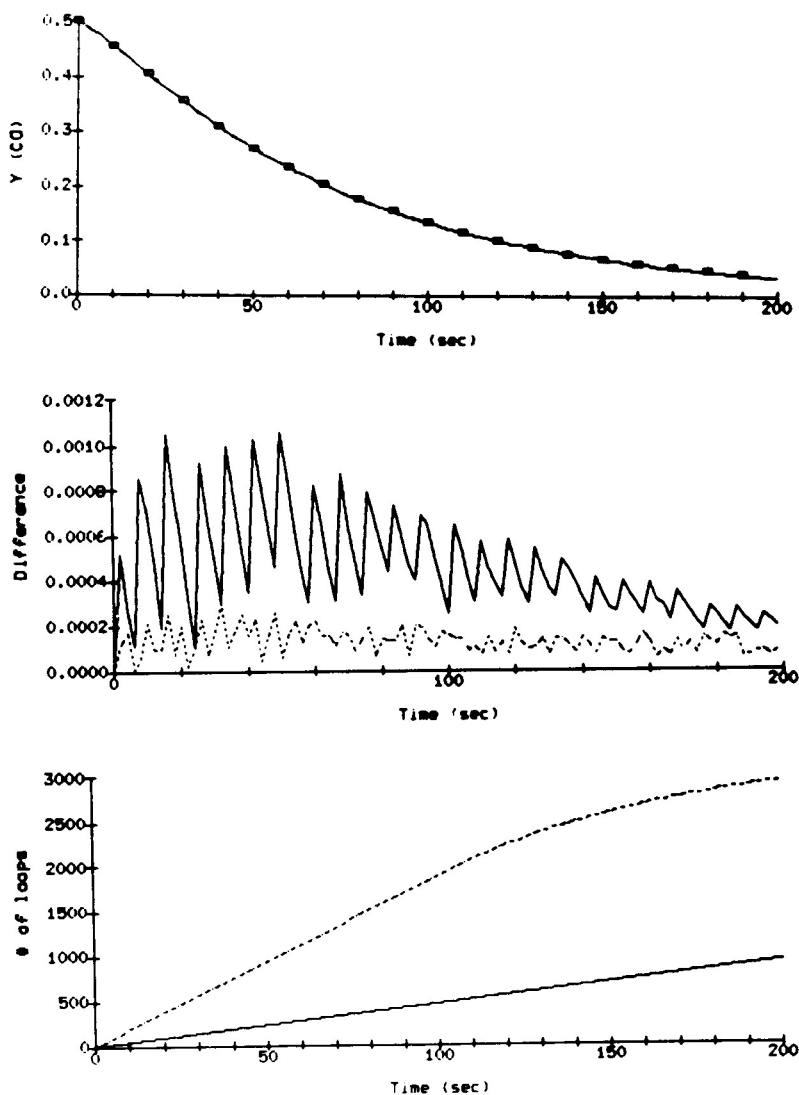


Fig. 4. Kinetics of CO release from $(\alpha\beta^{\text{CO}})_2$. Starting concentration $(\alpha\beta^{\text{CO}})_2 = 0.1$ mM (tetramer basis). Rate constants $k(121) = 0.09$ and $k(221) = 0.014 \text{ sec}^{-1}$. Top: Time course of the reaction calculated by (\square) differential equations and by HBKIN with a tolerance of $(\text{---}) 10^{-2}$ and $(\text{- -}) 10^{-4}$. Middle: Difference between the differential equation curve and the HBKIN curves. Bottom: Total number of loops required to simulate the reaction.

reaction time) even when the reaction had virtually reached its equilibrium, in order to retain accuracy along the time axis.

Figure 6 shows the kinetics of CO release from $(\alpha\beta^{\text{CO}})_2$ at total hemoglobin concentrations ranging from 1 to 0.0001 mM. The reported values for the kinetic constants (Gibson, 1973) and for the equilibrium constants of the dimerization

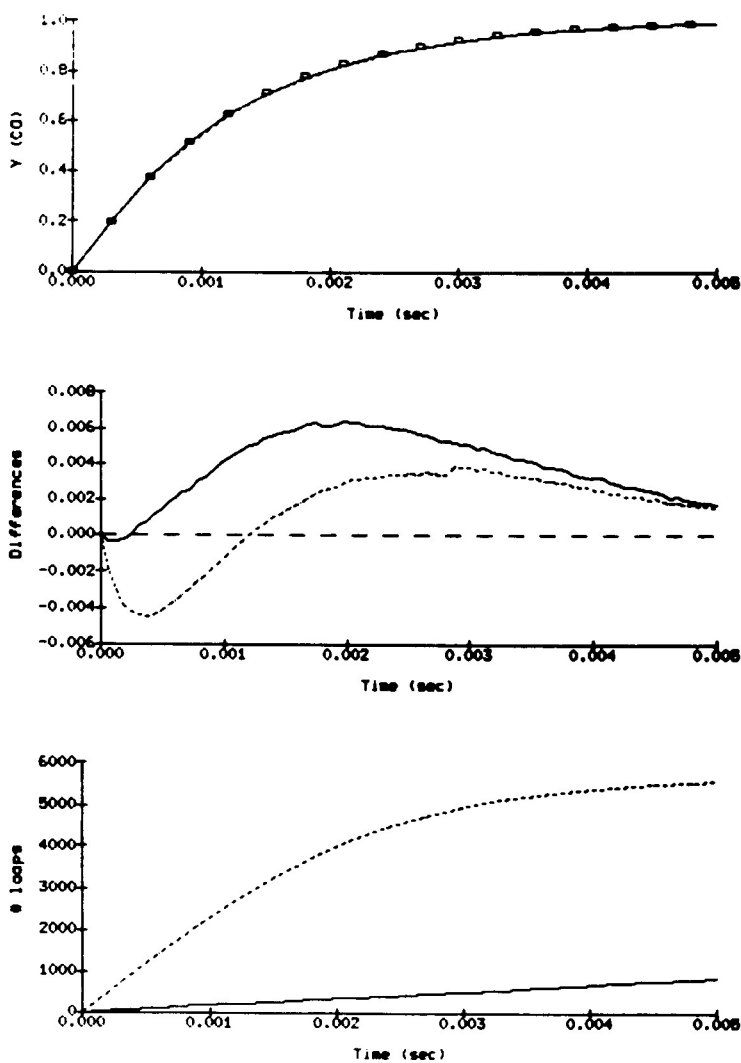


Fig. 5. Reaction of deoxygenated Hb with excess CO. Starting concentrations $[\text{deoxy Hb}] = 0.01 \text{ mM}$ and $[\text{CO}] = 1 \text{ mM}$. Rate constants $k(112) = 1.76 \times 10^3$, $k(212) = 1.2 \times 10^4$, $k(224) = 8.8 \times 10^2$, and $k(424) = 2.4 \times 10^4 \text{ mM}^{-1} \text{ sec}^{-1}$. Key is the same as in Fig. 3.

reaction (Turner *et al.*, 1981) were used (see legend). We assumed that the dimerization constants for the species $(\alpha\beta^{\text{CO}})_2$ and for $(\alpha\beta)(\alpha\beta^{\text{CO}})$ are the same as those for fully liganded HbCO and for deoxygenated hemoglobin, respectively. Since the dissociation rate of CO from the dimer $(\alpha\beta^{\text{CO}})$ is not available in the literature, we assumed either that the dimer $(\alpha\beta^{\text{CO}})$ formed in the dissociation reaction did not release CO (top panel), or that the dimer released CO with the same rate as the fully liganded tetramer (bottom panel).

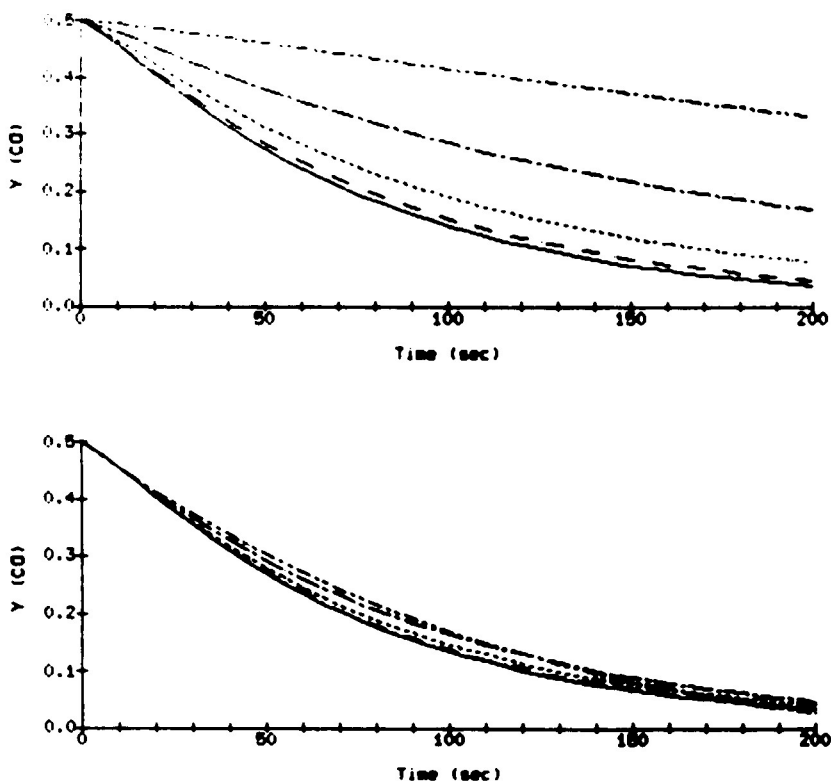


Fig. 6. Kinetics of the release of CO from $(\alpha\beta^{CO})_2$ at hemoglobin concentrations of (—) 1, (---) 0.1, (···) 0.01, (- · - ·) 0.001, and (- · · -) 0.0001 mM. Rate constants $k(121) = 9 \times 10^{-2}$ and $k(221) = 1.4 \times 10^{-2} \text{ sec}^{-1}$; $K(225) = 400$ and $K(125) = K(115) = 4 \times 10^7 \text{ mM}^{-1}$; for bottom panel only, $k(521) = 9 \times 10^{-3} \text{ sec}^{-1}$.

Figure 7, solid lines, shows the reaction of deoxygenated Hb with subsaturating ligand, where the pseudomonomolecular approximation (Olson, 1981) was not applicable. Dashed lines represent the same reaction, but for an "off" rate constant of 0.1 sec^{-1} .

4. DISCUSSION

The scheme of hemoglobin reactions in Fig. 1 can handle several aspects of the reaction of hemoglobin with ligands, for example, the functional differences between α and β chains, the dependence of the reactivity of a single chain on the quaternary conformation of the protein, the dimerization of tetramers into dimers and subsequent random reassociation of dimers to form tetramers, and the presence of a dimer/dimer interface [and thus that the two compounds $(\alpha\beta^L)(\alpha^L\beta)$ and $(\alpha\beta)(\alpha^L\beta^L)$ are different]. The presence of the ten tetrameric species in Fig. 1 was

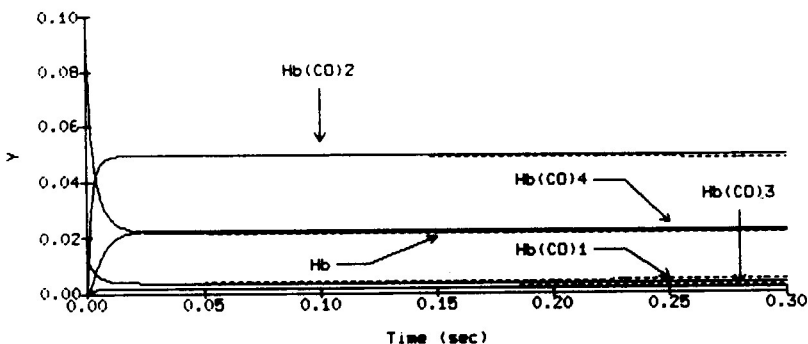


Fig. 7. Reaction of deoxygenated hemoglobin with subsaturating CO. Starting concentrations [deoxy Hb] = 0.1 mM and [CO] = 0.2 mM. Rate constants $k(112) = 1.76 \times 10^3$, $k(212) = 1.2 \times 10^4$, $k(224) = 8.8 \times 10^2$, and $k(424) = 2.4 \times 10^4 \text{ mM}^{-1} \text{ sec}^{-1}$; for dashed lines only, $k(121) = k(221) = k(242) = k(442) = 0.1 \text{ sec}^{-1}$.

shown experimentally in a hemoglobin solution at equilibrium (Perrella *et al.*, 1983), but the presence of dimers was inferred theoretically.

The HBKIN program was specially designed to compute this scheme, and thus it is a dedicated one, i.e., it can work only for hemoglobin or for closely related systems. More general programs to compute biological systems have been developed (KINSIM; Barshop *et al.*, 1983), but the size of the hemoglobin scheme greatly exceeds the limits of the KINSIM program (20 species, 20 reactions, and eight output expressions). Moreover, some reactions of hemoglobin are disproportionate (i.e., the dimerization reactions), and require a dedicated program section. Another approach to the hemoglobin scheme (Berger *et al.*, 1978) failed to account for the dimerization problem, and could run conveniently only on very large and fast computers due to the stiffness problem arising from taking a constant value for Dt over all of a progress curve.

The Euler method of numerical integration can deal efficiently with the complexity of the Hb reaction scheme, provided that appropriate time increments are selected as a compromise between two opposing needs: (1) minimizing the roundoff and discretization errors and the errors derived from assuming that the variations of the elements of the system are linear within the time intervals; and (2) shortening the total computer time. The algorithm devised here to avoid stiffness is intermediate between the flux-tolerance method (Barshop *et al.*, 1983) and Gear's predictor-corrector algorithm (Gear, 1971), and is fast, strictly obeys the mass-conservation principle, and never allows the solution to pass through zero, as may occur when applying Gear's method. Indeed, the test shown in the flowchart of Fig. 2 to catch negative values is usually activated only when exceedingly large values for tolerances are selected.

One advantage of this system is the possibility to consider, with minimal loss of computer time, more than one simultaneous reaction, for example, the dimerization reactions within a slow kinetics. Dimers do not show cooperativity and thus their functional properties are different from those of their parent tetramers (Mills *et al.*, 1976). Further, they can randomly reassociate to tetramers, changing their

distribution. Figure 6 shows the overall effect of decreasing hemoglobin concentration, and thus of increasing fraction of dimers, on the kinetics of CO release from partially liganded hemoglobin. Since no data are available for the dissociation of CO from dimers, the top and bottom panels show the time courses of the reaction assuming, respectively, that the dimer ($\alpha\beta^{\text{CO}}$) does not release CO, and that it does release CO with a rate constant equivalent to the dissociation of the fourth CO molecule from fully liganded HbCO (Sharma *et al.*, 1976), typical of a noncooperative process. This example thus shows that the failure to consider the dimerization reactions and randomized reassociation of dimers may lead to inaccurate results, especially when simulating the reactions of dilute hemoglobin and for relatively slow rate constants.

Another advantage of this system is the possibility to avoid the pseudomonomolecular approximations, for example, when ligands are not in excess, or in the presence of backward bimolecular reactions. Figure 7 shows the time courses for the single species in the reaction of deoxygenated Hb with half-saturating CO. The solid lines represent the simple bimolecular process using the reported "on" rate constants (Gibson, 1973), and the dashed lines represent the same reactions assuming an "off" rate constant of 0.1 sec^{-1} for all the intermediate species. This value is much greater than the reported one (Sharma *et al.*, 1976), but shows the possibility of managing without stiffness both "on" and "off" reactions simultaneously.

The widespread availability of microcomputers can greatly facilitate evaluating and computing even more complicated schemes than that shown in Fig. 1, provided that software support is adequate. The HBKIN program provided a powerful tool in the everyday work in our laboratory, both as a program standing alone to simulate time courses of the reactions from known rate constant values and starting concentrations, and as a module in a larger nonlinear curve-fitting program to estimate the values of the rate constants from a progress curve. The FORTRAN source of the program is available upon request.

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