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Model Studies on *RNA*-Replication I.**

The Quasiequilibrium Assumption and the Analysis of a Simplified Mechanism

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A simplified mechanism of *RNA*-replication by a specific polymerase is analysed by direct numerical integration and by means of the "quasiequilibrium approximation". The quasiequilibrium approximation is formulated in precise mathematical terms for three simple, two step reactions which describe approach towards equilibrium, irreversible transformation and unlimited growth.

(Keywords: Polymerization kinetics; Quasiequilibrium; RNA-replication)

Modelluntersuchungen zur RNA-Replikation, I. Annahme eines Quasigleichgewichts und Analyse eines vereinfachten Mechanismus

Ein vereinfachter Mechanismus der *RNA*-Replikation durch eine spezifische Polymerase wird durch direkte numerische Integration und mit Hilfe der Annahme von „Quasigleichgewicht" analysiert. Die Quasigleichgewichtsannahme wird an Hand von drei Beispielen einfacher Zweistufenreaktionen mathematisch definiert. Die drei Beispiele beschreiben: (1) die Annäherung an den Gleichgewichtszustand, (2) den irreversiblen Abbau und (3) das unbeschränkte Wachstum einer Verbindung.

1. Introduction

Polynucleotide replication—*in vitro* and *in vivo*—is an enormously complicated many step polymerization process. Commonly, two catalysts, an enzyme and a polynucleotide template are involved***.

** Dedicated to Prof. Dr. *G. Kainz* on the occasion of his 60th birthday.

*** Exceptions of this rule are enzyme-free template induced oligonucleotide synthesis[1] and template-free enzyme catalyzed "*de novo*" *RNA* synthesis[2–4].

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Despite the complexity of the problem the first extensive kinetic studies were reported more than ten years ago already by *Spiegelman*[5]. In his investigations *RNA* of the bacteriophage $Q\beta$ and the template specific enzyme $Q\beta$ *RNA* polymerase were used. More recently, systematic kinetic studies were performed on the replication of synthetic polynucleotides—poly (A), poly (U) polymerized by *RNA* polymerase from *E. coli* in a stirred flow reactor[6]—as well as on the $Q\beta$ system[3,4]. These kinetic investigations are essentially consistent with a simplified many step mechanism (Fig. 1) which will be the subject of the analysis reported here.

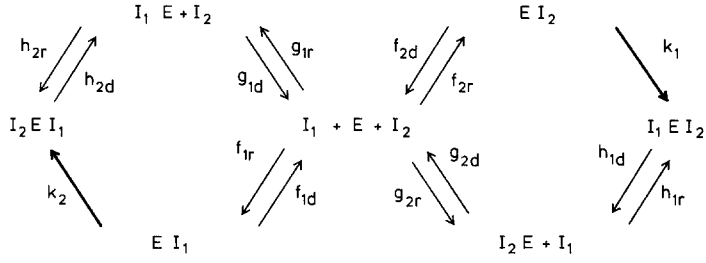

Fig. 1. A cyclic mechanism of *RNA* replication

The mechanism of *RNA* replication shown in Fig. 1 consists of two classes of reactions: (1) the binding of polynucleotides to proteins or polynucleotide-protein complexes and the dissociation of these polynucleotide-protein-complexes and (2) the polymerization process as such. All reactions of class (1) are considered to be reversible whereas the reactions of class (2) are assumed to occur irreversibly for practical purposes. This condition is very well fulfilled in realistic biological systems and in testtube experiments when the concentration of pyrophosphate is sufficiently small to prevent *RNA* degradation by pyrophosphorolysis.

At the same time the investigations reported here aim towards a second goal: the proposal and test of an approximative analysis of complex many-step reaction mechanisms. This procedure which we call characteristicly "quasiequilibrium approximation" is not new. It has been used frequently in chemical relaxation kinetics[7] and is based on the assumption that some reaction steps proceed at faster rates than the others. Recently, the quasiequilibrium approximation has been applied also to autocatalytically growing systems[8,9]. In this paper we supplement a mathematical analysis which justifies the generalization

of the approximation to irreversible reaction steps and to conditions where no stationary states exist. In a way the quasiequilibrium approximation is complementary to the steady state approximation which was analysed recently by *Noyes*[10]. He proposed a very general procedure which allows to check the validity of the steady state assumption in complex many-step reaction mechanisms. This approximation is based on the assumption that reaction intermediates are present at small and practically constant concentrations. Clearly, this method cannot be applied to autocatalytically growing systems in which all components including the intermediates grow.

2. The Quasiequilibrium Approximation

In this section we shall introduce and study the quasiequilibrium approximation by means of three exactly solvable examples.

2.1. Approach Towards Equilibrium

We start with two consecutive first order reactions:

$$\mathrm{A} \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} \mathrm{B} \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} \mathrm{X} \tag{1}$$

Following the analysis by *Eigen* and *DeMaeyer*[7] we introduce deviations from equilibrium concentrations as variables α, β and ζ ($[\mathrm{A}] = a$, $[\mathrm{B}] = b$, $[\mathrm{X}] = x$ and hence the conservation relation $a + b + x = c_0 = const.$ holds):

$$\alpha = a - \bar{a},\ \beta = b - \bar{b} \text{ and } \zeta = x - \bar{x} \tag{2}$$

From mass conservation we obtain $\alpha + \beta + \zeta = 0$. The equilibrium concentrations are denoted by bars:

$$\bar{a} = \frac{c_0}{1 + K_1 + K_1 K_2},\ \bar{b} = \frac{K_1 c_0}{1 + K_1 + K_1 K_2} \text{ and } \bar{x} = \frac{K_1 K_2 c_0}{1 + K_1 + K_1 K_2} \tag{3}$$

The equilibrium constants are defined in conventional manner as

$$K_1 = k_{12}/k_{21} \text{ and } K_2 = k_{23}/k_{32} \tag{4}$$

The dynamics of the system is described by the linear differential equation

$$\frac{\mathrm{d}\alpha}{\mathrm{d}t} = \dot{\alpha} = -(k_{12} + k_{21})\,\alpha - k_{21}\,\zeta \tag{5a}$$

$$\frac{\mathrm{d}\zeta}{\mathrm{d}t} = \dot{\zeta} = -k_{23}\,\alpha - (k_{23} + k_{32})\,\zeta \tag{5b}$$

which can be written most conveniently in vector notation

$$\dot{\mathbf{y}} = A\,\mathbf{y} \tag{5}$$

Herein $\mathbf{y}$ is a column vector (α, ζ) and A the 2×2 matrix.

$$A = \begin{pmatrix} -\tau_1^{-1} & -k_{21} \\ -k_{23} & -\tau_2^{-1} \end{pmatrix}.$$

For convenience we introduce relaxation times for the individual reaction steps

$$\tau_1^{-1} = k_{12} + k_{21} \text{ and } \tau_2^{-1} = k_{23} + k_{32} \tag{6}$$

The solution curves of (5) are of the general form

$$\alpha(t) = c_{11}\,e^{\lambda_1 t} + c_{12}\,e^{\lambda_2 t} \tag{7 a}$$

$$\zeta(t) = c_{21}\,e^{\lambda_1 t} + c_{22}\,e^{\lambda_2 t} \tag{7 b}$$

The reciprocal time constants λ_1, λ_2 are simply the eigenvalues of A. In order to calculate the coefficients c_{ij} we need the eigenvectors of A and the initial conditions $\alpha(0)$ and $\zeta(0)$. Eigenvalues and eigenvectors are determined by the matrix equation

$$A\,U = U\,\Lambda \text{ or } \Lambda' = U^{-1}\,A\,U, \tag{8}$$

where Λ is a diagonal matrix containing the eigenvalues

$$\Lambda = \begin{pmatrix} \lambda_1 & 0 \\ 0 & \lambda_2 \end{pmatrix}$$

and U contains the corresponding eigenvectors of A as columns

$$U = \begin{pmatrix} u_{11} & u_{12} \\ u_{21} & u_{22} \end{pmatrix}$$

Now, we are in a position to write down an expression for the coefficients c_{ij}:

$$c_{ij} = u_{ij}\,\{(U^{-1})_{j1}\,\alpha(0) + (U^{-1})_{j2}\,\zeta(0)\} \tag{9}$$

The elements of the inverse matrix of U are simply given by

$$U^{-1} = \begin{pmatrix} \frac{u_{22}}{\Delta} & -\frac{u_{12}}{\Delta} \\ -\frac{u_{21}}{\Delta} & \frac{u_{11}}{\Delta} \end{pmatrix} \tag{10}$$

with $\Delta = u_{11}u_{22} - u_{12}u_{21}$. Without losing generality we may put $u_{11} = u_{22} = 1$ and derive the following expressions for the coefficients of equation (7):

$$c_{11} = \frac{1}{1 - u_{12}\,u_{21}} \{\alpha(0) - u_{12}\,\zeta(0)\} \tag{9a}$$

$$c_{12} = \frac{1}{1 - u_{12}\,u_{21}} \{-u_{21}\,\alpha(0) + \zeta(0)\} \tag{9b}$$

$$c_{21} = \frac{u_{21}}{1 - u_{12}\,u_{21}} \{\alpha(0) - u_{12}\,\zeta(0)\} \tag{9c}$$

$$c_{22} = \frac{1}{1 - u_{12}\,u_{21}} \{-u_{21}\,\alpha(0) + \zeta(0)\} \tag{9d}$$

In practice the eigenvalues of A are obtained as the roots of a quadratic equation:

$$\lambda_{1,2} = \frac{1}{2}\left\{\tau_1^{-1} + \tau_2^{-1} \pm (\tau_1^{-1} - \tau_2^{-1})\sqrt{1 + \frac{4\,k_{21}\,k_{23}}{(\tau_1^{-1} - \tau_2^{-1})^2}}\right\} \tag{11}$$

The eigenvectors are calculated conveniently from the equations

$$u_{12} = -\frac{k_{23} + k_{32} + \lambda_2}{k_{23}} \tag{12a}$$

$$u_{21} = -\frac{k_{12} + k_{21} + \lambda_1}{k_{21}} \tag{12b}$$

Now, we apply the quasiequilibrium approximation and assume that one reaction step, let us say the step $A \rightleftharpoons B$ proceeds much faster than the other. Accordingly, we have

$$k_{12},\ k_{21} > k_{23},\ k_{32} \text{ and hence } \tau_1^{-1} > \tau_2^{-1}.$$

Then, the eigenvalues of A according to equation (11) are close to*

$$\lambda_1^Q = -\tau_1^{-1} \text{ and } \lambda_2^Q = -\left(\frac{k_{23} K_1}{1+K_1} + k_{32}\right) \tag{13}$$

and the corresponding eigenvectors are given by

$$U^Q = \begin{pmatrix} 1 & -\dfrac{1}{1+K_1} \\ 0 & 1 \end{pmatrix} \tag{14}$$

The solution curves of (5) are of the form

$$\alpha^Q(t) = \alpha(0) \cdot \exp(\lambda_1^Q t) - \frac{1}{1+K_1} \zeta(0) \cdot \exp(\lambda_2^Q t) \tag{15a}$$

and

$$\zeta^Q(t) = \zeta(0) \cdot \exp(\lambda_2^Q t) \tag{15b}$$

Equations (13) to (15) simply express the fact that in accord with our assumptions the process $A \rightleftharpoons B$ occurs instantaneously and is at quasiequilibrium when reaction $B \rightleftharpoons X$ progresses.

The establishment of quasiequilibrium can be verified by a straight forward calculation of the ratio

$$\frac{\beta^Q(t)}{\alpha^Q(t)} = -\left(1 + \frac{\zeta^Q(t)}{\alpha^Q(t)}\right) = \frac{\alpha(0)\exp(\lambda_1^Q t) + \dfrac{K_1}{1+K_1}\zeta(0)\exp(\lambda_2^Q t)}{\alpha(0)\exp(\lambda_1^Q t) - \dfrac{1}{1+K_1}\zeta(0)\exp(\lambda_2^Q t)} \tag{16}$$

For $t \gg |\lambda_1^Q|^{-1}$ we can neglect the first terms in numerator and denominator and find

$$t \gg |\lambda_1^Q|^{-1} \Rightarrow \frac{\beta^Q(t)}{\alpha^Q(t)} = K_1 \tag{16a}$$

Thus, the quotient β/α becomes independent of initial conditions and approaches K_1 for $t \gg |\lambda_1^Q|^{-1}$. It stays then at this value during the

* By the superscript "Q" we denote the results obtained within the frame of the quasiequilibrium approximation.

approach towards final equilibrium which occurs exponentially with the time constant $|\lambda_2^Q|^{-1}$ (we verify immediately that $\beta/\alpha = K_1$ implies $b/a = K_1$).

What happens if the quasiequilibrium condition is not fulfilled perfectly but still $|\lambda_1| \gg |\lambda_2|$ holds? By means of equations (7), (9) and (12) we find

$$t \gg |\lambda_1|^{-1} \Rightarrow \frac{\beta(t)}{\alpha(t)} = -\frac{k_{32} + \lambda_2}{k_{23} + k_{32} + \lambda_2} = K \tag{17}$$

Thus β/α and, hence, also b/a approach a constant value as before, but this value differs from K_1. We shall come back to this question when we discuss the next example (section 2.2).

2.2. Irreversible Consecutive Step

In the second example we consider the slower reaction step to be irreversible:

$$\mathrm{A} \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} \mathrm{B} \overset{k}{\rightarrow} \mathrm{X} \tag{18}$$

Formally, this reaction system is obtained from the previous example in the limit $k_{32} \to 0$ (and by putting $k_{23} = k$). Thus $a(t)$ and $b(t)$ do not approach some finite equilibrium value but vanish at infinite time

$$\lim_{t\to\infty} a = 0, \quad \lim_{t\to\infty} b = 0 \quad \text{and} \quad \lim_{t\to\infty} x = c_0 \tag{19}$$

By analogy with the previous example we define new variables:

$$\alpha = a, \ \beta = b \text{ and } \zeta = x - c_0 \tag{20}$$

The differential equation is of the same type as (5) with a matrix A defined by

$$A = \begin{pmatrix} -\tau_1^{-1} & -k_{21} \\ -k & -k \end{pmatrix} \tag{21}$$

The eigenvalues of A in this example are

$$\lambda_{1,2} = -\frac{1}{2}\left\{\tau_1^{-1} + k \pm (\tau_1^{-1} - k)\sqrt{1 + \frac{4kk_{21}}{(\tau_1^{-1} - k)^2}}\right\} \tag{22}$$

and the coefficients of the eigenvectors are given by

$$U = \begin{pmatrix} 1 & -\dfrac{k+\lambda_2}{k} \\ -\dfrac{\tau_1^{-1}+\lambda_1}{k_{21}} & 1 \end{pmatrix} \tag{23}$$

Applying the quasiequilibrium approximation for $A \rightleftharpoons B$ to the two-step mechanisms (18) we find

$$\lambda_1^Q = -\tau_1^{-1} \text{ and } \lambda_2^Q = -\frac{kK}{K+1} \tag{24}$$

(since we have just one equilibrium constant in this example we drop the index: $K = k_{12}/k_{21}$). The eigenvectors are the same as in the previous example

$$U^Q = \begin{pmatrix} 1 & -\dfrac{1}{1+K} \\ 0 & 1 \end{pmatrix} \tag{25}$$

and, accordingly, the solution curves in this case are indentical with those given in equation (15).

In order to study the approach towards a possibly existing quasi-equilibrium we define a time dependent ratio of concentrations

$$R(t) = \frac{b(t)}{a(t)} = \frac{\beta(t)}{\alpha(t)} = -\left(1 + \frac{\zeta(t)}{\alpha(t)}\right) \tag{26}$$

Under the assumption of the quasiequilibrium approximation this ratio is given precisely by equation (16). Thus, for times substantially longer than the reciprocal first eigenvalue, $t \gg |\lambda_1^Q|^{-1}$, the ratio approaches exactly the equilibrium constant K:

$$t \gg |\lambda_1^Q|^{-1} \Rightarrow \frac{b(t)}{a(t)} = K \tag{27}$$

Let us consider now the complete system (18). We may still assume that the two eigenvalues λ_1 and λ_2 are substantially different. The ratio of concentrations, b/a, can be expressed in most general form by the expression

$$R(t) = -\frac{(c_{11}+c_{21})e^{\lambda_1 t} + (c_{12}+c_{22})e^{\lambda_2 t}}{c_{11}e^{\lambda_1 t} + c_{12}e^{\lambda_2 t}} \tag{28}$$

At time long enough, $t \gg |\lambda_1|^{-1}$, $R(t)$ approaches a constant value ($\tilde{K}$) which is independent of initial conditions:

$$t \gg |\lambda_1|^{-1} \Rightarrow R(t) = \tilde{K} = -\frac{c_{12} + c_{22}}{c_{12}} = -\left(1 + \frac{1}{u_{12}}\right) = -\frac{\lambda_2}{k + \lambda_2} \quad (29)$$

$\tilde{K}$, however, need not coincide with the true equilibrium constant K. We may call $\tilde{K}$ therefore an effective or quasiequilibrium constant.

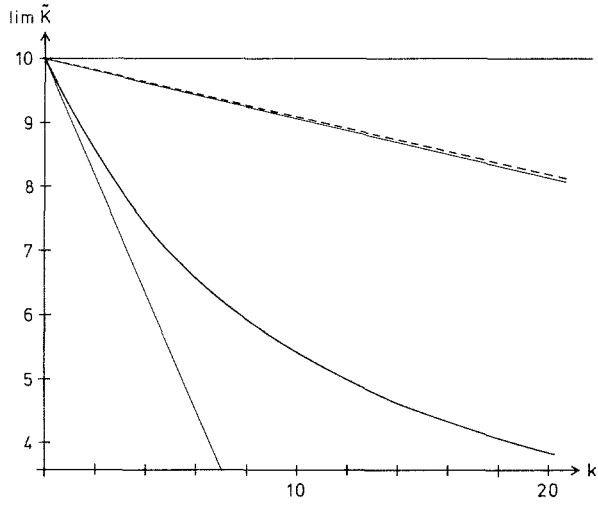

Fig. 2. The dependence of the effective equilibrium constant $\tilde{K}$ on the rate konstant k. We show two examples of simple two-step mechanisms as analysed in section 2.2 and 2.3: ———— (full line), the exponentially growing system (32) and its linearization according to equation (40) and — — — — (broken line), the irreversibly decaying system (18), the linearization of which is described in equation (31). For $k \to 0$ the effective equilibrium constant converges smoothly towards the true value of K

Indeed, we find a systematic deviation towards smaller values, $\tilde{K} < K$, with increasing values of the rate constant k (Fig. 2). This finding can be verified analytically: We use a power series expansion for the square root in equation (22) and collect all terms up to the order k^2/τ_1^{-2}. For the eigenvalues λ_2 we obtain

$$\lambda_2 \simeq \frac{K}{1+K}\left(-k + k_{21}\frac{k^2}{\tau_1^{-2}}\right) \quad (30)$$

which inserted into (29) yields

$$\tilde{K} \simeq K \frac{\tau_1^{-2} - kk_{21}}{\tau_1^{-2} + kk_{12}} \simeq K\left(1 - \frac{k}{\tau_1^{-1}} + \ldots\right) \tag{31}$$

For small values of k the effective equilibrium constant $\tilde{K}$ thus decreases linearly with the ratio k/τ_1^{-1} which can be verified easily by means of Fig. 2.

We have seen that the concept of a quasiequilibrium can be extended to mechanisms containing irreversible reaction steps. Then, the concentrations of reactants involved in the quasiequilibrium soon reach constant ratios and decrease synchronously. In case of the simple two-step mechanism it was possible to derive an analytical expression for the effective equilibrium constant $\tilde{K}$ which converges asymptotically to the true constant K at vanishing values of k.

2.3. Exponentially Growing System

In our third example we make an attempt to generalize the concept of a quasiequilibrium to an autocatalytically growing system. The most simple example of this type consists of a reversible first order reaction superimposed by catalytic action of B on the formation of A:

$$\mathrm{A} \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} \mathrm{B} \tag{32 a}$$

$$\mathrm{B} + \mathrm{X} \xrightarrow{k} \mathrm{A} + \mathrm{B} \tag{32 b}$$

We assume pseudo first order conditions for reaction (32 b); this means that the concentration of X is constant due to some buffering mechanism. For short we can write:

$$\begin{array}{ccc} & \overset{k_{12}}{\rightleftharpoons} & \\ \mathrm{A} & & \mathrm{B} \\ \uparrow & \underset{k_{21}}{} & \downarrow \\ & k & \end{array} \tag{32}$$

In this simple unconstrained reaction the concentrations $a(t)$ and $b(t)$ grow without limit. They are determined by the following linear differential equation:

$$\dot{a} = -k_{12}\, a + (k_{21} + k)\, b \tag{33 a}$$

$$\dot{b} = k_{12}\, a - k_{21}\, b \tag{33 b}$$

It is again of the same type as equation (5). The matrix A in this case is defined by

$$A = \begin{pmatrix} -k_{12} & k_{21}+k \\ k_{12} & -k_{21} \end{pmatrix} \tag{33 c}$$

The eigenvalues of A by the same token are obtained as the roots of a quadratic equation

$$\lambda_{1,2} = -\frac{1}{2}\tau_1^{-1}\left\{1 \pm \sqrt{1+\frac{4\,k_{12}\,k}{\tau_1^{-2}}}\right\} \tag{34}$$

For $k>0$ we have always one positive and one negative root. This means that the differential equation is characterized by one exponentially decreasing and one exponentially growing mode. The corresponding eigenvectors are given by the matrix

$$U = \begin{pmatrix} 1 & \dfrac{k_{21}+\lambda_2}{k_{12}} \\ \dfrac{k_{12}+\lambda_1}{k_{21}+k} & 1 \end{pmatrix} \tag{35}$$

We may apply the quasiequilibrium approximation for $A \rightleftharpoons B$ to our mechanism and obtain

$$\lambda_1^Q = -\tau_1^{-1} \text{ and } \lambda_2^Q = k\frac{K}{K+1} \tag{36}$$

The eigenvectors in this case are of the form

$$U^Q = \begin{pmatrix} 1 & \dfrac{1}{K} \\ -1 & 1 \end{pmatrix} \tag{37}$$

By a straight forward calculation we can verify that the ratio b/a converges to the equilibrium constant K. The approximation is good for periods longer than the reciprocal first eigenvalue:

$$t \gg |\lambda_1^Q|^{-1} \Rightarrow \frac{b\,(t)}{a\,(t)} = K \tag{38}$$

As in the previous example we release the quasiequilibrium approximation. The second eigenvalue up to terms of the order k^2/τ_1^{-2} is given by

$$\lambda_2 \simeq \frac{K}{K+1}\left(k - k_{12}\frac{k^2}{\tau_1^{-2}}\right) \tag{39}$$

As in the foregoing case we can define and calculate an effective equilibrium constant

$$t \gg |\lambda_1|^{-1} \Rightarrow \frac{b(t)}{a(t)} = \tilde{K} = K\left(1 - K\frac{k}{\tau_1^{-1}} + \ldots\right) \tag{40}$$

Thus, $\tilde{K}$ decreases linearly with the ratio kK/τ_1^{-1} for small values of k which again can be easily verified by inspection of Fig. 2. Note, that the tangent of this linear dependence is different from that in the preceding example by a factor K.

It is of a certain importance to stress the fact that the analysis of the growing system is very general. The ratio b/a becomes constant in any case: since λ_1 and λ_2 are of opposite sign the contribution of the second eigenvector will always outweigh that of the first at times longer that the period which is necessary for almost complete decay of the first mode. Again we were able to derive an analytical expression for the effective equilibrium constant $\tilde{K}$, which represents this constant ratio b/a. $\tilde{K}$ converges asymptotically to the value of the true constant K for vanishing k.

The procedure described here can be extended to more complex many step equilibria preceding a slow reaction step. The results obtained thereby will be reported elsewhere[11]. A specific example of a successful application of the quasiequilibrium approximation to a complex many step mechanism is described in the forthcoming sections.

3. A Mechanism of *RNA* Replication

The simplified mechanism of *RNA* replication which will be analysed now is shown schematicly in Fig. 1. Accordingly we distinguish free *RNA* molecules—plus and minus strand denoted by I_1 and I_2 respectively—free enzyme molecules E and various polynucleotide protein complexes. In particular, we have the complexes $E \cdot I_1$ and $E \cdot I_2$ which are ready for the synthesis of the complementary strands. *RNA* polymerization is considered to be a single, practically irreversible and slow "over all" reaction step. At the end of the polymerization reaction

a ternary complex, $I_2 \cdot E \cdot I_1$ or $I_1 \cdot E \cdot I_2$ respectively, is formed. These ternary complexes are assumed to dissociate in sequence into the complexes $I_2 \cdot E$ or $I_1 \cdot E$ and finally into free enzyme and polynucleotide molecules. The newly synthesized *RNA*-molecules leave the ternary complex first[5]. $I_1 \cdot E$ differs from $E \cdot I_1$ as well as $I_2 \cdot E$ differs from $E \cdot I_2$ because the 3′ end of the polynucleotide is attached to the enzyme in $E \cdot I_1$ and $E \cdot I_2$ whereas the 5′ end is bound in the other two complexes, $I_1 \cdot E$ and $I_2 \cdot E$. The latter two complexes, hence, are not ready for replication. Enzyme reactivation can occur either via a rearrangement of the complex—interconversion of 3′ and 5′ end of the *RNA*—or via a dissociative mechanism. We refer to the latter case. For further details see Ref.[4]. Thus both polynucleotides have to fall off before the enzyme can start *RNA* polymerization anew.

The individual reaction step as well as the symbols used for the rate and equilibrium constants are:

$$I_1 + E \underset{f_{1d}}{\overset{f_{1r}}{\rightleftharpoons}} E \cdot I_1; \qquad F_1 = \frac{f_{1r}}{f_{1d}} \tag{41}$$

$$I_2 + E \underset{f_{2d}}{\overset{f_{2r}}{\rightleftharpoons}} E \cdot I_2; \qquad F_2 = \frac{f_{2r}}{f_{2d}} \tag{42}$$

$$I_1 + E \underset{g_{1d}}{\overset{g_{1r}}{\rightleftharpoons}} I_1 \cdot E; \qquad G_1 = \frac{g_{1r}}{g_{1d}} \tag{43}$$

$$I_2 + E \underset{g_{2d}}{\overset{g_{2r}}{\rightleftharpoons}} I_2 \cdot E; \qquad G_2 = \frac{g_{2r}}{g_{2d}} \tag{44}$$

$$I_2 \cdot E + I_1 \underset{h_{1d}}{\overset{h_{1r}}{\rightleftharpoons}} I_1 \cdot E \cdot I_2; \qquad H_1 = \frac{h_{1r}}{h_{1d}} \tag{45}$$

$$I_1 \cdot E + I_2 \underset{h_{2d}}{\overset{h_{2r}}{\rightleftharpoons}} I_2 \cdot E \cdot I_1; \qquad H_2 = \frac{h_{2r}}{h_{2d}} \tag{46}$$

$$E \cdot I_2 + \sum_{\lambda=1}^{4} \nu_\lambda^{(1)} A_\lambda \xrightarrow{k_1} I_1 \cdot E \cdot I_2 \tag{47}$$

$$E \cdot I_1 + \sum_{\lambda=1}^{4} \nu_\lambda^{(2)} A_\lambda \xrightarrow{k_2} I_2 \cdot E \cdot I_1 \tag{48}$$

The last two reactions are assumed to proceed irreversibly. A_λ, $\lambda = 1, \ldots, 4$, are the four nucleoside triphosphates, *ATP*, *UTP*, *GTP* and *CTP*, $\nu_\lambda^{(1)}$ and $\nu_\lambda^{(2)}$ are the corresponding stoichiometric coefficients. We assume that the concentrations of nucleoside triphosphates are buffered and hence, they do not enter as variables into the rate equations.

For the variable concentrations of the individual molecular species we use:

$$[I_1] = x_1,\ [I_2] = x_2,\ [E] = e \tag{49}$$

$$[E \cdot I_1] = y_1,\ [E \cdot I_2] = y_2,\ [I_1 \cdot E] = z_1,\ [I_2 \cdot E] = z_2 \tag{50}$$

$$[I_2 \cdot E \cdot I_1] = w_1,\ [I_1 \cdot E \cdot I_2] = w_2 \tag{51}$$

Additionally, we define the following total concentrations:

$$x_1^0 = x_1 + y_1 + z_1 + w_1 + w_2 \tag{52}$$

$$x_2^0 = x_2 + y_2 + z_2 + w_1 + w_2 \tag{53}$$

$$e_0 = e + y_1 + y_2 + z_1 + z_2 + w_1 + w_2 \tag{54}$$

4. Analysis of the Equilibrium Between the Free Macromolecules and Complexes

In order to study the equilibrium of complex formation we put $k_1 = k_2 = 0$ and investigate the system of nine variables under the condition

$$\dot{x}_1 = \dot{x}_2 = \dot{y}_1 = \dot{y}_2 = \dot{z}_1 = \dot{z}_2 = \dot{w}_1 = \dot{w}_2 = \dot{e} = 0 \tag{55}$$

From (55) we obtain six linearly independent equations

$$\bar{y}_1 = F_1 \bar{x}_1 \bar{e} \tag{56}$$

$$\bar{y}_2 = F_2 \bar{x}_2 \bar{e} \tag{57}$$

$$\bar{z}_1 = G_1 \bar{x}_1 \bar{e} \tag{58}$$

$$\bar{z}_2 = G_2 \bar{x}_2 \bar{e} \tag{59}$$

$$\bar{w}_1 = H_2 \bar{x}_2 \bar{z}_1 \tag{60}$$

$$\bar{w}_2 = H_1 \bar{x}_1 \bar{z}_2 \tag{61}$$

We use them together with the three conservation laws (52) to (54) to evaluate the nine equilibrium concentrations $\bar{x}_1$, $\bar{x}_2$, $\bar{y}_1$, $\bar{y}_2$, $\bar{z}_1$, $\bar{z}_2$, $\bar{w}_1$, $\bar{w}_2$ and $\bar{e}$ as functions of the total concentrations x_1^0, x_2^0 and e_0. Thereby,

we apply a combined analytical and numerical approach used previously for simple two step equilibria[12].

Successive elimination of variables somewhat tedious but straight forward leads to two equivalent equations of fifth degree[13]:

$$a_5\bar{x}_1^5 + a_4\bar{x}_1^4 + a_3\bar{x}_1^3 + a_2\bar{x}_1^2 + a_1\bar{x}_1 + a_0 = 0 \tag{62}$$

and

$$b_5\bar{x}_2^5 + b_4\bar{x}_2^4 + b_3\bar{x}_2^3 + b_2\bar{x}_2^2 + b_1\bar{x}_2 + b_0 = 0 \tag{63}$$

Herein, the coefficients are functions of the three total concentrations and the following three combinations of equilibrium constants

$$A = F_1 + G_1 \tag{64}$$

$$B = F_2 + G_2 \tag{65}$$

$$T = F_1G_2 + F_2G_1 \tag{66}$$

Note, that the coefficients b_k can be obtained from a_k, $k = 0, \ldots, 5$, by substitution of x_1^0, x_2^0, A and B for x_2^0, x_1^0, B and A and vice versa.

$$a_0 = (x_1^0B)^2 \text{ and } b_0 = (x_2^0A)^2 \tag{67 a}$$

$$a_1 = x_1^0\{B[x_1^0(AB + 2T) + x_2^0(AB - T) - e_0(AB + T) + A - 2B] - T\}$$

and (67 b)

$$b_1 = x_2^0\{A[x_1^0(AB - T) + x_2^0(AB + 2T) - e_0(AB + T) - 2A + B] - T\}$$

$$\begin{aligned} a_2 = {} & x_1^0[x_1^0T(T + 2AB) - e_0T(T + 3AB) + AB(A - 2B) - T(A + 4B)] + \\ & + x_2^0[(e_0 - x_1^0)T + B](T - AB) + \\ & + e_0[e_0ABT + AB(B - A) + T(A + B)] + \\ & + B(B - A) + T \end{aligned}$$

and (67 c)

$$\begin{aligned} b_2 = {} & x_1^0[(e_0 - x_2^0)T + A](T - AB) + \\ & + x_2^0[x_2^0T(T + 2AB) - e_0T(T + 3AB) - AB(2A - B) - T(4A + B)] + \\ & + e_0[e_0ABT + AB(A - B) + T(A + B)] + \\ & + A(A - B) + T \end{aligned}$$

$$\begin{aligned} a_3 = {} & x_1^0[(x_1^0 - 2e_0)AT - 2(T + 2AB)]T + \\ & + x_2^0T(T - AB) + e_0T(e_0AT + T + 3AB) + \\ & + AB(B - A) + T(A + 2B) \end{aligned}$$

and (67 d)

$$b_3 = x_1^0 T(T - AB) + \\ + x_2^0[(x_2^0 - 2e_0)BT - 2(T + 2AB)]T + \\ + e_0 T(e_0 BT + T + 3AB) + AB(A - B) + T(2A + B)$$

$$a_4 = [(e_0 - x_1^0)2AT + T + 2AB]T$$

and (67 e)

$$b_4 = [(e_0 - x_2^0)2BT + T + 2AB]T$$

$$a_5 = AT^2 \text{ and } b_5 = BT^2 \tag{67 f}$$

The conventional method of evaluation—calculate either $\bar{x}_1$ from equation (62) or $\bar{x}_2$ from (63) and determine the remaining variables from equations (56) to (61) as well as (52) to (54)—fails here because of numerical difficulties. The equation

$$\bar{x}_1 = \frac{\bar{x}_2 B(e_0 - x_2^0 + \bar{x}_2) - (x_2^0 - \bar{x}_2)}{A(x_2^0 - \bar{x}_2) - \bar{x}_2 T(e_0 - x_2^0 + \bar{x}_2)} \tag{68}$$

and the corresponding equation for $\bar{x}_2$ are particularly ill-conditioned in many cases. The generally applicable formalism which we used in our calculations consists in a different strategy: all roots of both equations (62) and (63) are computed. Solutions for $x_1 - x_1^{(k)}$, $k = 1, \ldots, 5$ — are assigned to the corresponding roots for x_2 by means of the relation.

$$\frac{x_1^0 - x_1^{(k)}}{x_1^{(k)}}(B + x_1^{(k)}T) = Q^{(k)} = \frac{x_2^0 - x_2^{(k)}}{x_2^{(k)}}(A + x_2^{(k)}T);\ k = 1, \ldots, 5 \tag{69}$$

Each correctly assigned pair $(x_1^{(k)}, x_2^{(k)})$ is characterized by a certain value $Q^{(k)}$. If $Q^{(k)}$ is the same for two pairs the unique, final assignment is made with the aid of the equation

$$x_2^0(1 + x_1^{(k)}A + x_2^{(k)}B + x_1^{(k)}x_2^{(k)}T) - \\ - x_2^{(k)}[1 + x_1^{(k)}A + x_2^{(k)}B + x_1^{(k)}x_2^{(k)}T - e_0(B + x_1^{(k)}T)] = 0. \tag{70}$$

Equilibrium is unique. Hence, there is only one physically meaningful solution which fulfils the condition that all nine concentrations are positive:

$\bar{x}_1 \geq 0$, $\bar{x}_2 \geq 0$, $\bar{y}_1 \geq 0$, $\bar{y}_2 \geq 0$, $\bar{z}_1 \geq 0$, $\bar{z}_2 \geq 0$, $\bar{w}_1 \geq 0$, $\bar{w}_2 \geq 0$ and $\bar{e} \geq 0$.

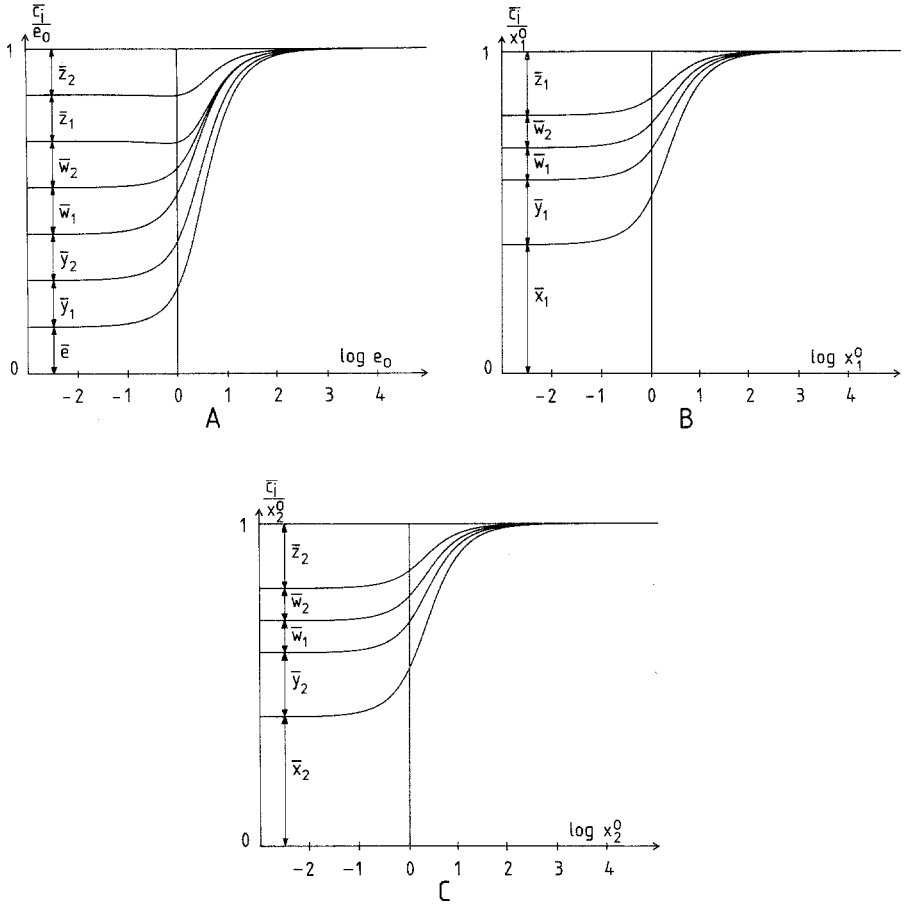

Fig. 3. The equilibrium mixtures of complexes and free macromolecules according to equations (49)–(61). The values of the individual equilibrium constants are: $F_1 = F_2 = G_1 = G_2 = H_1 = H_2 = 1$. We plot relative concentrations, e.g. $\bar{e}/e_0$, $\bar{y}_1/e_0$, $\bar{y}_2/e_0$ etc. or $\bar{x}_1/x_1^0$, $\bar{y}_1/x_1^0$ etc. or $\bar{x}_2/x_2^0$, $\bar{y}_2/x_2^0$ etc. respectively. The conservation relations (52) to (54) enable us to sum up all relative values yielding 1. Each equilibrium concentration is shown as the vertical distance between two plotted lines. The total concentrations applied are **A**: $x_1^0 = x_2^0 = 1$, $10^{-3} < e_0 < 10^5$, **B**: $x_1^0 = e_0 = 1$, $10^{-3} < e_0 < 10^5$ and **C**: $x_2^0 = e_0 = 1$, $10^{-3} < x_1^0 < 10^5$. Due to the particular choice of the equilibrium constants (equal to unity) all complexes are present at comparable amounts. Note that the plots against x_1^0 and x_2^0 become identical in case we interchange the indices "1" and "2"

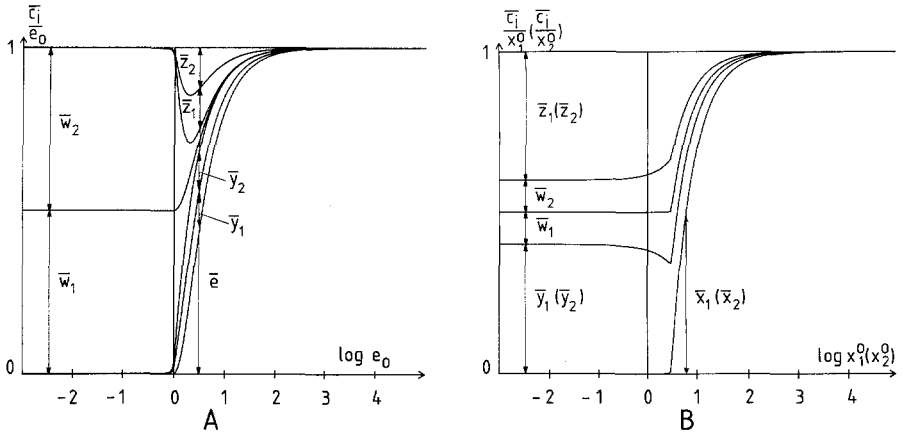

Fig. 4. The equilibrium mixtures of complexes and free macromolecules according to equations (49)-(61). The values of the individual equilibrium constants are: $F_1 = F_2 = G_1 = G_2 = H_1 = H_2 = 10^3$ (for a description of the plots see Fig. 3). The total concentrations applied are **A**: $x_1^0 = x_2^0 = 1$, $10^{-3} < e_0 < 10^5$ and **B**: $x_2^0(x_1^0) = 1$, $e_0 = 3$, $10^{-3} < x_1^0(x_2^0) < 10^5$ (we make use of the fact that the plots against x_1^0 and x_2^0 are identical in case we interchange the indices "1" and "2" since $F_1 = F_2$, $G_1 = G_2$ and $H_1 = H_2$, and save the second sketch). Due to the high values of the equilibrium constants all enzyme is bound in the ternary complexes at excess polynucleotide concentrations. The same applies vice versa for the polynucleotides at excess enzyme concentration. Here we find binary and ternary complexes at comparable amounts

These relations are used to pick out the equilibrium state in the actual numerical computations.

Some characteristic examples of equilibrium mixtures of polynucleotides, enzyme and their complexes are shown in the Figs. 3 to 6. In our first example all equilibrium constants are chosen equal unity*. Hence, all molecular species are present in equal amounts when the total concentrations are low and $x_1^0 = x_2^0$. The different graphs in Fig. 3 can be interpreted easily by the influence of excess E, I_1 or I_2 on complex formation. Example 2 (Fig. 4) differs from the first case by larger but still equal equilibrium constants: $F_1 = F_2 = G_1 = G_2 = H_1 = H_2 = 10^3\,[c^{-1}]$. Therefore, higher aggregates are strongly favoured already at low concentrations. In the remaining two examples we show the influence of different equilibrium constants on the distribution of molecular species (Figs. 5 and 6).

* Throughout this contribution we use arbitary concentration [c] and time units [t]. According to equations (41) to (48) all equilibrium constants are of dimension [c^{-1}], all recombination rate constants of dimension [$t^{-1}\,c^{-1}$], all dissociation rate constants as well as k_1 and k_2 of dimension [t^{-1}].

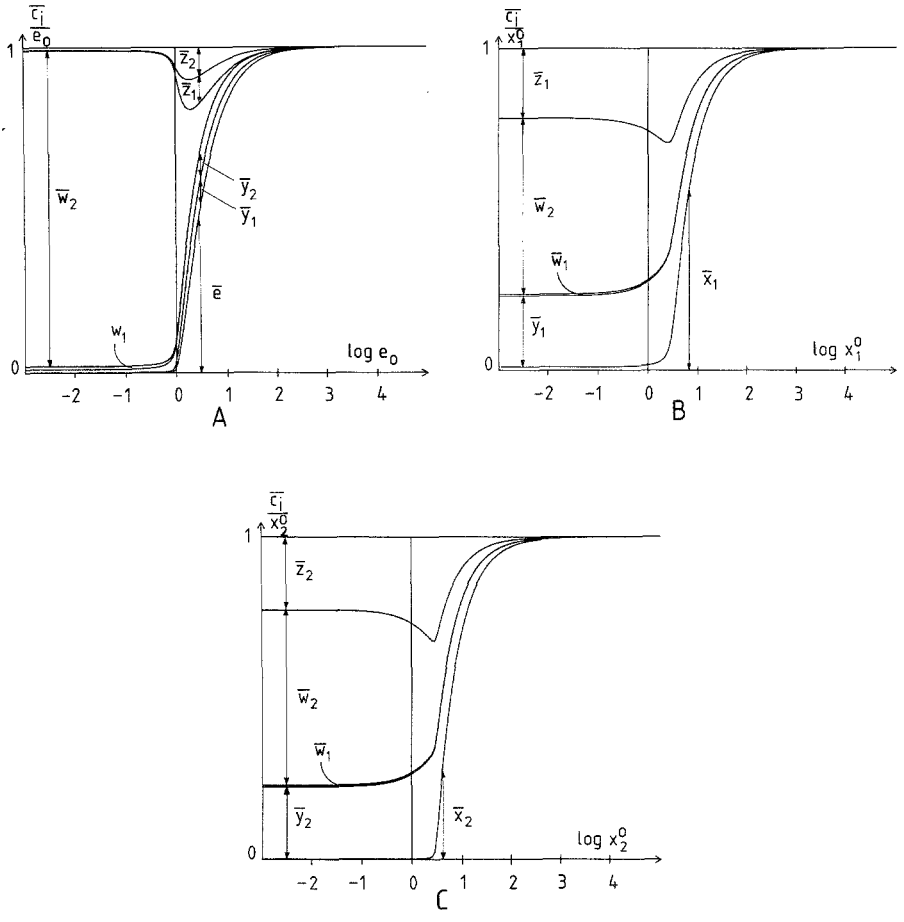

Fig. 5. The equilibrium mixtures of complexes and free macromolecules according to equations (49)-(61). The values of the individual equilibrium constants are: $F_1 = G_1 = H_2 = 10$ and $F_2 = G_2 = H_1 = 100$, i.e. all equilibrium constants referring to one particular cycle in Fig. 1, replication of plus or minus strand, are chosen equal (for a description of the plots see Fig. 3). The total concentrations applied are **A**: $x_1^0 = 1$, $x_2^0 = 1$, $10^{-3} < e_0 < 10^5$, **B**: $x_2^0 = 1$, $e_0 = 3$, $10^{-3} < x_1^0 < 10^5$ and **C**: $x_1^0 = 1$, $e_0 = 3$, $10^{-3} < x_2^0 < 10^5$. The most important feature of this case concerns the ratio of the two ternary complexes $\bar{w}_1/\bar{w}_2$. A straight forward calculation yields $\bar{w}_1/\bar{w}_2 = (G_1 \cdot H_2)/(G_2 \cdot H_1) = 10^{-2}$ in our example

Hence we find little $I_2 \cdot E \cdot I_1$ only at equilibrium

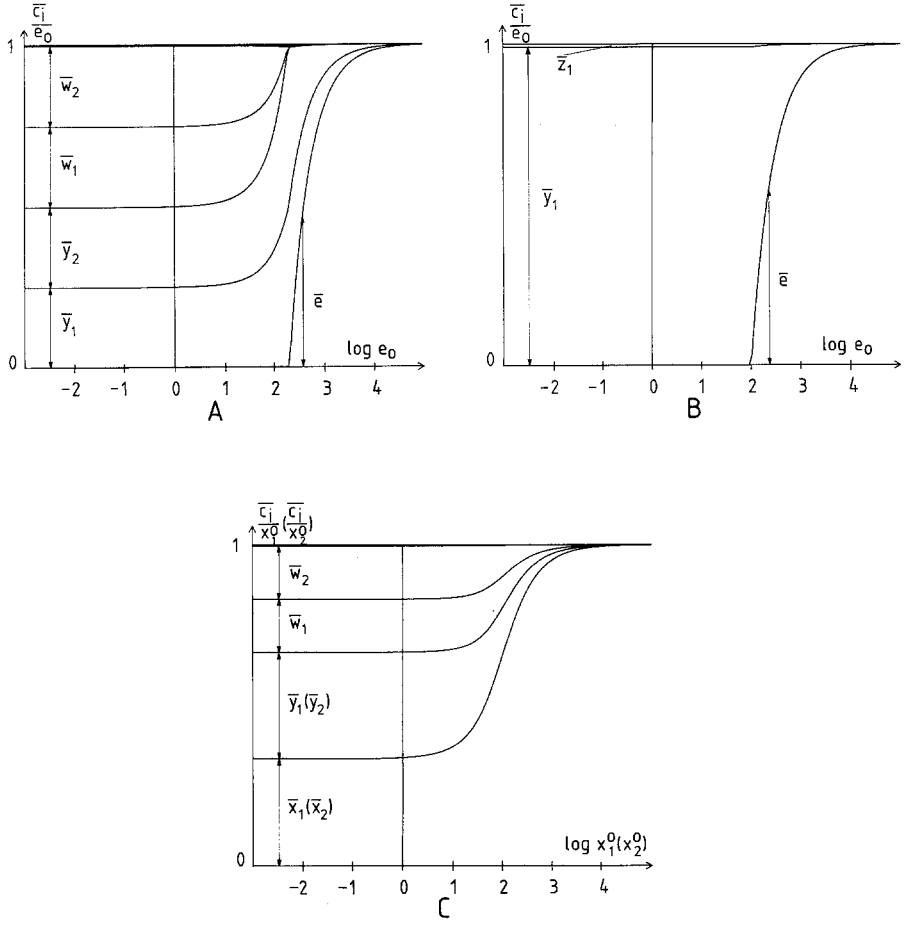

Fig. 6. The equilibrium mixtures of complexes and free macromolecules according to equations (49)-(61). The values of individual equilibrium constants are: $F_1 = F_2 = 10^4$, $G_1 = G_2 = 10^2$ and $H_1 = H_2 = 1$ (for a description of the plots see Fig. 3). The total concentrations applied are **A**: $x_1^0 = x_2^0 = 100$, $10^{-3} < e_0 < 10^5$, **B**: $x_1^0 = 100$, $x_2^0 = 10^{-2}$, $10^{-3} < e_0 < 10^5$ and **C**: $x_2^0(x_1^0) = 10^2$, $e_0 = 50$, $10^{-3} < x_1^0(x_2^0) < 10^5$ (for the symmetry between "1" and "2" see Fig. 4). This choice of equilibrium constants disfavours strongly complexes $I_1 \cdot E$ and $I_2 \cdot E$. Hence, $\bar{z}_1$ and $\bar{z}_2$ are detectable only under extreme conditions, e.g. **B**

5. Analysis of the Growing System

In this section we shall analyse the differential equation which corresponds to the mechanism of *RNA* replication described by the reactions (41) to (48). It is of the following explicit form:

$$\dot{x}_1 = f_{1d}\, y_1 + h_{1d}\, w_2 + g_{1d}\, z_1 - x_1 (f_{1r}\, e + g_{1r}\, e + h_{1r}\, z_2) \tag{71 a}$$

$$\dot{x}_2 = f_{2d}\, y_2 + h_{2d}\, w_1 + g_{2d}\, z_2 - x_2 (f_{2r}\, e + g_{2r}\, e + h_{2r}\, z_1) \tag{71 b}$$

$$\dot{y}_1 = f_{1r}\, x_1\, e - y_1 (f_{1d} + k_2) \tag{71 c}$$

$$\dot{y}_2 = f_{2r}\, x_2\, e - y_2 (f_{2d} + k_1) \tag{71 d}$$

$$\dot{z}_1 = h_{2d}\, w_1 + g_{1r}\, x_1\, e - z_1 (h_{2r}\, x_2 + g_{1d}) \tag{71 e}$$

$$\dot{z}_2 = h_{1d}\, w_2 + g_{2r}\, x_2\, e - z_2 (h_{1r}\, x_1 + g_{2d}) \tag{71 f}$$

$$\dot{w}_1 = k_2\, y_1 + h_{2r}\, x_2\, z_1 - h_{2d}\, w_1 \tag{71 g}$$

$$\dot{w}_2 = k_1\, y_2 + h_{1r}\, x_1\, z_2 - h_{1d}\, w_2 \tag{71 h}$$

$$\dot{e} = f_{1d}\, y_1 + f_{2d}\, y_2 + g_{1d}\, z_1 + g_{2d}\, z_2 - e (f_{1r}\, x_1 + f_{2r}\, x_2 + g_{1r}\, x_1 + g_{2r}\, x_2) \tag{71 i}$$

Additionally, we note that the time dependence of total concentrations is described by the following, fairly simple expressions:

$$\dot{x}_1^0 = k_1\, y_2 \tag{72 a}$$

$$\dot{x}_2^0 = k_2\, y_1 \tag{72 b}$$

and

$$\dot{e}_0 = 0 \tag{72 c}$$

The very last equation is self-evident since enzyme molecules are neither synthesized anew nor degrated and hence their total number is conserved during the reactions (41) to (48). Similarly, we can easily visualize equations (72 a) and (72 b): polynucleotide synthesis according to our mechanism occurs exclusively through the reactions (47) and (48). In these two processes the rates are proportional to the concentrations of the complexes $E \cdot I_2$ and $E \cdot I_1$, i.e. y_2 and y_1 respectively.

Numerical integration of the differential equation (71) can be performed by standard *Runge-Kutta* technique without special difficulty. Some examples are shown in Figs. 7 and 8. In order to simplify the forthcoming analysis we started the integration in an equilibrated mixture of free molecules and complexes. In this way we skip the relaxation towards quasiequilibrium—the process corresponding to λ_1 in section 2. Let us consider now the solution curves obtained thereby as

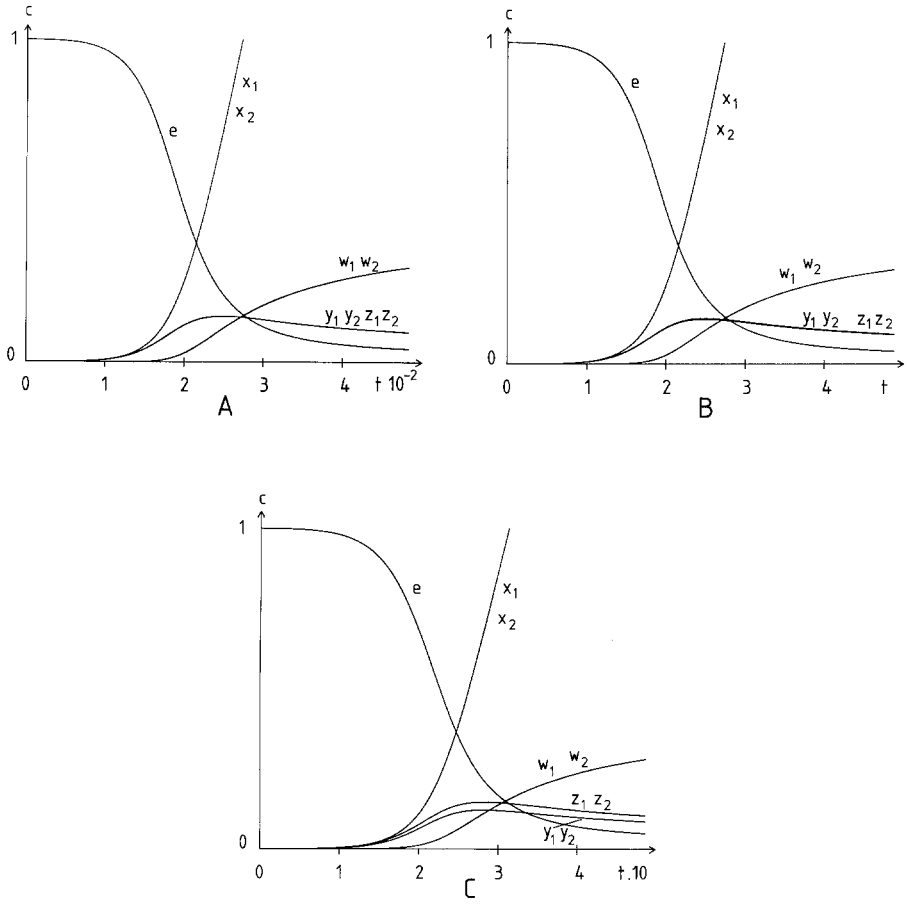

Fig. 7. Numerical integration of equation (71). The rate constants chosen are: $f_{1r} = f_{1d} = f_{2r} = f_{2d} = g_{1r} = g_{1d} = g_{2r} = g_{2d} = h_{1r} = h_{1d} = h_{2r} = h_{2d} = 10^3$. For the irreversible step we use **A**: $k_1 = k_2 = 0.1$, **B**: $k_1 = k_2 = 10$ and **C**: $k_1 = k_2 = 100$. Initial concentrations: $x_1^0 = x_2^0 = 10^{-3}$, $e_0 = 1$. The integration starts from an equilibrated mixture of complexes and free macromolecules. This case corresponds to a choice of equilibrium constants described in Fig. 3. Note that the graphs in **A** and **B** are almost identical except a stretch in the time axis!

functionals of the rate constants for the irreversible reaction steps, k_1 and k_2. For the sake of simplicity we assume that these two constants are equal: $k_1 = k_2 = k$. Then, the solution curves for a given set of rate constants for the reversible reactions depend on a single parameter, k, only. The two different cases studied in some detail here correspond to the equilibria investigated previously (Figs. 3 and 5).

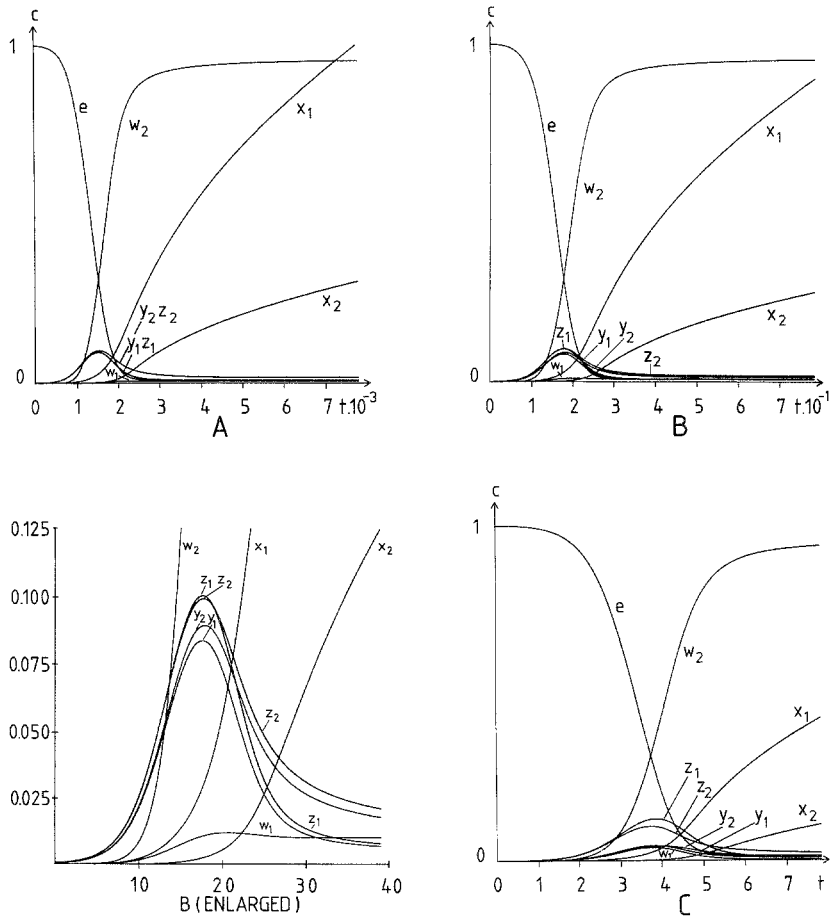

Fig. 8. Numerical integration of equation (71). The rate constants chosen are: $f_{1r} = g_{1r} = h_{2r} = 100$, $f_{2r} = g_{2r} = h_{1r} = 1\,000$, $f_{1d} = g_{1d} = h_{1d} = f_{2d} = g_{2d} = h_{2d} = 10$. For the irreversible step we use **A**: $k_1 = k_2 = 10^{-2}$, **B**: $k_1 = k_2 = 1$ and **C**: $k_1 = k_2 = 10$. Initial concentrations: $x_1^0 = x_2^0 = 10^{-3}$, $e_0 = 1$. The integration starts from an equilibrated mixture of complexes and free macromolecules. This case corresponds to a choice of equilibrium constants described in Fig. 5. Note that the graphs in **A** and **B** are almost identical except a stretch in the time axis!

At low values of k we observe that a change in k leads to a change in the time axis only. The concentrations of the molecular species present at a given time do not depend on k when we use the dimensionless unit $\tau = t \cdot k$ for the calibration of the time axis. We shall see later on, that this condition implies also that the quasiequilibrium approximation is

valid. At higher values of k scaling of the time axis does no longer lead to coincidence of the solution curves for different k values.

The two examples chosen are characterized by completely different distributions of free molecules and complexes during the the replication process. Nevertheless, the curves $x_1^0(t)$ and $x_2^0(t)$ are of the same general class in both cases (Fig. 9). Provided we start from sufficiently low initial concentrations $x_1^0(0)$ and $x_2^0(0)$ we distinguish three phases of growth: a phase of exponential growth, a phase of linear growth and a parabolic phase of saturation. The exponential phase of growth is characterized by excess enzyme: $e_0 > x_1^0$ and x_2^0. Every newly synthesized *RNA* molecule instantaneously enters the replication process. In the linear phase of growth the concentration of template, x_1^0 and x_2^0 exceeds that of the enzyme. Consequently, all enzyme molecules are engaged in the replication process which then proceeds at a constant rate. In the parabolic phase, finally, enzyme reactivation determines the rate of *RNA*-synthesis. These three phases have been observed *in vitro* in experimental studies on the $Q\beta$-bacteriophage *RNA*—specific *RNA* replicase system. For further details see[4], a short and easy to read version has been published recently[14].

Now, we shall apply the quasiequilibrium approximation to our example of a many-step reaction. Thereby we assume that *RNA*-polymerization is so slow that the equilibrium between different complexes and free molecules is hardly disturbed by the progress of replication. For this purpose we use equations (72 a) and (72 b). The concentrations of the reproductive complexes $E \cdot I_1$ and $E \cdot I_2$, y_1 and y_2, which enter the differential equation are calculated at equilibrium. For this goal we make use of the formalism described in the previous section. Numerical integration of equation (72 a, b) is straight forward. At each discrete step the equilibrium concentration are evaluated anew. In this way we obtained solution curves for our two test examples (Fig. 10). These curves when plotted against dimensionless time units ($\tau = t \cdot k$) coincide with those obtained by direct integration at the limit of low values of k. This finding can be verified easily by inspection of equations (72 a) and (72 b) when we assume $k_1 = k_2 = k$:

$$\frac{dx_1^0}{dt} = ky_2 \Rightarrow \frac{dx_1^0}{kdt} = \frac{dx_1^0}{d\tau} = y_2$$

and

$$\frac{dx_2^0}{dt} = ky_1 \Rightarrow \frac{dx_2^0}{kdt} = \frac{dx_2^0}{d\tau} = y_1 \text{ with } \tau = t \cdot k$$

Indeed, we see that the quasiequilibrium approximation is valid over a wide range of rate constants.

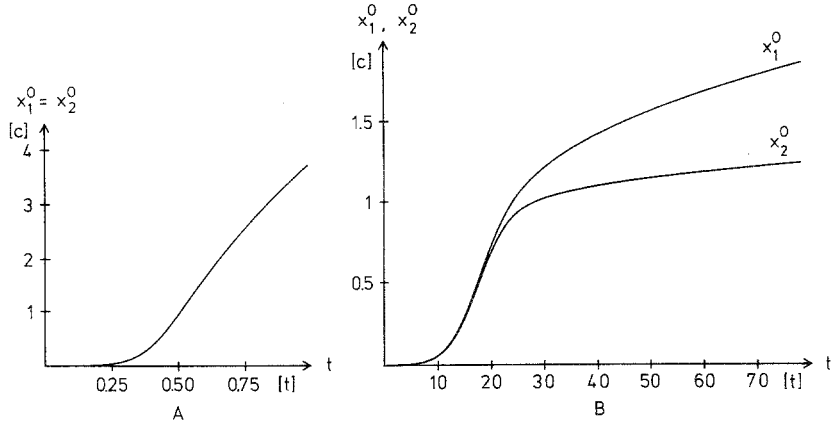

Fig. 9. Numerical integration of equation (71). The graphs show the time dependence of total concentrations x_1^0 and x_2^0. **A** corresponds to the conditions described in Fig. 7 with $k_1 = k_2 = 50$ and **B** corresponds to the conditions described in Fig. 8 with $k_1 = k_2 = 1$. Note that the curves can be subdivided in three phases of growth: exponential, linear and parabolic

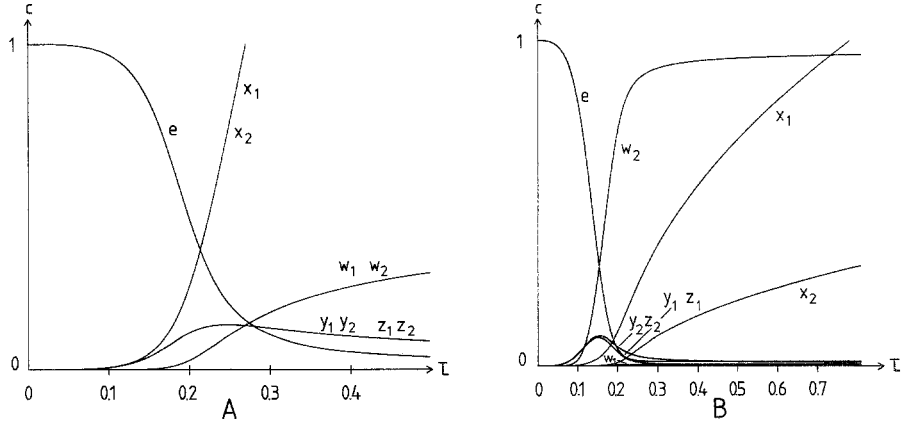

Fig. 10. Numerical integration of equation (72) under the condition of quasiequilibrium. **A** corresponds to the conditions described in Figs. 3 and 7, **B** to the conditions described in Figs. 5 and 8. The time axis is scaled in dimensionless units $\tau = t/k$; $k_1 = k_2 = k$. Note that plot **A** is almost identical with **7 A** and **7 B** and that plot **B** is almost identical with **8 A** and **8 B**

6. Conclusion

The simplified many-step mechanism of *RNA*-replication introduced in Fig. 1 reproduces the experimental results[4] although the polymerization process is described only as a single, over all reaction step. At excess of low molecular weight compounds, *ATP*, *UTP*, *GTP* and *CTP*, three phases of growth behaviour, namely exponential, linear and parabolic growth, of total *RNA* concentration are observed, if we go from low to high *RNA* concentration.

In order to study complex many step mechanisms approximations are inevitable. The concept of a fast pre-equilibrium preceding a slow reaction step, called quasiequilibrium approximation appears to be appropriate to describe *RNA* polymerization. The quasiequilibrium approximation is a standard method of analysis in relaxation kinetics[7] where one studies per definitionem the approach towards the equilibrium state. By means of simple consecutive first order reactions we were able to demonstrate that the quasiequilibrium approach can be extended to open, in particular to exponentially growing systems. Application of the quasiequilibrium to a mechanism of *RNA*-replication (Fig. 1) shows that this approach is valid over a wide range of rate constants.

The studies presented here were performed on the unconstrained system. This means that we assumed the existence of an inexhaustible reservoir of nucleoside triphosphates (*ATP*, *UTP*, *GTP* and *CTP*). These conditions are approximately fulfilled by the experimental setup used in most investigations[3–5]. There are, however, other equally important conditions like those encountered in flow reactors[6]. They can be described properly by constraints acting on the system growing without limits[8]. Investigations on the validity of the quasiequilibrium approximation in open systems with constraints will be subject of a forthcoming paper.

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